EFFECT OF HYSTERECTOMY ON THE OVARIAN CYCLE OF THE RAT

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Summary. The normal periodicity of the dioestrous cycle of the unmated rat was not permanently altered by hysterectomy, but when the operation was performed during or shortly after oestrus, the dioestrous interval was prolonged to a variable extent. The average duration of this period was longer than that of pseudopregnancy, but the vaginal smear and ovarian changes were very similar in both conditions. Subsequent dioestrous cycles were of normal length (4 to 5 days). The effect of hysterectomy on the current cycle probably resulted from cervical stimulation during the operation. Hysterectomy after sterile mating caused a similar prolongation of the current cycle and all subsequent cycles of pseudopregnancy in the hysterectomized rat. No ovarian degeneration was observed in rats killed up to 4 months after hysterectomy. Interspecific differences in the ovarian response to hysterectomy are briefly discussed.

INTRODUCTION

As far as we have been able to ascertain, the reaction of the ovary to hysterectomy has been studied in fourteen mammalian species, and it is quite clear that it differs greatly from one to another. A profound effect has been observed in the unmated adult guinea-pig (Loeb, 1923, 1927; Rowlands, 1961), cow and sheep (Wiltbank & Casida, 1956), and in the unmated or pregnant pig (Du Mesnil du Buisson & Dauzier, 1959; Spies, Zimmerman, Self & Casida, 1960), where the luteal phase may be so prolonged as to exceed the gestation period of the species. In contrast, the operation has no effect on the ovaries of the ferret (Deanesly & Parkes, 1933), the macaque (Burford & Diddle, 1936) or the opossum (Hartman, 1925). Changes in the ovarian cycle, similar in nature to those observed in the guinea-pig but much less pronounced, have been described in the pseudopregnant rat (Bradbury, 1937; Hechter, Fraenkel, Lev & Soskin, 1940; Bradbury, Brown & Gray, 1950) and rabbit (Asdell & Hammond, 1933; Loeb & Smith, 1936; Gillard, 1937; Mishell & Motyloff, 1941; Heckel, 1942; Chu, Lee & You, 1946). This operation during pregnancy in the rat (Bradbury, 1937; Bradbury et al., 1950), rabbit (Micare, 1940; Greep, 1941; Chu et al., 1946) and hamster (Klein, 1938) curtails rather than prolongs the life of the corpus luteum, especially when performed in the second half of gestation.
Apart from the changes that occur soon after operation, it has been shown that hysterectomy leads eventually to ovarian degeneration involving increased follicular atresia in the rat (see Spurny, 1959) and some other species, e.g. mouse (Hall, 1934), rabbit (Sessums & Murphy, 1933; Mishell & Motyloff, 1941; Tenney, Parker & Robbins, 1958), dog (Cheval, 1934, 1935) and cat (Baidin, 1939). These long-term effects have been revealed during investigations conducted with particular reference to the occurrence of ovarian cysts in women after hysterectomy (see Reynolds, 1949), and they have not been related to the more immediate effects on the ovarian cycle except by Sessums & Murphy and Mishell & Motyloff (loc. cit.), who showed that degenerative changes began within about 3 months after hysterectomy in the rabbit. The interval before ovarian degeneration is discernible in the rat is also 3 months or more, according to Hauptstein & Bühler (1936) and Winter (1932), but Spurny (1959) found some fibrosis and follicular atresia after only 20 days, increasing in the following weeks. Tietze (1952) on the other hand, found no disturbance of the vaginal cycle and no abnormalities of the follicular mechanism in the rat 6 to 8 months after hysterectomy, and Westman (1929) found no ovarian abnormalities in mice examined up to 10 months after prepubertal hysterectomy. It was not our intention to investigate the long-term effects of hysterectomy in the rat, except in so far as they may interfere with the cycle during the post-operative period covered by our observations.

Changes in the oestrous cycle that have been recorded after hysterectomy in the unmated rat are also contradictory. The dioestrous interval of the current cycle is often prolonged, presumably as a result of unavoidable manipulation of the cervix, but the effect is not maintained in subsequent cycles. This was noted by Durrant (1926) and confirmed by Bradbury (1937) and by Bradbury et al. (1950). Mavromati (1932) and Fels (1954) found that hysterectomy was followed by a prolonged oestrus associated with ovarian cysts, and it may be that the irregular cycles recorded by Winter (1932) and the suppression of the cycle reported by Takakusu (1924) were due to ovarian abnormalities caused by the act of removing the uterus rather than its subsequent absence.

As is well known, the ovarian cycle of the rat includes an active luteal phase after mating with a sterile male. The resulting pseudopregnancy is slightly prolonged in hysterectomized animals (Bradbury, 1937; Hechter et al., 1940; Bradbury et al., 1950). The present investigation was designed to confirm this observation and to relate the altered vaginal cycle to the histology of the ovaries, with regard to the growth and regression of the corpora lutea and their status, and to the general condition of the ovaries at successive stages of the investigation.

METHODS

The rats were of the Wistar strain bred in the Institute and weighed 190 to 200 g at the start of the experiment. Vaginal smears were made once daily to determine the oestrous rhythm before operation. Hysterectomy was performed under tribromoethanol (‘Avertin’) anaesthesia, administered intraperitoneally in a 2.5% solution at the rate of 1 ml/100 g body weight. The uterus was exposed through a ventral mid-line incision and ligated near the cervix. The
main uterine artery and vein and their ovarian branches were conserved, but
the uterine branches were ligated or cauterized before the mesometrium was
cut between the main vessels and the uterine horns in the way described by
Mayer, Tuchmann-Duplessis, Quelet & Sahy (1954). The body of the uterus
was cut as near to the cervix as possible. Only in some of the earlier experiments
were the Fallopian tubes ligated before cutting near to the utero-tubal junction.
The control operation consisted of the same anaesthetic procedure and laparo-
tomy and cutting the mesometrium without disturbing the blood supply.
Vaginal smears were again taken daily after operation.

Pseudopregnancy, induced by allowing the females to mate with a vasecto-
mized male, was dated from the recovery of a vaginal plug. Rats were killed by
chloroform and the ovaries immediately removed and fixed in Bouin’s fluid.
Serial sections were cut 5 µ in thickness, and every tenth section mounted and
stained with haematoxylin and eosin. The volume of the corpus luteum was
calculated as \( V = A \times 2D/3 \) where \( A \) is the area of the largest section, which
was measured by means of a Swift eyepiece circular graticule, and \( D \) is the
diameter calculated from the number of sections through which the corpus
luteum extends.

RESULTS

HYSTERECTOMY AT DIFFERENT STAGES IN THE DIOESTROUS CYCLE

During oestrus

The dioestrous interval was prolonged in five rats hysterectomized during
oestrus. Two were killed for examination of the ovaries, one on the 11th and
one on the 13th day of the prolonged cycle. Each pair of ovaries contained only
one set of large corpora lutea, varying in volume from 0.5 to 0.8 cu.mm. Older
corpora lutea were still distinguishable, but few of them exceeded 0.1 cu.mm.
Oestrus recurred 19, 20 and 29 days, respectively, after hysterectomy in the
remaining three rats, and the normal 4- to 5-day cycle was then resumed.

During metoestrus

Twenty-seven unmated rats were hysterectomized on the day after oestrus.
The onset of the next oestrus was delayed in eighteen of them, whereas the
normal dioestrous cycle was unaffected in the remaining nine. Six of those in
which the cycle was prolonged were observed through several subsequent
cycles which were of normal duration. The remainder (twelve rats) were killed
during or immediately after the prolonged cycle induced by hysterectomy.
Throughout this cycle, the vaginal smear was of the mucified type similar to
that of pseudopregnancy in intact rats. When killed, eight of these rats were
still in this condition (see Table 1). The ovaries were histologically similar to
those of intact pseudopregnant rats. Variation in the size of the corpora lutea
within each animal was of the order normally observed in the rat, but there
was considerable variation in the average luteal volume between individuals
(see Table 1). They were similar in size to those produced after mating with a
sterile male at a considerable time after hysterectomy or sham operation (see
Table 3, Pseudopregnancy VI).
In the eight animals in which the prolonged cycle was completed, its average duration was 18.0 ± 1.9 days, significantly longer ($P < 0.01$) than the average duration of pseudopregnancy (13.0 ± 0.45 days) recorded for fifteen rats of the same stock (see Table 2). The dioestrous cycles following the prolonged cycle were of normal duration in all six rats examined.

<table>
<thead>
<tr>
<th>Days after hysterectomy</th>
<th>Vaginal smear</th>
<th>Corpora lutea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Av. vol. ± S.E. (cu./mm)</td>
</tr>
<tr>
<td>5</td>
<td>Mucified</td>
<td>11 0.773 ± 0.025</td>
</tr>
<tr>
<td>12</td>
<td>'</td>
<td>11 0.588 ± 0.025</td>
</tr>
<tr>
<td>12</td>
<td>'</td>
<td>15 0.783 ± 0.022</td>
</tr>
<tr>
<td>13</td>
<td>'</td>
<td>11 0.626 ± 0.041</td>
</tr>
<tr>
<td>14</td>
<td>'</td>
<td>10 0.649 ± 0.021</td>
</tr>
<tr>
<td>14</td>
<td>'</td>
<td>15 0.654 ± 0.031</td>
</tr>
<tr>
<td>18</td>
<td>'</td>
<td>12 0.490 ± 0.022</td>
</tr>
<tr>
<td>18</td>
<td>'</td>
<td>14 0.671 ± 0.028</td>
</tr>
<tr>
<td>12</td>
<td>Pro-oestrus</td>
<td>10 0.609 ± 0.020</td>
</tr>
<tr>
<td>18</td>
<td>Oestrus</td>
<td>13 0.731 ± 0.042</td>
</tr>
<tr>
<td>9*</td>
<td>'</td>
<td>11 0.445 ± 0.021</td>
</tr>
<tr>
<td>28*</td>
<td>'</td>
<td>11 0.667 ± 0.038</td>
</tr>
</tbody>
</table>

* Ovulation had recurred.

**During early dioestrus**

A further six rats were hysterectomized on the 2nd day after oestrus. The operation had no effect on the recurrence of oestrus in five of them, but in the sixth animal there was a delay of 21 days before the normal dioestrous cycle was resumed.

These results show that the proportion of rats in which the cycle is prolonged decreases as the interval between oestrus and hysterectomy is increased. The response is probably related to the decreasing sensitivity of the uterine cervix to mechanical stimulation (Long & Evans, 1922; Greep & Hisaw, 1938).

**Hysterectomy after sterile mating**

Fifteen rats were examined daily through at least three dioestrous cycles before mating with a vasectomized male. After the resulting pseudopregnancy (I) and several further dioestrous cycles, a second pseudopregnancy (II) was induced in the same way, and eight of the rats were hysterectomized and seven sham-operated on the 5th day. Daily examination of the animals was continued through three further pseudopregnancies (III, IV and V) separated by three or four dioestrous cycles. Except for one rat which died after pseudopregnancy (V), they were killed during or soon after a further pseudopregnancy (VI) for histological examination of the ovaries.
Table 2 shows that the pseudopregnancy (II) in which the operations were performed was significantly longer than the pseudopregnancy (I) induced before surgical interference ($P < 0.001$ in the hysterectomized animals; $P < 0.05$ in the sham-operated controls). The mean duration of Pseudopregnancy II was not significantly longer in the hysterectomized than in the control-operated animals; however, the prolongation occurred in all the hysterectomized rats but in only three of the seven controls. Pseudopregnancies III, IV and V were significantly longer ($P < 0.001$) in the hysterectomized than in sham-operated animals. In the former group, these pseudopregnancies were similar in duration to Pseudopregnancy II, whereas in the controls they were similar to Pseudopregnancy I.

An intrinsic effect of hysterectomy was therefore seen only in Pseudopregnancies III, IV and V. This effect must be distinguished from a non-specific effect due to surgical interference; the latter was seen in the control animals in Pseudopregnancy II and in some unmated rats.

The mean volume of the corpora lutea of Pseudopregnancy VI in the

### Table 2

**EFFECT OF Hysterectomy AND SHAM OPERATION ON THE DURATION OF PSEUDO-PREGNANCY**

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Before operation (I)</th>
<th>Operation on the 5th day (II) Hysterectomy</th>
<th>Post-operative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(III)</td>
<td>(IV)</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>11</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>Mean and S.E.</td>
<td>12.9 ± 0.46</td>
<td>19.4 ± 0.96</td>
<td>17.0 ± 0.50</td>
</tr>
</tbody>
</table>

**Sham operation**

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Before operation (I)</th>
<th>Operation on the 5th day (II) Hysterectomy</th>
<th>Post-operative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(III)</td>
<td>(IV)</td>
</tr>
<tr>
<td>9</td>
<td>13</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>11</td>
<td>12</td>
<td>14</td>
<td>13</td>
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<tr>
<td>12</td>
<td>13</td>
<td>21</td>
<td>13</td>
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<tr>
<td>13</td>
<td>14</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>14</td>
<td>12</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>15</td>
<td>14</td>
<td>23</td>
<td>15</td>
</tr>
<tr>
<td>Mean and S.E.</td>
<td>13.3 ± 0.42</td>
<td>16.9 ± 1.47</td>
<td>13.1 ± 0.40</td>
</tr>
</tbody>
</table>

Roman numerals refer to successive pseudopregnancies.
Hysterectomy and the ovarian cycle

Hysterectomized and sham-operated rats, together with that of intact rats in pseudopregnancy and pregnancy, is shown in Table 3. In the three groups of pseudopregnant animals, the corpora lutea reached maximum size by the 5th day after mating and were maintained until ovulation recurred. The corpora lutea of the hysterectomized animals were maintained for a longer time, but did not grow larger than those of the sham-operated animals. In contrast, the corpora lutea of pregnancy suddenly increased in size between the 10th and 13th day, and became about three times as large as those of pseudopregnancy. The ovaries of the hysterectomized rats further resembled those of normal pseudopregnant animals in that the regression of corpora lutea of the preceding dioestrous cycle was not accelerated as it was in pregnancy.

**Table 3**

**MEAN VOLUME OF CORPORA LUTEA IN A SET (CU.MM)**

<table>
<thead>
<tr>
<th>Days after mating</th>
<th>Pseudopregnancy</th>
<th>Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In intact rats</td>
<td>After sham operation</td>
</tr>
<tr>
<td>5</td>
<td>0.916 ± 0.026</td>
<td>0.643 ± 0.020</td>
</tr>
<tr>
<td>10</td>
<td>0.861 ± 0.025</td>
<td>0.561 ± 0.030</td>
</tr>
<tr>
<td></td>
<td>0.900 ± 0.031</td>
<td>0.639 ± 0.015</td>
</tr>
<tr>
<td>13</td>
<td>1.062* ± 0.070</td>
<td>0.592* ± 0.038</td>
</tr>
<tr>
<td></td>
<td>0.956* ± 0.067</td>
<td>0.814* ± 0.021</td>
</tr>
<tr>
<td>15</td>
<td>0.748* ± 0.028</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0.602 ± 0.016</td>
<td>0.745 ± 0.047</td>
</tr>
<tr>
<td>19</td>
<td>0.794* ± 0.019</td>
<td>1.766 ± 0.050</td>
</tr>
</tbody>
</table>

* Ovulation had recurred and new corpora lutea were present.

The length of the dioestrous cycles between Pseudopregnancies II to VI was unaffected in both the hysterectomized and sham-operated rats. This supports the conclusion that hysterectomy as such has no effect on the cycle in the unmated rat.

**DISCUSSION**

The results show that the periodicity of the dioestrous cycle in the rat is unaffected by hysterectomy and that the regression of the corpora lutea, which are inactive in this condition, is not delayed. Pseudopregnancy, on the other hand, is about 4 days longer in the hysterectomized rat. Although this period of prolongation is very short compared with that recorded for the unmated guinea-pig, it is permanent in the sense that each successive pseudopregnancy is similarly prolonged. It is associated simply with a delay in the onset, rather than a decrease in the rate, of regression of the corpora lutea. This limited
response, as compared with that of the guinea-pig where the corpora lutea grow after hysterectomy to the size of those of pregnancy, may be due to the corpora lutea of the rat being less sensitive to stimulation. The difference between the two species may on the other hand be due to a difference in the nature of the stimulus that hysterectomy evokes.

The way in which the uterus regulates the persistence of the corpora lutea remains obscure. In the rat, the effect of hysterectomy on the corpus luteum is so brief, yet so consistently recurrent in each succeeding pseudopregnancy, that it would seem more readily explained on the hypothesis that hysterectomy has an 'oestrogen-sparing' effect (Heckel, 1942; see also Hechter et al., 1940) than by the assumption that the uterus functions as an endocrine organ (see Tenney et al., 1958; Reynolds, 1949). The role of oestrogen in the maintenance and regression of the corpora lutea will be clarified when more information is available about oestrogen and progesterone production by the follicles in the stages leading up to ovulation.

The great diversity in the response that has been observed in different species suggests that substantially different mechanisms are involved. Thus, experiments showing that endometrial extracts may reverse the effect of hysterectomy on the corpora lutea (e.g. Tenney et al., 1958; Hechter et al., 1940; Sessums & Murphy, 1953) show that the effect is blood-borne in the rat and rabbit. Du Mesnil du Buisson (1961), on the other hand, has shown that in the sow conservation of a sufficient length of uterine horn, one near the ovary leads to the regression of the corpora lutea in that ovary while those in the other ovary are maintained. This, together with the observations of Spies et al. (1960), that the effect of hysterectomy is not reversed by uterine transplants, suggests that nervous mechanisms are also involved in this species.

No evidence of the occurrence of ovarian degeneration was observed in these rats when they were examined 3 to 4 months after hysterectomy. They had mated with sterile males shortly before they were killed, and all had become pseudopregnant; this in itself is proof of normal ovarian function. Their condition was not comparable, therefore, with that of the hysterectomized rats used by Spurny and others (see Introduction) in which excessive follicular atresia and other abnormalities interfered with the periodicity of the oestrous cycle. The differences that have been noted in this respect are difficult to explain, but they may be related to strain differences in the time at which breeding activity normally ceases.

ACKNOWLEDGEMENT

We are grateful to Mr P. N. O'Donoghue, who prepared and examined the vaginal smears.

REFERENCES


Hysterectomy and the ovarian cycle


