STUDIES ON THE MECHANISM OF DECIDUALIZATION. I.

The oestrogen surge of pseudopregnancy and progravidity and its role in the process of decidualization

By

M. C. Shelesnyak, Peretz F. Kraicer and Gerard H. Zeilmaker

ABSTRACT

The concept of an oestrogen surge as a prerequisite for successful decidualization of the progravid uterus and consequently nidation in the rat was tested. Experiments were designed to see whether administration of an oestrogen antagonist at specific times would block decidualization (and nidation); whether the ovary was the source of the oestrogen, and whether a single dose of oestrogen, in the absence of the ovary (if the ovary is the source) would allow the uterus to respond to a decidualizing stimulus. It was found that when given prior to the surge, the oestrogen antagonist prevented decidualization and interfered with nidation. The ovary is thus the source of the oestrogen; and a single injection of oestradiol can act as a substitute for the surge.

Studies in this laboratory on the mechanism of ovum-implantation were initially directed towards the search for a metabolite which played a role in inducing decidualoma formation in the progravid uterus (Shelesnyak 1952, 1957). It was postulated that the deciduoma of Loeb (1907) was a satisfactory model system


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Mr. Zeilmaker participated in the first studies in these investigations, particularly those dealing with the spayed animals and those in which exogenous oestrogen was administered, during the early part of 1960 at which time he was a guest of the Department at the Weizmann Institute.
and »that the elucidation of a metabolic deciduoma-inducing factor (DIF) will permit a sharper focussing of the search for a factor associated with the blastocyst in the process of nidation« (Shelesnyak 1957, page 270).

A role for histamine in the induction of decidualization was established (Shelesnyak 1960). This information allowed the development of a method for inducing decidualization in the pseudopregnant or progravid rat which is more physiological than the classic traumatic technique (Shelesnyak & Kraicer 1961). The use of this technique made a critical examination of the processes of transformation of uterine stromal cells into decidual cells possible. Thus the genesis of decidual tissue in the pseudopregnant rat can be studied under controlled conditions (Shelesnyak & Kraicer 1960 a).

Indirect evidence was accumulated which suggested a theory of the mechanism of nidation which included an oestrogen surge as a requisite for the sensitization of the progestational uterus, so that the endometrium would be rendered capable of responding to the stimulus which provokes decidualization. This oestrogen surge is required during the period of progesterone predominance and is different from the oestrogen required for priming the uterus for proliferation.

The investigations reported here were undertaken to test the concept of the oestrogen surge. Indirect evidence suggested that the time of the pulse or surge occurred during the progestational phase (Shelesnyak & Kraicer 1960 a).

Experiments were designed to answer three questions:
1. Whether blocking oestrogen by anti-oestrogenic agents at specific times influenced decidualization;
2. whether the ovary was the source of the oestrogen; and
3. if the ovary was the source, whether in the absence of the ovary a single administration of exogenous oestrogen at the requisite time would allow sensitization of the uterus and subsequent decidualization.

METHODS AND RESULTS

Young adult female albino rats from the Weizmann Institute colony were used. They were approximately 100 days old and ranged in weight from 160 to 190 g. Animals showed at least two consecutive, normal four-day oestrous cycles prior to mating or induction of pseudopregnancy.

Pseudopregnancy was induced by faradic stimulation of the cervix uteri on the days of proestrus and oestrus. Day one of pregnancy and of pseudopregnancy was the day of appearance of the leucocytic vaginal smear. By this convention, the day on which the sperm is observed in the vaginal smear is designated as day zero of pregnancy.

Ovaries were removed through dorsolateral incisions using ether anaesthesia.
Intramuscular injections of 4 mg progesterone (0.1 ml of 4% solution in peanut oil) were administered daily from the time of ovariectomy until the day before post-mortem examination.

Deciduomata were induced by intraperitoneal injection of 1 ml of 2% aqueous solution of pyrathiazine hydrochloride at 10.00 h on day 4 of pseudopregnancy (Shelesnyak & Kraicer 1961). An oestrogen antagonist, MER-25 (Lerner et al. 1958; Segal & Nelson 1958) was injected intramuscularly as 0.1 ml of a fine suspension in peanut oil. Oestradiol-17 was injected intramuscularly; 2 μg were given in 0.1 ml of peanut oil.

Vaginal smears were examined daily between 09.00 and 10.00 h throughout the experiment. At post-mortem examination on day 8 of pregnancy or of pseudopregnancy the uteri of the pregnant rats were examined for implantation sites; uteri of the pseudopregnant rats were examined for the presence of decidual reaction. Decidual response was graded according to the scoring system described previously (Shelesnyak & Kraicer 1961).

**Series 1: The effect of anti-oestrogen (MER-25) on decidual induction**

The influence of blocking oestrogen with MER-25 was investigated by administration of the anti-oestrogen on day 3 or 4 of pregnancy or pseudopregnancy. The protocols and results for these experiments are presented in Tables 1 and 2.

### Table 1.
The effect of a single injection of anti-oestrogen MER-25 during progestation on nidation in the rat.

<table>
<thead>
<tr>
<th>Time of injection</th>
<th>Dose of MER-25 (mg/♀)</th>
<th>No. Without implants</th>
<th>Resorption sites (♀♀ with)</th>
<th>Normal sites (♀♀ with)</th>
<th>Number of normal implants per ♂</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.00 h on Day 4</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>8, 1, 7, 7, 8, 9, 11, 13</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>7, 7, 8, 8, 9, 10, 11</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>8, 3, 6, 8, 8, 9, 10, 10, 11</td>
</tr>
<tr>
<td>10.00 h on Day 3</td>
<td>0</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>7, 7, 8, 8, 9, 9, 13</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>4, 6, 9, 10</td>
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<tr>
<td></td>
<td>10</td>
<td>8</td>
<td>2</td>
<td>3</td>
<td>3, 4, 6, 7</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

* Animals killed on Day 8 of pregnancy.
Table 2.

<table>
<thead>
<tr>
<th>Treatments*</th>
<th>MER-25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of injection</td>
<td>Dose (mg/♀)</td>
</tr>
<tr>
<td>10.00 h on Day 4 of pseudopregnancy</td>
<td>10</td>
</tr>
<tr>
<td>10.00 h on Day 3 of pseudopregnancy</td>
<td>20</td>
</tr>
<tr>
<td>10.00 h on Day 4 of pseudopregnancy</td>
<td>5</td>
</tr>
<tr>
<td>10.00 h on Day 3 of pseudopregnancy</td>
<td>10</td>
</tr>
</tbody>
</table>

* Decidual induction by i. p. injection of pyrathiazine, 1 ml of 2 per cent w/v, at 10.00 h of Day 4 of pseudopregnancy.
** Animals killed 96 hours after pyrathiazine injection.
*** Maximum possible DIS = 4.0.

Administration of MER-25 on day 4 of pregnancy or pseudopregnancy did not influence nidation or decidualization. Post-mortem examination of the uteri on day 8 of pregnancy revealed normal nidation sites (Table 1). Examination of the pseudopregnant uteri 96 hours after the decidualizing stimulus was injected, revealed normal, massive decidual reactions (Table 2). Normal leucocytic configuration of vaginal smears persisted throughout the experiment.

Ten or 20 mg of MER-25 injected on day 3 prevented decidualization and interfered with nidation. Five mg was less effective. Vaginal smears were normal throughout the experiment.

Series 2: The ovary as the source of the oestrogen

The role of the ovary as the source of the oestrogen which was susceptible to blockade by MER-25 was investigated by ablation of the ovary. By performing the ovariectomy at different times during day 3 an attempt could be made to fix or establish the time of the assumed oestrogen surge. The results are presented in Table 3.

In animals ovariectomized at 12.00 h of day 3 of pseudopregnancy, the response to pyrathiazine induction of decidualization was abolished. In animals ovariectomized at 24.00 h this response was unaffected. Ovariectomy at 16.00
**Table 3.**
Effect of ovariectomy on Day 3 of pseudopregnancy on induction of deciduoma.

<table>
<thead>
<tr>
<th>Time of ovariectomy during Day 3 of pseudopregnancy</th>
<th>Treatments</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hour</td>
<td>Proportion of rats bearing deciduomata</td>
<td>Proportion of uterine cornea with massive deciduomata</td>
</tr>
<tr>
<td>12.00</td>
<td>22</td>
<td>0/12</td>
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<tr>
<td>16.00</td>
<td>18</td>
<td>6/12</td>
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<tr>
<td>20.00</td>
<td>14</td>
<td>8/11</td>
</tr>
<tr>
<td>24.00</td>
<td>10</td>
<td>12/12</td>
</tr>
</tbody>
</table>

* Decidual induction by i.p. injection of 1 ml (2 per cent w/v) pyrathiazine at 10.00 h on Day 4 of pseudopregnancy.
** Massive deciduomata = more than half of uterine horn full of decidual tissue.
*** Maximum possible DIS = 4.0.

**Table 4.**
Effect of oestradiol (2 μg) on non-traumatic induction of decidualization in ovariectomized pseudopregnant rats. Ovariectomy done on Day 3 between 08.00 and 10.00 h.

<table>
<thead>
<tr>
<th>Time in injection of 2 μg oestradiol during Day 3 of pseudopregnancy</th>
<th>Treatments</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hour</td>
<td>Proportion of ♀♂ bearing deciduomata</td>
<td>Proportion of uterine cornea with massive deciduomata</td>
</tr>
<tr>
<td>12.00</td>
<td>22</td>
<td>5/11</td>
</tr>
<tr>
<td>16.00</td>
<td>18</td>
<td>7/12</td>
</tr>
<tr>
<td>20.00</td>
<td>14</td>
<td>8/12</td>
</tr>
<tr>
<td>24.00</td>
<td>10</td>
<td>7/11</td>
</tr>
<tr>
<td>Pooled results</td>
<td>27/46</td>
<td>16/92</td>
</tr>
</tbody>
</table>

* Decidual induction by i.p. injection of 1 ml of 2 per cent w/v of pyrathiazine at 10.00 h of Day 4 of pseudopregnancy.
or 20.00 h allowed decidualization, but to a lesser degree; fewer uteri responded and massive reactions were observed only one-third as frequently as in the group spayed at 24.00 h.

**Series 3: Sensitization of the uterus in the absence of the ovary**

Since both oestrogen antagonist MER-25 and ovariectomy prevented decidual induction, restoration of uterine sensitivity was attempted using exogenous oestrogen. Oestradiol, in a single dose was administered at various times to rats which had been ovariectomized before 12.00 h. The results obtained are presented in Table 4.

Administration of 2 µg of oestradiol allowed decidualization in 60% of the rats. No relationship was observed between the time of injection (between 12.00 and 24.00 h) and the number of uteri which gave a response or the degree of decidualization. Some vaginal cornification was observed on the day following the administration of oestradiol.

**DISCUSSION**

In a recent publication (Shelesnyak & Kraicer 1960 a) evidence for the existence of an oestrogen surge was reviewed under the following three headings:

1. Studies on endometrial mast cells and histamine content of the uterus;
2. vaginal, ovarian, and pituitary changes indicative of persistence of periodicity during progestation; and
3. the oestrogen induction of ovum implantation after a period of delayed nidation.

That oestrogen is a prerequisite for nidation, was first proposed in the analysis of histamine-oestrogen-progesterone interrelationships associated with decidual cell reaction and ovum implantation in the rat (Johnson & Shelesnyak 1958). Subsequently it was also postulated that the essential oestrogen was secreted as a surge or pulse during progestation (Shelesnyak 1959). The occurrence of a pulse or discharge of oestrogen secretion received a measure of support from the observation that sensitivity to a decidual-inducing stimulus is transient in the untraumatized rat endometrium (Kraicer & Shelesnyak 1959; Shelesnyak & Kraicer 1960 b). Such a pulse may well be a manifestation persistence of cyclic follicular activity which was observed during pregnancy (Nelson 1929; Swezy & Evans 1930).

The hormonal requirements for deciduomata have been of great interest since Loeb's original observation of the dependence of uterine reaction on the presence of functional corpora lutea (Loeb 1907). The traumatic induction of deciduomata became a standard method in studies on the progestational hormones and on the decidualization process. The essential hormonal requirements
for decidual response to traumatic stimulus were shown to be limited to adequate progesterone (Rothchild et al. 1940; Chambon 1954). This dependence on progesterone alone was at variance with the essential role of oestrogen in nidation (Krebhiel 1941; Mayer & Meunier 1959), and indicated a difference between the decidual response to trauma and to the ovum.

Development of a technique for the non-traumatic induction of decidualization has allowed exploration of the relationship between decidualization and the postulated oestrogen surge. It was found that the time of endometrial sensitivity to a non-traumatic stimulus was as sharply delimited in time as the nidatory decidual response; traumatic stimulation, however, was effective over a longer period, suggesting less specificity and less parallelism with the nidatory decidualization.

The experiments reported here indicate that blockade of oestrogen on day 3 prevents the induction of decidualization (in the presence of amounts of progesterone sufficient to maintain decidual development). Chemical interference with oestrogen by an oestrogen antagonist, MER-25, prevented the induction of decidualization in pregnant and pseudopregnant rats. On day 4, MER-25 had no effect on the induction of the decidual reaction. These results suggest that the oestrogen acts in some way to initiate processes prerequisite to successful decidual induction. If the MER-25 is given before oestrogen has acted, decidualization cannot be induced. MER-25 administered after the oestrogen has acted, no longer antagonizes decidual induction.

Finn & Emmens (1961) have recently reported studies with dimethylstilboestrol, a compound with both oestrogenic and anti-oestrogenic effects, on the trauma induced decidualoma in the rat. Their findings are in agreement with the oestrogen surge concept which we have proposed (Shelesnyak 1959) but their report, which appeared in abstract form, did not present sufficient detail to enable comparisons to be made.

It was shown that the oestrogen surge occurs after 12.00 h of day 3 of pseudopregnancy. Animals ovariectomized at 12.00 h of day 3 of pseudopregnancy failed to respond to the decidual inducing stimulus. When ovariectomy was performed 12 hours later, the uterus responded normally. It is concluded that the ovary is the source of the oestrogen surge.

If animals are ovariectomized before noon of day 3 of pseudopregnancy, the uterine sensitivity to decidual induction can be restored by exogenous oestrogen administered at the time when the oestrogen surge would have occurred. A dose of 2 μg of oestradiol restored responsiveness equally well at any time from between 10 to 22 hours before decidual induction.

In conclusion, there is a surge or discharge of oestrogen, or a surge of oestrogen secretion by the ovary, which is a prerequisite for uterine decidualization. This oestrogen surge takes place during the later half of day 3 of pseudopregnancy and probably also during pregnancy, and precedes the
time of peak endometrial sensitivity to decidual induction by 12 to 20 hours. This oestrogen is essential for successful nidation.

ACKNOWLEDGEMENTS

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