EFFECTS OF CONCURRENT LACTATION ON LITTER SIZE AND PRENATAL MORTALITY IN AN INBRED STRAIN OF MICE

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Summary. Analyses of recorded data showed that fewer young were born in second litters than in first litters of JU/Fa mice and that the reduction in the size of the second litters occurred only when lactation was concurrent with gestation.

The effect of concurrent lactation on prenatal mortality in second pregnancies was experimentally tested. 46% of lactating females and 7% of non-lactating females, mated in the post-partum oestrus, lost whole litters. Dissections of lactating and non-lactating pregnant females showed that concurrent lactation caused a significant decrease in the number of live embryos. This was the consequence of the extremely high post-implantation mortality—48.3% of implanted embryos—in lactating females. The excess deaths occurred mainly in the 'middle' period, i.e. from 5 to 7 days after implantation.

Progesterone was injected into nineteen mated lactating females from the 5th to the 17th day of lactation. The doses given were equivalent to 2.5 mg/day over this period. Whole-litter losses did not occur and the incidence of 'middle' post-implantation mortality was reduced to 'non-lactating' levels. Implantation, which was markedly delayed in untreated lactating females, occurred 1 or 2 days after the first injection in all but one treated female. It is therefore suggested that concurrent lactation causes progesterone deficiency during pregnancy in JU mice.

INTRODUCTION

The variation of litter-size with parity in the mouse is well-known. The size of the first litter is sub-maximal and litter size increases until a maximum is reached in the second pregnancy or later. This maximum is maintained for a variable number of pregnancies and is followed by a continuous decline in litter size as the mouse ages. The pattern is similar in the rat and the initial increase in litter size is also characteristic of the pig (see Biggers, Finn & McLaren, 1962, for review). The number of eggs shed, as measured by corpora lutea counts, increases sharply during early reproductive life in the mouse (MacDowell & Lord, 1925, 1927) and in the pig (Perry, 1954), and this is probably the cause of the observed increase in litter size after the first pregnancy, in these animals.

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In the course of an experiment on the effect of crossing inbred strains of mice on fertility (McCarthy, 1963) it was found that the litter size of one strain, JU/Fa, as measured by the number of living young at birth, was greater in the first pregnancy than in the second. This finding was confirmed when recorded data were analysed. The cause of this unusual decrease in litter size was investigated by further analysis of the records and by experimental tests of the effects of concurrent lactation and the administration of exogenous progesterone.

**Table 1**

A COMPARISON OF MEAN LITTER SIZE IN FIRST AND SECOND PREGNANCIES IN JU MICE (1960–62)

<table>
<thead>
<tr>
<th>JU Stock</th>
<th>Diet</th>
<th>Pregnancy</th>
<th>No. litters</th>
<th>Mean litter size</th>
<th>Difference first minus second</th>
</tr>
</thead>
<tbody>
<tr>
<td>1960–61</td>
<td>A</td>
<td>First</td>
<td>42</td>
<td>8-0 ± 0-3</td>
<td>1-3 ± 0-5*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Second</td>
<td>42</td>
<td>6-7 ± 0-4</td>
<td></td>
</tr>
<tr>
<td>1961–62</td>
<td>B</td>
<td>First</td>
<td>42</td>
<td>9-1 ± 0-3</td>
<td>1-9 ± 0-5**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Second</td>
<td>42</td>
<td>7-2 ± 0-4</td>
<td></td>
</tr>
<tr>
<td>1962 (i)</td>
<td>B</td>
<td>First</td>
<td>25</td>
<td>9-9 ± 0-4</td>
<td>2-1 ± 0-7**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Second</td>
<td>25</td>
<td>7-8 ± 0-5</td>
<td></td>
</tr>
<tr>
<td>1962 (ii)</td>
<td>B</td>
<td>First</td>
<td>23</td>
<td>9-0 ± 0-3</td>
<td>1-8 ± 0-8*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Second</td>
<td>23</td>
<td>7-2 ± 0-5</td>
<td></td>
</tr>
</tbody>
</table>

*P is the probability that a difference occurred by chance.
*P<0.05; **P<0.01.

**METHODS AND RESULTS**

**ANALYSIS OF RECORDS**

The JU strain is listed in the Standardized Nomenclature for Inbred Strains of Mice, 1960 (Cancer Research, vol. 20). It was developed in this laboratory and records were available for 3 years after it had passed its twentieth consecutive full-sib mating. The stock was maintained by full-sib matings, caged in pairs, so that males were continuously present. Litters were weaned at 21 days of age. Records of litter size were analysed for the years 1960–62. All females which had borne at least two litters were included in the analysis. For the purpose of analysis the records were divided into three periods of approximately 1 year. This was necessary because the diet was changed between the first and second period. Two groups of mice were included in the 1962 period: (i) those from the stock, and (ii) mice specially bred for other purposes and kept in a different room.

The sizes of first and second litters were compared within each period. The results are shown in Table 1. The number of young obtained from the second pregnancy was significantly lower in each comparison. Although the size of the first litter was apparently influenced by diet, the ranking of litter size with respect to pregnancy did not change. An analysis of variance showed no significant heterogeneity between the sizes of second litters in the four periods and further analysis was conducted on the pooled data.
Second litters were born 20 to 38 days, or 40 to 100 days, after the births of first litters. The seventy-four litters born in the former interval were assumed to have resulted from post-partum inseminations and the fifty-three litters in the latter interval from inseminations after the weaning of first litters. The mean litter sizes of these two groups of second litters were $6.5 \pm 0.3$ and $8.1 \pm 0.4$, respectively, the difference between them, $1.6 \pm 0.5$ being significant at the 1% level. Thus, reduction in the size of second litters occurred when the second litter resulted from insemination at the post-partum oestrus, and when the mother was consequently suckling the first litter concurrently with the gestation of the second. This suggests that concurrent lactation was responsible for the reduced size of the second litters, though there is no evidence from previous studies that lactation has any effect on litter size in the mouse (Bruce & East, 1956; Roberts, 1956). It is possible, however, that it was the time of mating and not the concurrent lactation that was responsible, and the experiments described in the next section were undertaken in order to decide between these two possibilities, as well as to determine the stage of gestation at which the reduction in litter size took place.

**DISSECTION OF PREGNANT FEMALES**

*Method*

Three groups of pregnant females were dissected. All were pregnant with their second litters, but the groups differed according to whether they were concurrently suckling or not, and whether the second litter resulted from post-partum or post-weaning insemination. These three groups were:

*Group 1*. Lactating females with second litters resulting from post-partum insemination and concurrently suckling their first litters,

*Group 2*. Non-lactating females with second litters resulting from post-weaning insemination and not concurrently suckling,

*Group 3*. Non-lactating females with second litters resulting from post-partum insemination but not concurrently suckling (young of first litter removed).

Females of these three sorts were obtained as follows. Virgin JU females were mated at 8 weeks of age, in single-pair matings, with males of the same strain, and the males were left with the females. Births were recorded daily, and post-partum mating was determined by the presence of a vaginal plug. Females which failed to mate in the post-partum oestrus were discarded. The first litters were removed at birth from some females, which were assigned to Group 3. The remaining females were allowed to suckle their litters for 21 days, after which the litters were weaned. These females were examined daily for pregnancy, by palpation, and for mating, as shown by the presence of a vaginal plug. Those that became pregnant (about 50%) were assigned to Group 1, since the pregnancy resulted from the post-partum mating. Those that mated after the first litter was weaned were assigned to Group 2.

The females of Groups 2 and 3 were dissected at 17 days of gestation, timed from the vaginal plug. Females of Group 1, however, could not be dissected at a fixed stage because of the variable delay in implantation. They were dissected when they were found to be pregnant, and the ages of the embryos were determined by the criteria of Grüneberg (1943). The age of these embryos ranged
from 10 to 19 days, with a mean of 14 days. The difference between the interval, in days, from post-partum mating to dissection and the developmental age of the live embryos at dissection provided an estimate of the delay in implantation associated with concurrent lactation. This method of estimating the duration of delay in implantation was valid, as the absence of vaginal plugs during the suckling period showed that mating did not occur during lactation.

At dissection, counts were made of (1) the number of corpora lutea, as a measure of the number of eggs shed, (2) the number of live embryos and (3) the number of dead implants. The difference between (1) and the total number of implants, given by the sum of (2) and (3), indicated pre-implantation losses. Post-implantation losses were indicated by (3).

Dead implants were classified on a morphological basis to indicate how soon after implantation death had occurred. Those represented by 'moles' were scored as 'early' deaths. Any implant which was represented by an undersized placenta without a live embryo was scored as a 'middle' death. Dead embryos of recognizable bodily form were scored as 'late' deaths.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Time of insemination</th>
<th>No. dissections</th>
<th>Mean age of live embryos (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Concurrent lactation</td>
<td>Post-partum</td>
<td>43</td>
<td>14</td>
</tr>
<tr>
<td>2. Non-lactating</td>
<td>Post-partum</td>
<td>36</td>
<td>17 (all)</td>
</tr>
<tr>
<td></td>
<td>and post-weaning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Non-lactating: first litter removed</td>
<td>Post-partum</td>
<td>28*</td>
<td>17 (all)</td>
</tr>
</tbody>
</table>

* Two females were not pregnant and are excluded.

The essential features of the three experimental groups of pregnancies are summarized in Table 2. The number of pregnant females dissected in each group is shown. Two females, from which first litters were removed at birth, did not become pregnant. The comparison of Groups 2 and 3 tested whether the time of mating affected the size of second litters. The comparison of Group 1 with either Group 2 or 3 tested whether concurrent lactation affected the size of second litters.

Delay in implantation

Implantation was delayed in all concurrently lactating females. The duration of the delay ranged from 5 to 17 days and the mean was 9-8 days. The distribution of the duration of delay and the relationship between the mean number of young suckled and the duration of delay are shown in Table 3. The weighted regression of the duration of the delay in implantation, in days, on the number
of young weaned was highly significant ($b = +1.4 \pm 0.30$, $P<0.001$). Although no correlation has been found between the number of young weaned and the prolongation of gestation in some studies (e.g. Bruce & East, 1956) there are many reports of a positive correlation between the two variables in mice and rats (see Enzmann, Saphir & Pincus, 1932; Weichert, 1940, 1942, for reviews).

**The components of litter size**

Table 4 shows the numbers of corpora lutea and live young and the percentage mortality, estimated from dissections of the three groups of females. There was no evidence that the time of mating influenced litter size, as measured by the number of live embryos at dissection. The number of live embryos in non-lactating females was not reduced when litters resulted from post-partum inseinations. The difference between the mean numbers of live young in Groups 2 and 3, $0.5 \pm 0.57$, was not significant.

### Table 3

<table>
<thead>
<tr>
<th>Delay in days</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12 to 13</th>
<th>14 to 15</th>
<th>16 to 17</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. litters</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>10</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Mean No. weaned</td>
<td>6.3</td>
<td>7.0</td>
<td>7.4</td>
<td>8.7</td>
<td>4.0</td>
<td>7.3</td>
<td>9.0</td>
<td>8.5</td>
<td>9.5</td>
<td>9.5</td>
</tr>
</tbody>
</table>

All the females in the experimental groups had mated in the post-partum oestrus, so that the thirty-six females in Group 2 did not become pregnant after post-partum insemination. Thus $36/79$ (46%) of all females that suckled their first litters lost whole litters during the suckling period. When first litters were removed from thirty females in Group 3 only two (7%) failed to become pregnant to post-partum insemination. It was thus concluded that concurrent lactation increased the incidence of whole-litter losses. Such losses may have occurred because of the failure of blastocysts to implant or because of whole-litter death in the post-implantation stages. Either of these events would be followed by remating when the first litter was weaned and this is how pregnancies in Group 2 were obtained. As the nature of these whole-litter losses was not ascertained, they were excluded from estimates of the proportions of pre-implantation or post-implantation mortality in litters of lactating females and do not appear in Table 4. Thus one or both of the latter estimates for Group 1 in Tables 4 and 5 is not truly representative of losses in lactating females and is biased downwards.

It is apparent from Table 4 that concurrent lactation caused a large reduction in the number of live embryos at dissection. The mean number of live embryos in concurrently lactating females was significantly less than that in non-lactating females. The differences between the mean number in Group 1 and
the mean numbers in Groups 2 and 3 were $3.2 \pm 0.54$ and $3.7 \pm 0.58$, respectively, both of which were significant at the 0.01% level. The large reduction in the number of live embryos in lactating females was not explained by either a decrease in the number of eggs shed or by an increase in pre-implantation mortality. This is clear from the fact that the mean number of total implantations in Group 1 was not significantly different from that in either group of non-lactating females. The average number of implantations in lactating females was $10.4 \pm 0.27$ and in two groups of non-lactating females was $9.8 \pm 0.35$ and $10.9 \pm 0.41$, respectively. Table 4 shows that post-implantation deaths occurred with an extremely high frequency—almost 50% of the implanted embryos—in concurrently lactating females. Consequently, the number of live embryos was much reduced in these females. The proportion of post-implantation mortality calculated for concurrently lactating females (48.3%) may be an underestimate because, firstly, counts were made at an earlier developmental stage in Group 1 than in the other two groups and, secondly, it does not include whole-litter losses which might have occurred in the post-implantation period.

**Table 4**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean No. corpora lutea</th>
<th>Mean No. total implants</th>
<th>Mean No. live embryos</th>
<th>Pre-implant. loss (%)&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Post-implant. loss (%)&lt;sup&gt;†&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lactating</td>
<td>$11.8 \pm 0.20$</td>
<td>$10.4 \pm 0.27$</td>
<td>$5.4 \pm 0.36$</td>
<td>9.2</td>
<td>48.3</td>
</tr>
<tr>
<td>2. Non-lactating</td>
<td>$11.7 \pm 0.24$</td>
<td>$9.8 \pm 0.35$</td>
<td>$8.6 \pm 0.37$</td>
<td>16.2</td>
<td>11.6</td>
</tr>
<tr>
<td>3. Non-lactating: first litters removed</td>
<td>$12.4 \pm 0.25$</td>
<td>$10.9 \pm 0.41$</td>
<td>$9.1 \pm 0.41$</td>
<td>11.8</td>
<td>17.0</td>
</tr>
</tbody>
</table>

<sup>*</sup> Expressed as a percentage of the count of corpora lutea.<br> <sup>†</sup> Expressed as a percentage of the total number implanting.

The nature of post-implantation mortality

The partitioning of post-implantation deaths, according to the stage of development reached by dead implants, in the second pregnancies of lactating females and of the two groups of non-lactating females, is shown in Table 5. The incidence of mortality in litters of lactating females was higher than in non-lactating females in all post-implantation stages. The most divergent estimates were found for the 'middle' period, 26% of total implants in lactating females dying at this stage of development, i.e. from 5 to 7 days after implantation. In contrast, the incidence of 'middle' deaths in non-lactating females was only 2%. The latter was consistent with other estimates of later post-implantation mortality in different strains of mice (Lyon, 1959; McCarthy, 1963).

At dissection the uteri of lactating females were usually found to be lined with extravasated blood. Although no histological study was attempted, it was apparent that placentae in lactating females were subject to internal necrosis. This was observed in the peripheral region of some placentae of apparently
Effects of lactation on pregnancy in mouse

healthy foetuses. Some surviving placentae of 'middle' deaths were observed to be totally necrotic when dissected under the binocular microscope, whereas in others necrosis was local. These features were noted in the majority of autopsies of pregnant lactating females.

The possible basis of the effects of lactation is suggested by the nature of mortality in litters of lactating females. The features associated with post-implantation death, including placental necrosis, were similar to the consequences of post-implantation ovariectomy in the rat (Huggett & Pritchard, 1945). Krehbiel (1941a) found that unilateral ovariectomy in mated lactating rats resulted in a high proportion of whole-litter losses and in a substantial decrease in the number of young born to females which did not lose the whole litter—a situation very similar to that observed in intact lactating JU mice. The similarity between the observed effects of ovariectomy in pregnant rats and the effects of lactation on fertility in JU mice suggests that the excessive prenatal mortality in second litters of JU mice might be due to progesterone deficiency. The following experiment was carried out to test this.

### Table 5

POST-IMPLANTATION DEATHS CLASSIFIED BY DEVELOPMENTAL AGE, AS A PERCENTAGE OF TOTAL IMPLANTATIONS, IN LACTATING AND NON-LACTATING FEMALES

<table>
<thead>
<tr>
<th>Group</th>
<th>Total No. implantations</th>
<th>Early deaths</th>
<th>Middle deaths</th>
<th>Late deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>1. Lactating</td>
<td>449</td>
<td>86</td>
<td>19.2</td>
<td>117</td>
</tr>
<tr>
<td>2. Non-lactating</td>
<td>352</td>
<td>30</td>
<td>8.5</td>
<td>7</td>
</tr>
<tr>
<td>3. Non-lactating: first litters removed</td>
<td>306</td>
<td>42</td>
<td>13.7</td>
<td>7</td>
</tr>
</tbody>
</table>

### TREATMENT WITH PROGESTERONE

JU females, which mated in post-partum oestrus, were allowed to suckle their first litters. Males were removed after post-partum mating. Females were subsequently assigned to one of three groups which were treated as follows:

(a) Nineteen females were injected with 5.0 mg of progesterone in oil ('Lutocyclin', CIBA) on the 5th day and on every 2nd day thereafter until the 17th day after post-partum mating (designated Treated in Table 6).

(b) Nineteen females were injected with equal volumes of vegetable oil at similar intervals (designated Injected controls in Table 6).

(c) Fourteen females were not injected (designated Uninjected controls in Table 6).

The first litters were weaned at 21 days of age and the females were killed and dissected on or after the 21st day post coitum. The components of litter size were recorded and the duration of delay in implantation was calculated as described above.
Effects of progesterone treatment

The proportion of females pregnant at dissection, the range of duration of delay in implantation and the incidence of post-implantation mortality in the three post-implantation stages are shown for the treated and control groups in Table 6. All progesterone-treated females were pregnant at dissection and there was a marked reduction in the duration of delay in implantation. Implantation occurred 1 or 2 days after the first injection of most females. The delay in implantation in treated females was longer than 3 days in only one case. In contrast, all the females in the oil-injected control group, except one, lost whole litters. No apparent explanation of the extremely high proportion of whole-litter losses in this group can be offered. A medium proportion of uninjected females were pregnant at dissection, as was observed in the previous experiment. Data on the six pregnant dissected females in the two control groups were pooled to get some control estimate of post-implantation mortality.

Table 6

<table>
<thead>
<tr>
<th>Group</th>
<th>No. in group</th>
<th>Pregnant</th>
<th>Delay in implantation</th>
<th>Post-implantation mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>1 to 5 days</td>
</tr>
<tr>
<td>Treated</td>
<td>19</td>
<td>19</td>
<td>100</td>
<td>8* to 14 days</td>
</tr>
<tr>
<td>Injected controls</td>
<td>19</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Uninjected controls</td>
<td>14</td>
<td>5</td>
<td>36</td>
<td></td>
</tr>
</tbody>
</table>

* Pooled estimates for controls.

'Middle' deaths in the treated females were rare. An estimate of 2·4% was made in nineteen litters. There were no symptoms of placental necrosis in any of the developing conceptuses, and uteri were free of extravasated blood. Compared with the controls, there was a high incidence of early post-implantation deaths, identified as 'moles' (Table 6). The control data in this experiment were, however, rather meagre, and comparison with the larger sample of untreated lactating females in the first experiment (Table 5) shows that the proportion of 'early' deaths in litters of treated females (18·2%) was not higher than in litters of untreated lactating females (19·2%). Treated females contained as many live embryos at dissection (8·6 ± 0·71) as did non-lactating females (8·6 ± 0·37, from Table 4).

Lactation continued after progesterone treatment. This was expected as there is no report in the literature that progesterone can inhibit lactation. Folley (1942) found that massive doses of progesterone, as high as 15 mg daily, had no observable effect on established lactation in the rat. All treated females reared their litters but three killed their litters on the last day of the suckling period. The latter may have occurred because the second litters had reached term
before the first litters were weaned. This resulted from the prevention of delayed implantation by treatment. The remaining sixteen treated females had $7.7 \pm 0.41$ young, on average, in the first litters at weaning.

**DISCUSSION**

*Effect of lactation on fertility*

The observation that the mean number of young born in the second litter was lower than in the first, was not typical of the pattern of variation in fertility of the mouse and was not made in five other inbred strains maintained by the author. The potential litter size of JU females in the second pregnancy, as measured by the number of corpora lutea, was greater than in the first pregnancy. The mean number of corpora lutea in second pregnancies (shown in Table 4) was about 12.0; whereas the mean number in first pregnancies was $11.0 \pm 0.15$ (McCarthy, 1963). The observed decrease in the number of live young in second pregnancies was due to the marked increase in post-implantation mortality in litters of concurrently lactating females, which comprised about half of all recorded second litters. Concurrent lactation was responsible for this excessive mortality and also for a high proportion of whole-litter losses. The removal of first litters at birth confirmed this.

The JU strain showed no decrease in the number of young born in the first litter during the course of inbreeding (Falconer, 1960). Yet inbreeding depression in fertility did occur and it is reflected by the incapacity of females to maintain litter size in the second pregnancy.

**Hormonal basis of the effects of concurrent lactation**

When the equivalent of 2.5 mg of progesterone/day was administered to JU females from the 5th to the 17th day of lactation, the effect of concurrent lactation on prenatal mortality in second pregnancies was negated. Treated females had as many live young, in second litters, as non-lactating females. The long delay of implantation, the whole-litter losses, and the high proportion of ‘middle’ post-implantation mortality, characteristic of pregnancies of untreated females, were eliminated. This suggests that progesterone production was suboptimal during pregnancies concurrent with lactation. This is not in agreement with previously published explanations of the influence of lactation on pregnancy. In other studies, the influence of concurrent lactation on pregnancy in mice and rats has been reflected only in delayed implantation. Whitten (1958) successfully prevented delayed implantation in lactating mice with small doses of oestrogen. Similar results have been obtained with lactating rats (Krehbiel, 1941b; Weichert, 1942). Such results are interpreted as indicating oestrogen deficiency induced by lactation. Yet, neither the mode of action of small doses of oestrogen in preventing delayed implantation nor the means by which oestrogen deficiency could arise are agreed upon. Whitten (1958) suggested that exogenous oestrogen releases the blastocyst from inhibition induced by excess progesterone and that the lack of oestrogen is caused by reduced secretion of gonadotrophin. Krehbiel (1941b) and Weichert (1942) suggested that the oestrogen acts synergistically with progesterone in sensitizing the uterus and that oestrogen deficiency is due either to lack of gonadotrophin (Krehbiel, 1941b).
1941b) or to removal of oestrogen in the milk (Weichert, 1942). Amoroso & Finn (1962) suggested the further possibility that small amounts of oestrogen may reinforce the activity of the corpus luteum.

Generally, the explanations in the literature have implied that the cause of delayed implantation is similar in mice and rats. There is evidence that the needs of embryo implantation are not similar in these two species and, further, that the hormonal patterns during lactation may be different. The hormonal requirements for implantation in these animals have been determined by ovariectomy followed by administration of exogenous hormones. In ovariectomized rats progesterone and oestrogen were necessary for implantation (Mayer, 1963; Nutting & Meyer, 1963). Progesterone alone was sufficient to induce implantation in mice which were ovariectomized 1 or 2 days after mating (Smithberg & Runner, 1956). Postponing hormone treatment delayed implantation in ovariectomized mice or rats but, whereas injection of progesterone alone prolonged the delay in rats (Cochrane & Meyer, 1957), it terminated the delay in mice (Smithberg & Runner, 1956). The latter authors found that implantation could be induced and pregnancy maintained in castrated pre-puberal mice by daily injections of 1·0 or 2·0 mg of progesterone, i.e., in the absence of exogenous or ovarian oestrogen. On the basis of these findings, a reduction in the secretion of progesterone would seem the most likely cause of delayed implantation in lactating mice, whereas a deficiency of either progesterone or oestrogen could cause the delay in lactating rats. There is some evidence that progesterone production is sub-optimal in lactating mice. Greenwald (1958) failed to produce deciduomata in lactating mice and concluded that progesterone production was inadequate to elicit and maintain deciduomata on finding that treatment with exogenous progesterone facilitated decidual response. In contrast to these negative results with lactating mice, deciduomata can be induced in lactating rats (Lyon & Allen, 1938; Krehbiel, 1941c). In the light of these considerations the prevention of delayed implantation in lactating mice by oestrogen may be achieved as suggested by Amoroso & Finn (1962), i.e., by reinforcing the action of the corpus luteum.

The effects of concurrent lactation on implantation and prenatal mortality in JU mice would seem best explained in terms of progesterone deficiency. It would be desirable to confirm the results of exogenous progesterone treatment at lower doses before speculating on how lactation induced the deficiency. Because the interaction of concurrent lactation and pregnancy results in such marked disorder of pregnancy in JU mice, it may be erroneous to extend the explanation of these findings to other strains of mice, but in view of the severity of the interaction, this strain may be useful in further analysis of the hormonal basis of delayed implantation in lactating mice.

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**Effects of lactation on pregnancy in mouse**

REFERENCES


