OLFATORY BLOCK TO PREGNANCY AMONG GROUPED MICE

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Summary. The incidence of the pregnancy-block caused by alien males was not augmented by increasing the numbers of males to which the female was exposed, but it was reduced by the presence of other females in proportion to their numbers.

When females were deliberately crowded during pregnancy an increased susceptibility to disease was noted, but there was no evidence of the hormonal disturbance otherwise associated with such stress.

A mild degree of stress was indicated even when the housing conditions were adequate, among mixed populations formed after mating, but not among pregnant females housed together in the absence of males.

Pregnancy failure was more than three times as frequent under inadequate physical conditions of housing during Days 1 to 3 after mating, than under standard control conditions.

INTRODUCTION

Olfactory stimuli cause significant modifications in the reproductive cycle of female mice. The oestrous rhythm is disrupted by the grouping of females. If the groups are small there is an increase in the incidence of spontaneous pseudopregnancies (Lee & Boot, 1955); if the groups are large many females become anoestrous (Whitten, 1959). In the unmated female, the odour of a single male is sufficient to cancel the established influence of the combined olfactory effects of many females, for Whitten (1959) showed that the introduction of only one male, caged, into a stock box containing as many as thirty females, most of which were anoestrous at the time of introduction, caused over 80% of them to come into heat within 4 days.

In the pregnant female the effect of male odour is more complex. After exposure of the recently-mated female to males other than the stud male, implantation does not take place, nor is the regular 5-day cycle interrupted; the female returns to oestrus as if mating had not occurred. If the males to which the female is exposed are of the same strain as the stud male, pregnancy fails in 25 to 30% of females, but if they belong to a different strain as many as 80% of pregnancies may be blocked (Parkes & Bruce, 1961). Once implantation is established the pregnant female no longer reacts to male odour, even of alien males (Bruce, 1961), but no test situation has been devised in which

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pregnancy fails regularly in all females. The strength of olfactory stimulation is
difficult to assess, and it may be that under the various conditions so far examined
the stimulus has been insufficient to elicit a maximum response.

Early experiments on the olfactory block to pregnancy in mice suggested that
the mere proximity of several males might provide a stronger stimulus than
physical contact with one male (Bruce, 1959). Under the particular conditions
then employed, the females were generally tested in pairs, and for the proximity
experiments they were caged inside a stock box containing several males each of
which contributed towards the total olfactory effect received by the females.
Olfactory stimulation of male origin had therefore been augmented, but, at the
same time, the physical conditions of the test were changed because the females
were confined instead of free, and for the period of exposure they were housed in
stock boxes. Scanty data both from tests on the olfactory block to pregnancy
(Bruce, 1960) and from experiments on the effect of fasting (Bruce, 1963)
suggest that a mutual reaction between females may help to stabilize pregnancy.
But conclusive evidence that community living among females produces the
same hormonal reaction after mating as in the unmated female is lacking.

Neither is information available about possible competition between the
effects on the pregnant female of male odour and female odour when both are
present. Ultimately both appear to affect pituitary luteotrophin, and hence
pregnancy indirectly, but with opposite effects. The relative sensitivity of the
mated female to each could therefore have ecological implications.

Attempts to clarify some of these points have been made in the experiments
described below.

MATERIALS AND METHODS

MICE

P Strain, randomly-bred albino, maintained as a closed colony for many years
and derived originally from a few pairs purchased from a dealer.

Dutch strain, randomly-bred piebald, derived from a few pairs purchased
from a dealer in 1959.

TO Strain, randomly-bred albino, derived from an inbred strain (Gledhill,
1959).

CBA Strain, inbred from the colonies maintained at the Medical Research
Council’s Radiobiological Research Unit, Harwell.

HOUSING

Metal boxes with containers for food and water inserted in the lid, and with
sawdust as floor covering, were used. No nesting material was supplied. The
boxes were of the following dimensions: stock box—wire mesh lid = 20 × 15 × 5
in.; small box—solid lid = 16 × 6 × 4 in. Diet 41B (Bruce, 1963) was used.

THE TEST

Young virgin females about 3 months old were paired with stud males and
examined daily for mating. When the vaginal plug was found (Day 0), the
female was separated from the stud male and housed alone or in groups as
indicated in the text; 24 hr later she was introduced into the box containing the test male (or males) or moved to an empty box (control). The female remained in the test situation for 3 days at the end of which she was returned to the box she had originally occupied on separation from the stud male. Grouped females were treated in the same way, remaining together as a group throughout. Vaginal smears were examined daily. As in previous work (Bruce, 1960), the

**Table 1**

<table>
<thead>
<tr>
<th>STUD → TEST</th>
<th>PROPORTION OF FEMALES RETURNING TO OESTRUS WHEN HOUSED WITH</th>
<th>ONE MALE</th>
<th>THREE MALES</th>
</tr>
</thead>
<tbody>
<tr>
<td>P male × P female → CBA male</td>
<td>43/57 75%</td>
<td>28/38 74%</td>
<td></td>
</tr>
<tr>
<td>TO male × P female → P male</td>
<td>33/60 58%</td>
<td>23/38 61%</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>SINGLE FEMALE</th>
<th>PROPORTION OF FEMALES RETURNING TO OESTRUS</th>
<th>CAGED FEMALE</th>
<th>FREE FEMALE</th>
<th>WEIGHTED MEAN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10/34</td>
<td>3/8</td>
<td></td>
<td>31</td>
</tr>
<tr>
<td>1</td>
<td>9/11</td>
<td>11/15</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9/11</td>
<td>6/15</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10/11</td>
<td>11/15</td>
<td>81*</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>9/10</td>
<td>11/16</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>14/15</td>
<td>14/15</td>
<td>93</td>
<td></td>
</tr>
</tbody>
</table>

* Test of homogeneity $\chi^2 = 2.29, P>0.5$.

return of oestrus within 7 days of the stud mating was regarded as indicating a blocked pregnancy.

**RESULTS**

**STRENGTH OF STIMULUS SUPPLIED BY GROUPED MALES**

One series of experiments had involved exposing the recently-mated female to three other mice. A comparison was therefore made between the behaviour of females each in contact with three males and that of females, in other tests, each in contact with a single male. Records were available for P strain females in two tests of discrimination: (1) stud P male then exposed to CBA males, or (2) stud TO male then exposed to P males (Table 1).
More pregnancies failed in the first situation than in the second (75 and 60% respectively) but in neither test was the effect of three males together greater than that of a single male. The observations were then extended to larger groups of males. To prevent the fighting which takes place when adults are grouped, males which had been reared together since weaning were used. These tests were carried out in stock boxes in order to avoid overcrowding of the largest group (sixteen males). An increase in the number of males to which the female was exposed again failed to raise the incidence of pregnancy-block significantly (Table 2 and Text-fig. 1).

Spontaneous failure of pregnancy in the P strain without the intervention of male mice is about 8% (Bruce, 1959; Parkes & Bruce, 1962), but in this particular experiment pregnancy failed in more than three times as many control females (31%). Unacceptable housing conditions appeared to be the most likely explanation, since the control females had also been placed in a stock box, each female alone, for the period of test instead of in the usual type of small box.

PREGNANCY-BLOCK AMONG GROUPED FEMALES EXPOSED TO A SINGLE MALE

The question of competition between the olfactory influences of males and females was examined by reversing the experimental conditions so that the females were grouped together before exposure to the male (Table 3 and Text-fig. 2). Each test male was tried with all sizes of group as far as possible; this precaution may have been unnecessary because alien strains were involved and because no difference between the pregnancy-blocking capacity of individual males was detected under the conditions of test, but it could be essential in tests carried out within a single strain. The suspicion concerning the acceptability of stock boxes arising from the previous experiment precluded the
further use of this size of box. The largest group examined therefore contained only eight females. This number could just be accommodated adequately in the small box which had been designed originally for six mice.

The presence of other females during the period of exposure protected against the influence of the male proportionally to the number of females in the group. With eight females together the incidence of pregnancy-block was less than half that in females exposed singly (24% and 57% respectively). Nevertheless, even with this number in the group, a significant number of pregnancies ($P<0.001$) failed by comparison with the control females (7% failures).

Four strains of females were examined; all showed the same trend (Table 3) and it is especially interesting to note that pregnancy was less easily disturbed among TO females than among females of the other three strains.

**SOCIAL STRESS AND PREGNANCY FAILURE**

A decrease in fertility among groups of sixteen mice (eight males and eight females) housed under crowded conditions for 1 week has been reported by Southwick & Bland (1959). Social stress might therefore have contributed to the number of pregnancy failures, at least among the females exposed to sixteen males. To investigate the possibility of the intervention of social stress in addition to olfactory stimulation among grouped mice, the fertility of the stud mating was recorded after housing the recently-mated females under a variety of conditions (Table 4). The groups, like those which had been exposed to males, were established permanently on finding the vaginal plug and removing the female from the stud male; 17 days later the females were isolated for the birth of the
### Table 3

**Protection against pregnancy-block afforded by grouped females**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Male Stud→Test</th>
<th>Proportion of females returning to oestrus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One male, one female</td>
<td>One male, two females</td>
</tr>
<tr>
<td>P</td>
<td>P→CBA</td>
<td>43/57 (69%)</td>
</tr>
<tr>
<td></td>
<td>TO→P</td>
<td>28/46 (70%)</td>
</tr>
<tr>
<td>TO</td>
<td>P→CBA</td>
<td>19/39 (33%)</td>
</tr>
<tr>
<td></td>
<td>TO→P</td>
<td>3/27 (9%)</td>
</tr>
<tr>
<td>CBA</td>
<td>P→CBA</td>
<td>15/22 (68%)</td>
</tr>
<tr>
<td>Dutch</td>
<td>TO→P</td>
<td>10/18 (56%)</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>57%*</td>
</tr>
</tbody>
</table>

*Test of homogeneity \(\chi^2 = 25.04, P<0.001\).*
litters and supplied with nesting material. Fertility was estimated as the proportion of matings resulting in live births. Vaginal smears were not taken in this experiment. While previous experience has shown that frequent change of box does not disturb the establishment of pregnancy (Parkes & Bruce, 1962), excessive soiling in addition to an unsuitable microclimate might constitute another form of social stress. To minimize the risk of this happening, the small boxes which contained sixteen females were changed daily. Even within 24 hr they became very dirty, ventilation was inadequate so that the sides and lid of the box were coated with moisture and the temperature in the sawdust was several degrees higher than that of the boxes housing a single female, the range per 24 hr being 76 to 86° F and 69 to 77° F, respectively.

It will be seen from Table 4 that the fertility of the stud matings was not

<table>
<thead>
<tr>
<th>Housing</th>
<th>Treatment</th>
<th>No. mated females</th>
<th>Litters born from stud mating</th>
<th>Pregnant females becoming sick*</th>
<th>Fertility of stud mating (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small box</td>
<td>Control female left with stud male&lt;br&gt;female isolated</td>
<td>16</td>
<td>15</td>
<td>8-3</td>
<td>1 86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28</td>
<td>23</td>
<td>8-8</td>
<td>1 78</td>
</tr>
<tr>
<td>Stock box</td>
<td>Mated females together</td>
<td>24</td>
<td>19</td>
<td>8-3</td>
<td>0 63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31</td>
<td>24</td>
<td>7-5</td>
<td>1 45</td>
</tr>
<tr>
<td>Small box</td>
<td>Mated females crowded with virgin females&lt;br&gt;(about equal numbers of each, sixteen females/box)</td>
<td>30</td>
<td>19</td>
<td>9-3</td>
<td>6</td>
</tr>
<tr>
<td>Stock box</td>
<td>Mated female + stud male grouped as pairs&lt;br&gt;twelve pairs/box&lt;br&gt;seventeen pairs/box</td>
<td>48</td>
<td>24</td>
<td>9-2</td>
<td>5 86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>5</td>
<td>9-2</td>
<td>1 78</td>
</tr>
</tbody>
</table>

* Streptobacillus moniliformis?

seriously impaired by crowding together the pregnant females, but that it was reduced as expected by the presence of males.

During this experiment several of the females, particularly in these two groups, became sick during pregnancy. The symptoms, general loss of condition, staring coat, and arrested pregnancy were compatible with infection by *Streptobacillus moniliformis* to which the pregnant mouse is especially susceptible and which has appeared from time to time among P mice in this Institute (Sawicki, Bruce & Andrewes, 1962). Bacteriological examination of the sick females could not be carried out, unfortunately, but a tissue extract from one of them caused sickness and abortion when injected into another pregnant female, thus implying that an infective agent rather than hormonal disturbance—the postulated effect of overcrowding (Christian & Lemunyan, 1958)—was the cause of the breakdown.

If the six females which became sick are omitted from the crowded females, the fertility of the remainder (nineteen out of twenty-four) returns to within
normal limits. Elimination of the sick females from the females crowded with males fails to restore the fertility of the remainder (twenty-nine out of fifty-nine). In the group as a whole less than half the females carried the original pregnancy to term, six became sick during pregnancy, eighteen were pregnant again from matings subsequent to that with the stud male, and the remaining twelve females (18%) were still not pregnant even after 17 days of cohabitation. It is reasonable to assume that in these females three or four successive pregnancies had been blocked (Bruce, 1962); none of the females had implantation scars in the uterus.

The fertility of grouped females adequately housed was not significantly lower than that of the control females and only one out of fifty-five became sick.

**DISCUSSION**

More efficient blocking by CBA males than by P males suggests that the difference between the odour ‘spectra’ (Parkes & Bruce, 1961) is greater between CBA and P than between TO and P, so that more females perceive the difference and react to it. To what extent strain odours are modified by inbreeding is not known, but it is evident that exposure to several males, whether of an inbred or outbred strain, is no more effective than exposure to a single male, the stimulus supplied by him receiving no reinforcement from the presence of other males. A broader odour spectrum is therefore no more effective than a narrower one, provided both are sufficiently different from that of the stud male. The olfactory pattern impressed upon the female by the ‘individual’ stud male determines her subsequent behaviour towards another male. This implies that the discrimination which operates in pregnancy block is of a very high order, and is different from that required to recognize females for, by contrast, the reaction of the mated female to other females is closely related to the size of the group, as in the effect on the oestrous cycle among unmated females. The mutual protection against the olfactory influence of the male is strong presumptive evidence that her reaction is indeed the same as that of the unmated female, namely an increase in the production of prolactin by the adenohypophysis (Lee & Boot, 1956) presumably as the result of inhibition of the hypothalamus. Twenty-four hours elapsed before the introduction of the male because the groups were formed when the vaginal plug was found. During this period sufficient luteotrophin may have been produced in some females to tide over the critical period of pituitary inhibition caused by the intervention of the male, so that some of the pregnancies which would normally have been blocked were carried to term. Nevertheless, even when as many as eight females were grouped together in the presence of males the pregnancy rate was not maintained at the level found in control females. The olfactory influence of the male is thus partially, but only partially, effective in the face of competition from the odour of other females. Table 4 also reveals the presence of strain differences in the reaction of the female. Pregnancy was less easily disturbed among TO females exposed to males either singly or in groups than among P females similarly exposed; at all levels tested, about twice as many pregnancies from the stud mating were carried to term (Table 3). Fewer females from the
CBA and Dutch strains were examined. These showed great variability but in general females of both strains appeared to possess about the same power of discrimination as those of the P strain.

Social stress among the crowded females, and to a lesser extent among the females grouped with males, is indicated by the lowered resistance to infection among these two groups, although, without the intervention of male mice, there was no overt failure of reproduction even among the crowded females. Increased susceptibility to disease in dense populations has been reported for meadow voles by Louch (1956) and for rats by Calhoun (1962) but, as only one of the pregnant females grouped together but not crowded became sick, population density *per se* does not appear to be the crucial factor initiating the stress in these experiments. Southwick & Bland (1959) found that among groups of from two to eight mice housed together for 1 week adrenal hypertrophy was also more likely to occur among mixed populations than among males alone.

The influence of the physical environment on the establishment of pregnancy in the deer mouse has been illustrated in experiments by Eleftheriou, Bronson & Zarrow (1962). The high spontaneous failure of pregnancy among control females housed alone in stock boxes during Days 1 to 3 of pregnancy suggests that even the far more domesticated laboratory mouse may be affected by the physical environment in unsuspected ways, as has been emphasized by Crov- croft (1962).

In comparing these results with those of other workers on the effects of social stress (e.g. Southwick & Bland, 1959; Christian & Lemunyan, 1958; Calhoun, 1962) distinction must be made between the results of crowding before or after mating. Only the establishment and maintenance of pregnancy are in question when the mated female is subjected to crowded conditions, but in very adverse circumstances mating and fertilization may also be affected. In the mouse several mountings are generally made before intromission, and ejaculation may last as long as 25 sec (McGill, 1962). Under severe crowding interruption before the completion of the act might occur. That this could provide a hazard was suggested by Louch (1956) who found that the number of matings among crowded meadow voles increased, but that few were completed. Such a crowding effect might occur with laboratory mice in addition to the olfactory pregnancy-block.

It seems likely that some degree of social stress may have occurred among the single females housed with sixteen males. The increased proportion of blocked pregnancies, although statistically similar to that in other groups in the experiment, was nevertheless the highest recorded, but it seems unlikely that among the smaller groups social stress reached an effective level as little infection occurred.

ACKNOWLEDGMENTS

I am indebted to Miss M. V. Mussett for the tests of statistical significance; to Dr E. V. Hulse of the Medical Research Council Radiobiological Research Unit, Harwell, for the supply of CBA mice; and especially to Professor A. S. Parkes, C.B.E., F.R.S. for much constructive criticism, to all of whom I extend my thanks.
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