Reprinted from *Biology of Reproduction*, Volume 22, No. 4, May 1980. Pages 777-780.

Variation in Length of the Estrous Cycle in Mice Due to Former Intrauterine Proximity to Male Fetuses[1]

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ABSTRACT

Female mouse fetuses occupying an intrauterine position between male fetuses exhibit longer estrous cycles in adulthood than do females formerly residing in utero next to other female fetuses. This difference in adult cycle length was observed consistently when the two types of females were maintained under a variety of social conditions. Thus, these data suggest a fundamental biasing of the intrinsic timing of the estrous cycle by former intrauterine proximity to males in this and possibly other species in which more than one fetus is present in the uterus.

INTRODUCTION

The mammalian estrous cycle encompasses a complex series of changes in the brain, pituitary and ovary, all synchronized by hormonal and environmental cues to promote ovulation and sexual receptivity. The length of this cycle varies considerably between species and, in most cases, variation among conspecifics is also observed (Alleva et al., 1971; Nequin et al., 1979; Schwartz, 1969). The present report documents one source of such individual variation in mice, namely, that there is a direct effect of intrauterine proximity to males on the length of a female's estrous cycle during later adult life.

In the present experiments, female mouse fetuses located in utero between two male fetuses (designated here as 2M females) and female fetuses adjacent to other female fetuses (0M females) were collected by cesarean delivery and fostered to dams that had just given birth naturally. The length of the estrous cycles of these two types of females was compared in adulthood while they were either 1) housed individually or grouped in the presence of a male or 2) housed individually or grouped in an environment free of males. This spectrum of housing conditions was deemed an experimental necessity because of the sensitivity of the mouse's estrous cycle to regulation by social (pheromonal and tactile) cues. It is well established now, for example, that female mice emit cues that prolong the estrous cycles of other females, while stimuli emanating from adult males accelerate cycling (Bronson, 1979; Vandenbergh, 1974; Whitten and Champlin, 1973). Thus, among females that are grouped together in the absence of males, prolonged and irregular cycles are usually observed, while adult females that are either grouped with males present or housed individually exhibit shorter and more regular cycles. The present experiments were designed to determine whether possible effects of intrauterine position on estrous cycle length would relate to differential sensitivity to extrinsic (social) cues or reflect a more fundamental influence on the intrinsic timing of the adult estrous cycle.

MATERIALS AND METHODS

One hundred and fifty adult CF-1 females were paired with males each morning between 0800–1000 h. Inseminated females bearing vaginal plugs (25–35 each day) were individually housed (Day 0 of pregnancy). On Day 19 of pregnancy at 0900 h, the females that had not yet begun to deliver (about 75% of the pregnant females) were killed by cervical dislocation and the fetuses were removed from the uterus. The sex of the pups was determined by observing the size of the anogenital space and the 0M and 2M females were saved. The pups were removed from the mothers that had just delivered naturally and 6 experimental pups from the same intrauterine position were

Accepted January 15, 1980.
Received October 1, 1979.
[1]This research was supported by grant HD03803-11 from NICHHD.
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fostered to each dam. The 0M and 2M females were weaned when 21 days old and kept in foster-litter groups until being assigned to an experiment. Animal rooms were maintained at 23 ± 1°C on a 14L:10D cycle with lights on at 0600 h. Separate rooms were available for the separation of males and females when necessary.

RESULTS

Our initial experiment was conducted with 60-day-old 0M and 2M females that had been housed since birth in a room containing males. A total of 20 females from each intrauterine position were placed in 30 × 30 × 15 cm cages that were divided in half by a wire-mesh partition. Five females from the same former intrauterine position were housed on one side of the screen and an adult male was housed on the other side to accelerate the females' cycles. One complete cycle was measured for each animal by daily vaginal lavage (1300–1400 h). A complete estrous cycle was considered to be the number of days between fully cornified vaginal smears, with the first observation of such a smear being counted as Day 1 of the cycle being studied. Under these housing conditions 0M females were found to have significantly shorter estrous cycles than did 2M females [0M females, mean cycle length in days ± SEM = 5.3 ± 0.1 vs 6.4 ± 0.5 for 2M females; $t(38) = 2.2$, $P<0.05$].

The next experiment involved a partial replication and an extension of the previous study in that it assessed estrous cycle length between another set of 0M and 2M females when housed individually or in like-groups as adults in the presence of a male. These females were 90 days old at the start of the experiment and had been housed since weaning in a room free of male odor. The barrier-type cages described above were used again in this experiment. Twenty females from each intrauterine position were housed as previously described in like-groups of 5 across a barrier from a male. Ten other females of each type were housed individually across the barrier from a male. The first complete estrous cycle subsequent to the first estrous smear was determined for all females. When group housed in the presence of a male, 90-day-old 0M females again had significantly shorter estrous cycles than did 2M females (Fig. 1A; $t(38) = 2.07$, $P<0.05$). When housed individually in the presence of a male, 0M females also exhibited shorter cycles than did 2M females, but with only 10 animals/group, this difference did not reach statistical significance [$t(18) = 2.04$, $P = 0.06$; Fig. 1A].

The final experiment examined estrous cycle length in 150-day-old 0M and 2M females when housed individually or in groups in the same male-free room in which they had been housed since weaning, thus eliminating any possible influence of male produced cues on the estrous cycles of these females. Because of the expectation of prolonged cycles, vaginal smears were obtained daily for 4 weeks from 15 females of each intrauterine position maintained in foster-litter groups of 5 per 18 × 29 × 13 cm cage. Another 16 females of each type were examined for the same length of time after being rehoused 1 per cage. Pilot studies had indicated that 60–90-day-old CF-1 females that had been raised in groups in a male-free environment would not exhibit regular estrous cycles when tested while individually housed. However, by 150 days of age, such females exhibited regular cycles in the absence of male stimulation when housed individually (cf. Vandenbergh et al., 1972). This experiment thus assessed the possibility that 0M and 2M females might have estrous cycles that were intrinsically different in length, independent of any potential differences due to regulatory cues emanating from conspecifics of one or both sexes. Each female's mean cycle length was first calculated and the data then were analyzed by t test. All females grouped in the absence of a male exhibited prolonged cycles, with no significant differences between 0M and 2M females being noted. When housed individually in the absence of males, however, 0M females again exhibited shorter estrous cycles than did 2M females [$t(30) = 2.1$, $P<0.05$; Fig. 1B].

Ovulation is known to be a relatively constant accompaniment of vaginal estrus when adult mice are housed with males, but this is not always true when adult females are housed away from males (Stiff et al., 1974). To verify that the individually-housed females studied in this experiment were experiencing ovulatory cycles, 7 isolated females of each type were killed on the morning of the first estrus that was observed after the 4 week test period; 7/7 0M females and 6/7 2M females had tubal ova at this time, indicative of normal, adult cycles.

DISCUSSION

Given the well established importance of social cues to the estrous cycle of the mouse,

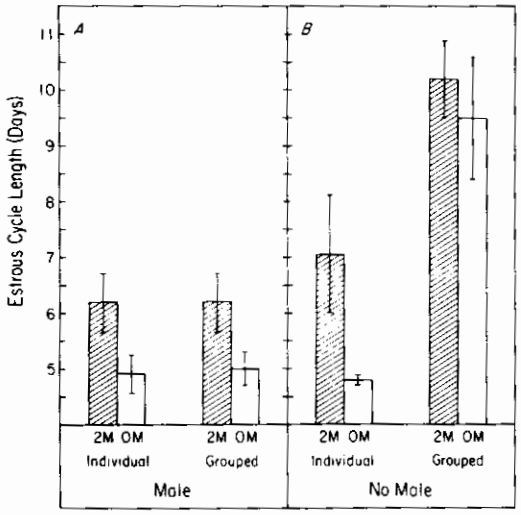

FIG. 1. Mean length of the estrous cycles (in days) of 0M and 2M females housed individually or grouped, 5/cage: A) when measured over one complete cycle with a male present on the other side of a wire-mesh barrier; B) when measured over a 4 week test period in a male-free environment.

individual variation in the length of this cycle could be traceable either to its intrinsic control, to its extrinsic regulation by social cues or to both factors. The present experiments were designed to separate these two potential sources of variation. To summarize the data presented in Fig. 1, grouping of adult female mice in the absence of a male, regardless of the female's former intrauterine position, resulted in cycles that were greatly prolonged. Thus, the production of and/or the sensitivity to the cues originating from females that interfere with the exhibition of regular cycles in mice is not related in adult females to former intrauterine position (Bingel, 1972; Drickamer, 1974; Ryan and Schwartz, 1977; Whitten, 1958). When cycle length was shortened and regularized either by isolation from other females or by male exposure, or both, adult 0M females exhibited shorter estrous cycles than did 2M females. This consistency reveals a fundamental biasing of the length of the adult estrous cycle by former intrauterine proximity to males and suggests an intrauterine effect on the intrinsic mechanisms that regulate cycle length.

Previously, both morphological and behavioral differences between 0M and 2M females have been reported. Specifically, 2M females have larger anogenital spaces at birth (a sensitive bioassay for androgen exposure), they are more aggressive in a variety of situations and urine-mark their environment at a higher rate; 0M females, on the other hand, are highly preferred by males that are allowed to choose between the two types of females (vom Saal and Bronson, 1978).

We recently determined that 2M female fetuses have significantly higher levels of testosterone in their blood and amniotic fluid than do 0M female fetuses. Importantly, the mother's circulation does not appear to be involved in this difference (vom Saal and Bronson, 1980). This finding supports the hypothesis that 0M and 2M females differ in their morphology, stimulus characteristics and behavior as a result of the 2M female fetuses having been exposed to elevated levels of androgens produced by contiguous male fetuses. It is well documented that exposure to elevated levels of testosterone within the first few days after birth via exogenous administration can have profound effects on a female mouse's reproductive capacity. Specifically, such females are incapable of ovulating during later life (Barraclough and Leathem, 1954; Gorski, 1979). It is interesting to note that the period during which the developing brain of a mouse becomes maximally sensitive to the influence of testosterone on the capacity to exhibit the "female" pattern of cyclic hormone production in adulthood is just after birth. Thus, this sensitive period occurs just after the time that females that develop next to male fetuses (about 70% of all females) cease being exposed to elevated levels of androgens produced by the male fetuses. The present as well as previous findings indicate that while female mice that develop in utero between male fetuses have adult estrous cycles that are prolonged, there is no decrement in the capacity to ovulate, mate, produce and raise normal numbers of healthy young when these females are housed singly (vom Saal and Bronson, 1978).

The present data extend the range of reproductively important processes that appear to be biased by former intrauterine position to now include a nonbehavioral effect on reproductive potential. While the full range of reproductive correlates of former intrauterine position probably is not yet realized, the picture that is emerging suggests that in utero proximity of female mice to male fetuses may yield variation

in reproductive potential during later life by way of several pathways. Whether such effects are common in other species in which more than one fetus is present in the uterus remains to be established.

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RECOMMENDED REVIEWS

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