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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Zoologia. — *Estrous cyclicity in Swiss mice from social conditions inhibiting estrus* (*). Nota di ROSANGELA CINQUETTI e LUCIANA RINALDI, presentata (**) dal Socio S. RANZI.

RIASSUNTO. — Le femmine di topo mantenute in gruppi isosessuali presentano irregolarità della ciclicità estrale o sono bloccate in anestro. L'estro si verifica rapidamente nelle femmine tolte dal gruppo e stabulate singolarmente.

Questa ricerca è stata condotta per esaminare l'andamento della ciclicità estrale dopo un lungo periodo di blocco provocato dalla stabulazione in grossi gruppi o dalla presenza di femmine adulte dalla pubertà. Un primo lotto, esaminato in settembre, era formato da femmine provenienti da gruppi numerosi; un secondo lotto, esaminato in giugno, era costituito da gruppi di sorelle coetanee tenute con la madre dalla nascita. Tutte le femmine erano della stessa età (circa 5 mesi) e provenivano dalla nostra colonia di topi Swiss. Allevamento ed esperimento sono avvenuti in presenza olfattoria di topi maschi.

In entrambi i lotti la ciclicità si è prontamente ristabilita. Tuttavia le femmine provenienti da grossi gruppi presentavano cicli di durata significativamente maggiore, maggior numero di cicli lunghi e aberranti, periodi più prolungati di metaestro e diestro. Le differenze tra i due gruppi potrebbero dipendere dalle precedenti condizioni di stabulazione, ma anche dalla stagione; sono quindi necessarie ulteriori indagini.

Estrous cycle length in mice depends on genetic background and is affected by environmental conditions. The importance of genetic factors is confirmed by differences observed among lines selected from a base population derived from inbred strains (Barkley and Bradford, 1981).

Environmental and social stimuli influence attainment of sexual maturity as well as estrous cyclicity in female mice (Vandenbergh, 1975). Vaginal opening and first estrus are accelerated by the presence of adult males or by their urine but are delayed by inhibitory substances from adult females (Vandenbergh, 1967, 1969); suppression of estrous cyclicity occurs in females housed in dense, all-female groups, but estrus resumes readily if female mice are exposed to the presence of adult males or only to their urine (Whitten, 1959; Marsden and Bronson, 1964). Irregular estrous cycles, mainly pseudopregnancies, also occur if female mice are housed in small groups (Lee and Boot, 1955). Reproduction in young females of *Peromiscus*, moreover, has been shown to be inhibited by substances from an adult female but this effect is not permanent, as young females begin to reproduce after the adult female is removed (Haigh, 1983).

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(**) Nella seduta del 20 giugno 1986.

The studies reported above suggest that estrous cyclicity in females previously subjected to reproduction inhibitory conditions resumes properly. To our knowledge, however, no studies have been concerned with the dynamics of estrous cyclicity in female mice after the inhibitory conditions have been removed.

The purpose of this paper is to report on a study on the pattern of estrous cyclicity in female mice from large isosexual groups or from small groups with their mother.

Materials and Methods

Animals.

All females were obtained from our countinuously maintained laboratory colony of Swiss Morini outbred mice. Food (Mil pellets) and water were supplied *ad libitum* and sawdust bedding was changed once each week. A bisexual colony room maintained at controlled temperature (23 $\pm$ 3 °C) was used for the experiment and animal rearing.

The experiment was carried out on 56 virgin female mice, about 5-months-old (the age of the most regular cyclicity in mice, according to Nelson *et al.*, 1982).

Group A consisted of 28 female mice randomly selected from 4 groups of about 30 females each and reared in large groups from weaning.

The 24 females of group B were randomly selected from 6 isosexual littermate groups kept with their mother until use in the experiment.

During the experiment and for a previous adjustment period of about 2 weeks, one female was housed in each compartment of wire-mesh cages (40 $\times$ 40 $\times$ 15 cm) divided equally into 4 living areas by wire-mesh barriers.

Vaginal smears were taken daily for 19 days between 10.00 and 11.00 h and immediately stained with Mayer Haemallum and Eosin. Due to space limitations, the group B females were examined from the middle of June, whereas the group A females were examined from the second half of September.

Criteria adopted for classifying cycles and indentifying cycle stages.

According to the criteria adopted by Barkley and Bradford (1981), cycles were classified as short ($<$ 7.0 days) and prolonged (7.0-9.0 days) cycles, pseudopregnancies (10.0-13.0 days) or periods of anestrus ($\geq$14.0 days). Moreover, we defined " regular cyclicity " a sequence of $\leq$ 9.0-day cycles whose length did not differ for more than 1 day. Cyclicity was considered " irregular " if the shortest and longest cycle differed by 2 or more days. Occurrence of one or more cycles $>$ 9 days in duration was considered as suggestive of " aberrant cyclicity ".

Since the criteria adopted for classifying estrous stages in mice described in literature (Allen, 1922; Barkley and Bradford, 1981; Nelson *et al.*, 1982) are different, we carried out a preliminary analysis on 28 female mice, to single out the vaginal smears to be associated with fertile stage. 12 females exhibiting vaginal smears ranging from EC^{+} (indicative of late proestrus stage) to C^{++} (late estrus) were mated with stud males overnight; this allowed us to state that all these vaginal smears were indicative of a fertile phase.

Consequently, the following criteria were used for identifying cycle stages: proestrus–nucleated or nucleated and cornified cells; estrus–cornified cells (C to C^{++}); metestrus–cornified cells and leucocytes; diestrus-leucocytes and nucleated cells associated or not associated with cornified cells.

Statistical analysis.

Most comparison was made by analysis of variance (single classification Anova for two groups). When the F-max test revealed inequality of variances, we calculated the approximative t-value, t'_s (Sokal and Rohlf, 1969). Differences between groups in the number of differently cycling females and frequency distribution of females exhibiting cycles 3, 4, 5 or 6 days in duration, long cycles (7.0-9.0 days) and aberrant cycles (> 9 days) were assessed by means of the chi-square test. Differences with P values < 0.05 were considered significant.

RESULTS

Cyclicity and analysis of cycles.

Groups A and B did not differ significantly as to cyclicity. However, the group B females completed a significantly greater mean number of cycles (mean $= 3.42 \pm 0.22$ SE) than did the group A females (2.75 ± 0.15) (F test, $P < 0.025$). The number of cycles completed by each female during 19 days was significantly different ($m \times n$ chi-square test; $P < 0.005$); it varied from 1 to 4 in group A, from 0 to 5 in group B. 64% of the group A females completed 3 cycles, whereas 58% of the group B females completed 4 cycles.

In group A, 14 out 28 (50%) females exhibited only short cycles (4 to 6 days in duration), 7 (25%) also completed long cycles, 2 (7.14%) both long and aberrant cycles, 2 (7.14%) only aberrant cycles. In 1 female (3.57%) a short cycle followed a diestrus interval ≥ 14 days.

The mean length of all cycles was 6.52 ± 0.416 days, that of the short cycles 5.24 ± 0.205 days. Aberrant cycles varied from 10 to 12 days and were characterized by metestrus + diestrus intervals of 7.0-8.5 days in duration. In 1 female, however, a cycle suggestive of pseudopregnancy was followed by a cycle with prolonged estrus.

In group B, 19 out 24 (79.16%) females completed only 3-to 6-day cycles, 3 (12.5%) females also long cycles, 1 (4.16%) exclusively long cycles; 1 female exhibited persistent cornification of the vaginal epithelium during the whole observation period. The mean length of all cycles was 4.6 ± 0.249 days, that of the short cycles 4.3 ± 0.136 days.

The group B females differed significantly from the group A females with respect to the shorter length of all cycles (F test, $P < 0.001$; Table 1), the number of females completing 3-4-5-and 6-day cycles and the absence of aberrant cycles.

Analysis of stage (Table 1).

In group A, as many as 14 out 28 (50%) females exhibited long and > 9-day cycles; hence, the mean length of all cycles differed markedly from that of the short cycles. In short cycles, estrus and diestrus lasted about the same time and together took up approximately 2/3 of the cycle length; the remaining time was equally shared between metestrus and proestrus.

TABLE I.

Estrous cycle composition.

	Mean length ± SE			
	Group A	Group B	Test	P
3- to 14- cycles				
Proestrus	0.95 ± 0.077	0.84 ± 0.051	t'_s	> 0.05
Estrus	1.95 ± 0.159	1.57 ± 0.208	F	> 0.05
Metestrus	1.10 ± 0.111	0.65 ± 0.065	t'_s	< 0.05
Diestrus	2.52 ± 0.283	1.55 ± 0.135	t'_s	< 0.05
Mean cycle length . .	6.52 ± 0.416	4.61 ± 0.249	F	> 0.001
Cycles < 7 days				
Proestrus	0.92 ± 0.071	0.85 ± 0.047	F	> 0.05
Estrus	1.54 ± 0.102	1.32 ± 0.173	t'_s	> 0.05
Metestrus	0.97 ± 0.094	0.67 ± 0.064	t'_s	< 0.05
Diestrus	1.80 ± 0.087	1.44 ± 0.098	F	< 0.05
Mean cycle length .	5.24 ± 0.205	4.29 ± 0.136	F	< 0.001

The longer mean length of all cycles in this group appeared to be due chiefly to diestrus and metestrus. When compared with the 14 females exhibiting only short cycles, the 13 females also exhibiting long and aberrant cycles spent significantly more time in diestrus (days 3.31 ± 0.511 versus 1.81 ± 0.109; t'_s test, $P < 0.05$); furthermore, a marked lengthening of estrus intervals (days 2.34 ± 0.316 versus 1.66 ± 0.110; t'_s test, $0.05 < P < 0.1$) must be pointed out.

In group B, > 7-day cycles only occurred in 4 out 24 (16.66%) females; hence, mean cycle length inclusive of all cycles slightly differed from the mean length of short cycles as well as the time spent in the various cycle stages. Estrus and diestrus intervals, similar in length, took up together nearly 2/3 of the cycle length; metestrus was the shortest stage. In the 4 females exhibiting long cycles, cycle lengthening resulted from an equivalent prolongation of estrus and diestrus periods.

Comparison between groups showed that the group A females spent significantly more time in diestrus (t'_s test on all cycles, F test on short cycles, $P < 0.05$) and metestrus (t'_s test on both all and short cycles, $P < 0.05$) than did the group B females.

DISCUSSION

Our study shows that estrous cyclicity resumes promptly and regularly in female mice once they are removed from housing conditions inhibiting reproduction. In both groups A and B estrous cycle length usually varied from 3 to 9 days and the proportion of females exhibiting aberrant cycles was low.

The results reported above, however, show a difference in estrus recurrence between groups A and B.

In the group A females, reared in large groups from weaning and observed from the second half of September, 5-day cycles were predominant, and long and aberrant cycles occurred in 50% of females.

The group B females, kept in their own litter with their mothers from birth until use in the experiment, carried out in June, exhibited shorter and more regularly recurrent estrous cycles, chiefly 4-day cycles. In this group, moreover, 3-day cycles occurred in 25% of females, long cycles in 16.66% only. Persistent cornification of the vaginal epithelium was observed in one female that, mated with a fertile male, proved to be sterile.

Females of the A group differed significantly from the group B females with respect to the number of cycles completed during 19 days, the longer average length of cycles and the proportion of 9-to 14-day cycles. Analysis of stages showed that in both groups estrus took up approximately 1/3 of cycle length and that the time spent in proestrus appeared to be the least variable; it also showed that the group A females exhibited significantly longer periods of metestrus and dietrus than did the group B females.

Since animals were all derived from the same colony, differences between groups A and B would not seem ascribable to genetic background. However, inequality of variances with respect to proestrus, metestrus and diestrus during all cycles and estrus and metestrus during short cycles between groups A and B suggests a great inherent variability in our colony.

The difference in estrus recurrence between groups A and B may depend on previous social conditions, but the importance of seasonal factors can not be ruled out. Further studies, however, are required to shed light on this problem.

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