Estrous Cyclicity in Mice Housed in the Presence or Absence of Males (36176)

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Only a small proportion of Charles River (CD*-1 strain) mice exhibit 4-day estrous cycles spontaneously (1). Consequently, further investigations aimed at comparing 4- and 5-day estrous cycle phenomena in this strain of mice, such as have been carried out in the rat (2), have not been possible. Whitten (3), however, has shown that female mice of other strains exhibit shorter estrous cycles when housed individually in the presence of a male than when housed individually without males. The present study was undertaken partly to determine whether such a technique might be a feasible means by which a large number of 4-day cyclic CD*-1 mice could be obtained for future studies.

It has also been reported (1) that a large proportion of singly-housed CD*-1 mice exhibit acyclicity and/or irregular cyclicity for varying periods of time following the start of daily vaginal smear examinations. Such non-cyclicity has been observed (Bingel, personal observations) despite the fact that the appearance of the cellular contents of the *first* vaginal smear obtained (11–13 days after the animal's arrival in the laboratory) from each mouse of a given shipment invariably suggests that the majority of animals probably are cycling regularly; that is, the proportion of smears characteristic of each stage of the estrous cycle approximates the percentage of each theoretically expected for a group of 4- and 5-day cyclic animals. The second question asked, therefore, was whether housing female mice in the presence of a male could prevent the disruption of estrous cyclicity that appears to develop following the start of daily vaginal smear examinations.

Materials and Methods. Seventy-five 6-week-old virgin female albino mice were obtained from Charles River Mouse Farms, placed in environmentally controlled quarters (L18:D6, lights on 3AM–9PM), and immediately divided into six groups, A–F (Day 1 of the experiment). The L:D ratio differed slightly from the one (L14:D10) used previously (1). Each mouse belonging to Groups A–D was housed individually with an adult male, 19, 16, 10, and two months old, respectively, but separated from him by a stainless steel mesh (mesh size: 5 mm square) cage divider to prevent mating from occurring. Mice belonging to groups E and F were also housed individually, without males; cage dividers were included in the cages of Group E mice. Vaginal smears were taken as previously reported (1), begining on Day 11.

From days 11–36 the mice were merely observed for the occurrence or non-occurrence of estrous cyclicity as indicated by the appearance of the cellular contents of the vaginal smears. Between Days 36 and 84 cyclic mice were autopsied at 10AM on a day of estrus after they had exhibited at least two consecutive cycles of the same length. Non-cyclic mice also were autopsied during this time period. Ovarian, uterine, and adrenal wet weights were determined and analyzed by group comparison (4).

Results. Since no differences were observed among Groups A–D, nor between Groups E and F, for any of the parameters studied, the data for Groups A–D and for Groups E and F, respectively, have been combined and are presented as data obtained from mice housed with males (A–D) and without males (E–F).

On the basis of the appearance of the contents of the first vaginal smear obtained from

TABLE I. Cycle Stage as Indicated by First Vaginal Smear (Day 11).

Group		Cycle Stage				Total
		Proestrus	Estrus	Metestrus	Diestrus[a]	
A–D with male	no. of mice	8	10	12	17	47
	% of total	17	21	26	36	
E–F without male	no. of mice	5	8	4	11	28
	% of total	18	29	14	39	

[a] All smears not characteristic of proestrus, estrus, or metestrus (1) were designated diestrus. Such smears represent the days of Diestrus I, II, and/or III for 4-, 5-, and 6-day mice.

each mouse, the animals were presumed to be in one of the four stages of the estrous cycle in the proportions indicated in Table I; chi-square analysis (4) indicated there was no significant difference between mice housed with males (A–D) and those housed without males (E–F). The percentage distribution among the four stages remained similar for the two groups on Day 12, but by Day 13, 68% of the mice housed without males were exhibiting diestrous (leukocytic) vaginal smears; chi-square analysis of these data indicated a highly significant difference between the two groups ($p < .005$).

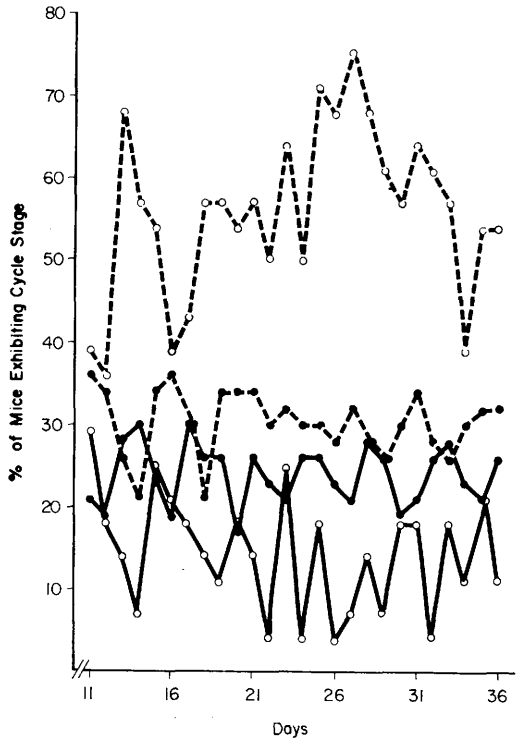

FIG. 1. Percentage of vaginal smears indicative of estrus or diestrus exhibited by mice housed with males ($n = 47$) or without males ($n = 28$). The percentage of proestrous and metestrous vaginal smears have been omitted since they closely approximate the values obtained for percentage of estrous smears for each group, respectively. A–D with male: ●—●, estrous smears; ●- -●, diestrous smears. E–F without male: ○—○, estrous smears; ○- - -○, diestrous smears.

The percentage of diestrous and estrous vaginal smears exhibited by the mice housed with males (A–D) and those housed without males (E–F) from Days 11–36 are shown graphically in Fig. 1. It can be seen that for mice housed with males, the percentages for both cycle stages range between 17 and 36. For mice housed without males, however, a marked difference can be seen between the percentage of animals exhibiting each of the two cycle stages; for the most part, smears characteristic of estrus were obtained from less than 25% of these animals on any given day, while 50% or more of these animals usually exhibited diestrous smears. Furthermore, comparison of the daily vaginal smear records of the individual mice comprising these two groups revealed that during this time period, 42 of the 47 mice housed with males were cycling regularly (4- or 5-day cycles), while only 13 of the 28 mice (46%) housed without males were exhibiting regularly recurring estrous cycles (4–6 days in length) ($X^2 = 14.26$, $p < .005$). Nevertheless, all

TABLE II. Cycle Length at Autopsy.

Group	Total No. of Mice	Cyclic			Non-cyclic[a,b]		
		4-Day	5-Day	6-Day	L	C	I
with male	47	37	7	—	2	1	—
without male	28	1	20	1	4	—	2

[a] These nine mice were still not exhibiting regular estrous cycles at the time of autopsy.

[b] L = predominantly leukocytic smears; C = predominantly cornified smears, although this animal did have newly ovulated ova in her oviducts at autopsy; I = irregular: smears containing predominantly leukocytes alternating at irregular intervals with smears containing predominantly cornified cells.

except nine mice eventually did exhibit regular estrous cycles and were autopsied on a day of estrus between Days 36 and 84 (Table II).

The majority of mice housed with males exhibited 4-day cycles (Table II), confirming results reported previously by Whitten (3), in contrast to the cycles five days in length exhibited by the majority of mice housed without males. No significant differences were found between these two groups with respect to their mean body and organ weights.

Discussion. The results of this study indicate that housing female mice individually with an adult male, but separated from him by a steel mesh cage divider, is an effective way of obtaining 4-day cyclic mice for study; that is, the predominance of 4-day cycles exhibited by mice housed with males and of 5-day cycles exhibited by mice housed alone, reported previously by Whitten (3) for three other strains, has been shown to hold true for CD*-1 mice as well. Similarity between the organ weights of cyclic mice housed with and without males indicates that whatever the differences are between the two groups causing the former to exhibit predominantly 4-day cycles and the latter 5-day cycles, such differences do not affect values for ovarian, uterine, and adrenal weights at estrus. Thus, further investigation for the purpose of comparing 4- and 5-day estrous cycle phenomena in the mouse, such as has been carried out in the rat (2), now appears feasible.

The results of this study also indicate that housing female mice with adult males prevents the acyclicity and/or irregular cyclicity that occurs for varying periods of time in many singly-housed mice following the start of daily vaginal smear examinations.

Summary. The majority of CD*-1 female mice housed individually with adult males exhibit 4-day estrous cycles. Regular cyclicity is observed in these animals from the time daily vaginal smear examinations are begun. The majority of mice housed individually without males exhibit 5-day cycles, but frequently only after periods of non-cyclicity following the start of vaginal smear examinations.

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