Spontaneous Anoestrus in Mice

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Summary

The length of oestrous cycles in a group of mature albino mice has been measured by the technique of daily vaginal smears. A high proportion of mice had prolonged cycles. It was shown that mice housed singly may experience abnormal cycles, the number being least when a wire loop is used for taking the smears. Housing mice in groups of four brought about an immediate cessation of normal cycling in the majority of mice. The introduction of a male was apparently sufficient stimulation for a rapid return to normal cycling.

INTRODUCTION

In studies of the influence of external stimuli on reproduction in mice, one response criterion is the length of the oestrous cycle. Since most experiments require large numbers of mice per treatment combination, the usual procedure is to have basic experimental units of four to five mice per cage, each cage in this Department measuring, 11 in. x 6 in. x 5½ in. This grouping is carried out soon after weaning. It is proposed to draw attention to a problem which may have an important bearing on the design of experiments in which the length of oestrous cycles is being investigated, particularly in groups of animals. It is characterised by the spontaneous occurrence of anoestrous periods which may in fact be pseudopregnancy (Lee and Boot, 1955, 1956) or simple anoestrus (Whitten, 1957). Cycles greater than 8 days in length were considered abnormal in the experiment to be described.

Vaginal smears were normally taken six days per week, using a metal spatula dipped in isotonic saline. For the purposes of this paper, cycle length was taken as the period between the last fully oestrous smears in successive cycles. This method was used because it is difficult to time the occurrence of oestrus and ovulation accurately when a number of successive positive smears are recorded.

PRELIMINARY OBSERVATIONS

A group of 39 mice (housed four per cage) represented the control animals in an experiment. These animals were smeared for approximately seven weeks, commencing when they were about 30 days old and continuing until the majority had been cycling for four weeks. Only 90 cycles, of which 55 per cent. were longer than 8 days, were recorded. It was therefore decided to determine to what extent the high incidence of abnormal cycles was related to the following factors: (a) the stimulus of taking the smear and the influence on it of the operator and the method of smearing; and (b) the number of mice per cage. When the results of this experiment became available, males were introduced and the opportunity was taken to examine the effect of the method of housing on time of mating.

MATERIALS AND METHODS

Ninety-six randomly bred female albino mice were weaned at 18-20 days and kept in large cages, approximately 30 per cage, for three months. They were then placed at random in normal-sized

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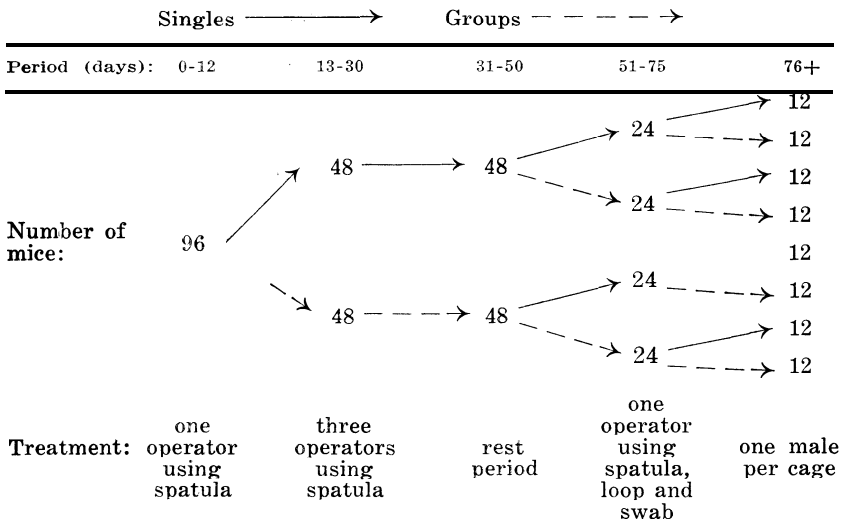
cages, one per cage, and kept in a room in which the temperature was 76 ±2° F. and air changes were at the rate of 14 per hour. The daily light: dark ratio was held constant at 12 : 12 hour. All observations were carried out in daylight. Large numbers of males were kept in cages in the room throughout the period of the experiment. It was impossible for the experimental mice to see the males, although there is little doubt they would have been exposed to male odours (Whitten, 1956).

An outline of the experimental design is shown diagramatically in Table I. The mice were confined singly (Singles) for two weeks

TABLE I.

Grouping of Mice and Treatment.



Note: Mice were run in groups of 30 from 18-20 days to 3½ months of age. They were then housed as singles for 14 days before day 0.

prior to the start of smearing. For the first twelve-day experimental period each mouse was smeared by a single operator using a metal spatula. Half the mice were then grouped four per cage (Groups) and the remainder left as singles. During the period 13-30 days three operators each smeared 16 mice as Singles and 16 as Groups. At the end of this period the mice remained housed for a rest period of 20 days. For the period 51-75 days the mice were rearranged so that the differences due to the previous method of housing could be examined. The following three methods of smearing were employed :-

(a) A metal spatula was dipped in saline and pushed well into the vagina; the fairly copious smear contained cells from the length of the dorsal wall. This method is rapid but the tip of the spatula often comes in contact with the cervix. There was no distension of the vagina.

(b) A fine wire loop was dipped in saline and gently placed in the vagina. A scraping was carefully made from the dorsal wall for a short distance in approximately the anterior and middle thirds. This is a slow and somewhat tedious method as the loop must be flamed between smears and only an

experienced operator can be expected to do it satisfactorily. It gives a most satisfactory smear, however, and the **loop** is unlikely to come in contact with the cervix.

(c) A non-irritant highly absorbent plastic material was cut into small swabs. A pair of fine forceps was used to introduce the swab. Unfortunately the swabs were often too big and caused distention of the vagina; also the forceps often came in contact with the cervix. The smears obtained with this method were scanty and were laborious to score.

At the completion of the period 51-75 days the mice were again rearranged to eliminate bias due to previous treatments and one active male was then placed in each cage. Each male was replaced by a fresh one ten days later. The date of birth of each litter was recorded.

Results and Discussion

The results obtained by the different operators and the different methods of smearing are set out in Tables II and III for the animals

TABLE II.

Numbers of Mice Showing Normal (N) and Abnormal (Abn) Cycles in the period 13-30 days.

Housing	Operators					
	A		B		C	
	N	Abn	N	Abn	N	Abn
Singles	9	7	11	5	8	8
Groups	0	16	5	11	1	15

Partition of χ^2

Source of variation	DF	X^2
Singles v. Groups	1	22.02***
Between operators	2	4.73
Interaction	2	3.37

TABLE III.

Numbers of Mice Showing Normal (N) and Abnormal (Abn) Cycles in the period 51-75 days.

Housing	Previous Housing											
	Singles						Groups					
	spatula		loop		swab		spatula		loop		swab	
	N	Abn	N	Abn	N	Abn	N	Abn	N	Abn	N	Abn
Singles	3	5	7	1	2	6	8	0	6	2	4	4
Groups	0	8	0	8	0	8	0	8	0	8	0	8

Partition of χ^2—Mice housed as singles only.

Source of variation	DF	X^2
Previous Singles v. Previous Groups	1	3.2
Between methods of smearing	2	7.33*
Interaction	2	4.36

housed in the two ways, the distribution of cycle lengths is shown in Figure 1, and the distribution of the birth of litters after joining on day 76 is set out in Table IV.

TABLE IV.

Birth of Litters after Joining on Day 76.

Housing	Number of litters born during period			
	96-98 days	99-101	after 101	Total
Singles*	20	21	5	46
Groups	17	18	13	48

*Two mice died.

Mice housed in groups experienced significantly more abnormal cycles than mice housed singly. The results obtained by different operators did not differ significantly, but results obtained by different methods of smearing differed significantly, fewer abnormal cycles occurring among the mice from which smears were taken by the loop method than among the other mice. The previous manner in which the mice were housed did not affect the results obtained by the different methods of smearing.

Although the mice housed in groups had earlier experienced more abnormal cycles than mice housed singly, there was no evidence of heterogeneity in the date of birth of the litters after day 76 ($\chi^2_{(2)} = 3.99$; $0.1 < P < 0.2$).

These results emphasise that there are important unknown factors influencing the oestrous behaviour of mice. Daily smearing with a spatula of mice housed singly brings about a refractory state which results in approximately 50 per cent. experiencing a prolonged dioestrous period after 2-3 weeks. In some mice there is also a prolonged series of successive positive smears. Thus 11.9 per cent. of 178 cycles observed over a 30 day period were of an abnormal nature. In the same group of mice after a three week rest period 8.8 per cent. of 159 cycles also were greater than 8 days in length. However it would seem that this difference was largely accounted for by a significantly smaller effect due to using a wire loop in one third of the animals in the second period.

The extraordinary effect of grouping mice, bringing about an almost immediate cessation of normal cycling, leads to special problems in reproductive studies. It is possible that such an effect occurs in many other mammals. Chitty and Austin (1957) have already shown that this factor is of importance in oestrous cycles in the field vole. Although phylogenetic differences resulting in greater variability in response to external stimuli and allowing a greater range of modifying factors, are likely to be of importance in this regard, it would seem desirable to examine the sheep, for example, because of its economic importance.

One point borne out by this experiment is the dominant part played by the male. While there is adequate proof of prolonged cycles with grouping, the addition of a male to a cage evidently overcomes this refractoriness. This would seem to suggest that the anoestrus is not due to pseudopregnancy. It also may be important to consider this result in interpreting the findings of many workers (see Radford and Watson, 1957, for details) on the influence of the ram on ovarian activity in the ewe. Perhaps the most fruitful line of investigation would be to determine the effect of the presence of

the ram on the length of the dioestrous period. This is suggested because the time of onset of oestrus and ovulation is ultimately determined by the period of progesterone production by the corpus luteum of the previous cycle, and this period is presumably controlled by pituitary hormone. On the assumption that external influences affecting ovarian function are mediated through the pituitary, an obvious quantitative measure is available.

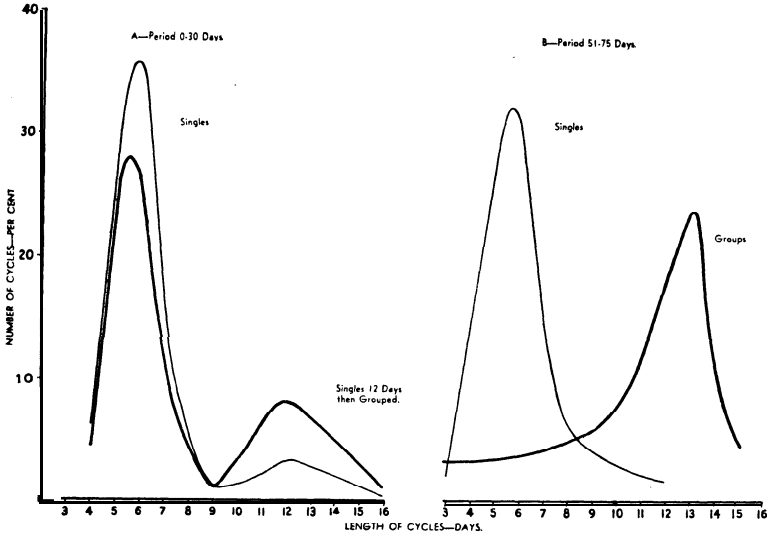


FIG. 1—Distribution of cycle lengths during observation periods 0-30 and 51-75 days.

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