THE SUPPRESSION OF OVARIAN CYCLICAL ACTIVITY IN GROUPS OF MICE, AND ITS DEPENDENCE ON OVARIAN HORMONES

M. D. CLEE, ELIZABETH M. HUMPHREYS* AND J. A. RUSSELL

Department of Physiology, University Medical School, Teviot Place, Edinburgh EH8 9AG

(Received 14th March 1975)

The oestrous cycle of the mouse is influenced by environmental conditions (Merton, 1937; Van der Lee & Boot, 1955, 1956; Dewar, 1959). Contact with intact, but not castrated, male mice shortens the oestrous cycle (Whitten, 1958; Bruce, 1965). This effect is due to an olfactory pheromone in the urine of male mice (Parkes & Bruce, 1962; Marsden & Bronson, 1964; Bronson & Whitten, 1968). Isolated females usually have cycles 4 to 6 days long, but, if the females are caged in groups, the cycles become more irregular (Andervont, 1944; Whitten, 1957, 1959). Whitten (1966) suggested that this effect might be due to a female pheromone. Champlin (1971) has shown that oestrus is less frequent in a single female placed in a cage previously soiled by a group of females.

In view of the finding of Kimura (1971) that ovariectomized mice have a reduced capacity to suppress ovarian cyclical activity, we performed two series of experiments to see whether the effect of female mice on the oestrous cycles of each other depends on ovarian hormones. In Exp. I the effect of the presence of ovariectomized females on the oestrous cycle of normal females was measured. Experiment II was performed to see if treatment of ovariectomized mice with oestrogen altered their influence on the oestrous cycle, and whether the effects of castrated male mice were similar to those of ovariectomized females.

Virgin female mice of the Schofield strain, weighing between 20 and 25 g, were grouped as follows. *Experiment I.* There were fifteen cages, each containing one normal mouse, in Group A; five cages, each containing four normal mice, in Group B; and eight cages, each containing one normal and three ovariectomized mice, in Group C. *Experiment II.* There were six cages, each containing three females which had been implanted with 8.2 to 11.7 mg oestradiol-17 β 40 days after ovariectomy and one normal female, in Group D; eight cages, each containing one normal female and three castrated males, in Group E; fourteen cages, each containing one normal female, in Group F.

The opaque plastic cages measured 15 $\times$ 30 $\times$ 11 cm high, and were kept 15 cm apart on racks in a room which contained no other animals, was lit naturally and kept at 22 $\pm$ 1°C. The first experiment was carried out during February and March, and the second in May. Adaptation periods of 10 days in Exp. I and 25 days in Exp. II were used.

Vaginal smears were taken daily between 09.15 and 10.15 hours. The

* Correspondence to Dr E. M. Humphreys.

proportion of each cell type was assessed on a 0 to +++ scale and the stage of the cycle then deduced (Allen, 1922; Bingel & Schwartz, 1969). Three parameters were measured. These were the number of complete oestrous cycles during 30 days, the length of each complete cycle and the proportion of each 30-day period spent either in pro-oestrus, oestrus and metoestrus, or in dioestrus. The results are shown in Tables 1, 2 and 3.

Table 1. The effect of different groupings of female mice on the number of complete oestrous cycles in 30 days

Group	No. of mice	No. of cycles in 30 days							No. of cycles/mouse (Mean $\pm$ S.E.M.)
		1	2	3	4	5	6	7	
A	15			1	1	10	3		5.00 $\pm$ 0.19
B	20	1	5	7	3	3	1		3.25 $\pm$ 0.28
C	8				2	6			4.75 $\pm$ 0.15
D	6	1	3		2				2.50 $\pm$ 0.50
E	8			2		4	1	1	4.87 $\pm$ 0.47
F	14			2		4	6	2	5.42 $\pm$ 0.32

Comparison of Groups by Student's t test:

A versus B, $P < 0.001$; D versus E, $0.005 > P > 0.001$;
 A versus C, $0.4 > P > 0.3$; D versus F, $P < 0.001$;
 B versus C, $0.005 > P > 0.001$. E versus F, $0.4 > P > 0.3$.

Table 2. The effect of different groupings of mice on the composition of the oestrous cycle

Group	No. of mice	No. of days/mouse (Mean $\pm$ S.E.M.)	
		Pro-oestrus + metoestrus + oestrus	Dioestrus
A	15	22.66 $\pm$ 0.96	7.34 $\pm$ 0.96
B	20	15.85 $\pm$ 1.26	14.15 $\pm$ 1.26
C	8	22.37 $\pm$ 0.77	7.63 $\pm$ 0.77
D	6	11.66 $\pm$ 1.33	18.34 $\pm$ 1.33
E	8	18.87 $\pm$ 1.45	11.13 $\pm$ 1.45
F	14	20.92 $\pm$ 0.93	9.08 $\pm$ 0.93

Comparison of Groups by Student's t test:

A versus B, $P < 0.001$; D versus E, $0.005 > P > 0.001$;
 A versus C, $0.9 > P > 0.8$; D versus F, $P < 0.001$;
 B versus C, $P < 0.001$. E versus F, $0.3 > P > 0.2$.

At the end of Exp. II the oestradiol implants were removed, dried and weighed. Oestradiol was absorbed at an average rate $\pm$ S.E.M. of 0.45 ± 0.02 μ g/day.

Similar conclusions may be made from all the methods of measuring ovarian cyclical activity used in this study, although cycle length, cycle frequency and the duration of components of the cycle are not directly comparable with each other. The finding that ovarian cyclical activity was suppressed in groups of

four normal female mice caged together confirms previous observations (Andervont, 1944; Whitten, 1959; Champlin, 1971), although the Schofield strain has not been used before. The suppressive effect was abolished by ovariectomy, except that a small effect on the length of the cycle remained (Table 3).

Table 3. The effect of different groupings of female mice on the length of the oestrous cycle

Group	Total no. of cycles	Cycle length	
		Mean $\pm$ S.E.M. (days)	% of mean for Group A
A	74*	4.76 $\pm$ 0.05	100
B	63†	5.96 $\pm$ 0.24	125
C	37*	5.17 $\pm$ 0.09	108
<hr/>			
			% of mean for Group F
D	15	6.43 $\pm$ 0.92	142
E	39	4.83 $\pm$ 0.15	107
F	75*	4.53 $\pm$ 0.09	100

* In each of these groups one of the cycles was more than 3 standard deviations from the mean and was not included in the analysis.

† Two cycles in this group were discarded for reasons given above.

Comparison of Groups by Student's t test:

A versus B, $P < 0.001$;	D versus E, $0.02 > P > 0.01$;
A versus C, $P < 0.001$;	D versus F, $P < 0.001$;
B versus C, $0.02 > P > 0.01$.	E versus F, $0.1 > P > 0.05$;
	A versus F, $0.05 > P > 0.025$.

Kimura (1971) has reported similar results. Experiment II showed that oestradiol treatment of ovariectomized mice restores the ability to suppress ovarian cyclical activity. The results of Exps I and II cannot be directly compared, because the oestrous cycles of the mice caged individually (Groups A and F) were longer during Exp. I (Table 3); this difference may be attributable to increased hours of daylight during Exp. II. The mean cycle lengths for each experimental group can be compared when calculated as percentages of the means for Groups A and F (Table 3). It is clear that, compared with intact females, ovariectomized mice given implants of oestradiol had an enhanced ability to suppress ovarian cyclical activity. The rate of absorption of oestradiol was just above the threshold dose required to produce oestrus in spayed mice (Terenius, 1971). This finding, together with the continual presence of cornified epithelial cells in the vaginal smears of the animals with implants, suggests a constantly high level of circulating oestrogen in these animals which may have been responsible for their enhanced suppressive effect.

How female mice suppress ovarian cyclical activity in other mice is not certain. It is likely that pheromones are involved (Champlin, 1971), although Dewar (1959) has suggested that mounting behaviour is important, and

Kimura (1971) has shown that direct contact with other mice may play a part. In the present experiments contact with ovariectomized mice had a small effect on cycle length only (Table 3). This suggests that ovarian hormones were not wholly responsible for the suppressive effect exerted by normal mice. Castrated males, however, did not have a significant effect.

Regardless of the mechanism of interaction, we conclude that the mutual suppression of ovarian cyclical activity is largely dependent upon the ovaries of the suppressing mice, and the suppressive effect can be restored to ovariectomized mice by oestradiol. Female mice may produce a pheromone under the influence of oestrogen which inhibits ovarian cyclical activity in other mice.

The oestradiol tablets were kindly provided by Organon Laboratories Ltd, Crown House, Morden, Surrey. This work was originally submitted as a thesis by one of us (M.C.), in support of the degree of B.Sc. (Hons.) in the Department of Physiology, University of Edinburgh.

REFERENCES

- ALLEN, E. (1922) The oestrous cycle in the mouse. *Am. J. Anat.* **30**, 297–371.
- ANDERVONT, H.B. (1944) Influence of environment on mammary cancer in mice. *J. natn. Cancer Inst.* **4**, 579–581.
- BINGEL, A.S. & SCHWARTZ, N.B. (1969) Pituitary LH content and reproductive tract changes during the mouse oestrous cycle. *J. Reprod. Fert.* **19**, 215–222.
- BRUCE, H.M. (1965) The effect of castration on the reproductive pheromones of male mice. *J. Reprod. Fert.* **10**, 141–143.
- BRONSON, F.H. & WHITTEN, W.K. (1968) Oestrus-accelerating pheromone of mice: assay, androgen-dependency and presence in bladder urine. *J. Reprod. Fert.* **15**, 131–134.
- CHAMPLIN, A.K. (1971) Suppression of oestrus in grouped mice: the effects of various densities and the possible nature of the stimulus. *J. Reprod. Fert.* **27**, 233–241.
- DEWAR, A.D. (1959) Observations on pseudopregnancy in the mouse. *J. Endocr.* **18**, 186–190.
- KIMURA, T. (1971) Modifications of the estrous cycle in mice housed together with normal or ovariectomized females. *Scient. Pap. Coll. gen. Educ. Tokyo* **21**, 161–166.
- MARSDEN, H.M. & BRONSON, F.H. (1964) Estrous synchrony in mice: alteration by exposure to male urine. *Science, N.Y.* **144**, 1469.
- MERTON, H. (1937) Studies on reproduction in the albino mouse. *Proc. R. Soc. Edinb.* **58**, 80–97.
- PARKES, A.S. & BRUCE, H.M. (1962) Pregnancy block in female mice placed in boxes soiled by males. *J. Reprod. Fert.* **4**, 303–308.
- TERENIUS, L. (1971) The Allen-Doisy test for estrogens reinvestigated. *Steroids* **17**, 653–661.
- VAN DER LEE, S. & BOOT, L.M. (1955) Spontaneous pseudopregnancy in mice. *Acta physiol. pharmac. neerl.* **4**, 442–444.
- VAN DER LEE, S. & BOOT, L.M. (1956) Spontaneous pseudopregnancy in mice. II. *Acta physiol. pharmac. neerl.* **5**, 213–214.
- WHITTEN, W.K. (1957) Effect of exteroceptive factors on the oestrous cycle of mice. *Nature, Lond.* **180**, 1436.
- WHITTEN, W.K. (1958) Modification of the oestrous cycle of the mouse by external stimuli associated with the male. Changes in the cycle determined by vaginal smears. *J. Endocr.* **17**, 307–313.
- WHITTEN, W.K. (1959) Occurrence of oestrus in mice caged in groups. *J. Endocr.* **18**, 102–107.
- WHITTEN, W.K. (1966) Pheromones and mammalian reproduction. *Adv. reprod. Physiol.* **1**, 155–177.