OESTRUS-ACCELERATING PHEROMONE OF MICE: ASSAY, ANDROGEN-DEPENDENCY AND PRESENCE IN BLADDER URINE

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Summary. Two methods for the assay of a pheromone produced by male mice were compared. Using these methods it was shown that the pheromone was present in urine from males of two inbred strains and from androgenized, spayed females, but not in urine from castrate males. It was present in urine collected directly from the bladder free from any accessory gland secretion.

All-female grouping of laboratory mice is often accompanied by a suppression of oestrous cycles. Exposure to the presence of a male results in both an induction and an acceleration of cycling and, hence, usually leads to a synchrony of oestrus among the members of a group (Whitten, 1958). This action of a male is independent of visual, auditory or tactile stimulation and, therefore, has been postulated as taking the form of an olfactory-mediated pheromone (Whitten, 1959, 1966 and unpublished material). Support for the pheromone hypothesis was noted when Marsden & Bronson (1964) mimicked the effect of a male by using only topical exposure to male urine. Finally, Bruce (1965) reported the loss of a male's capacity to influence oestrous cycles following castration, thus indicating the androgen-dependency of the pheromone.

The present study was initiated with three objectives: (1) to obtain an efficient assay of the urinary pheromone, (2) to confirm that its production is androgen-dependent, and (3) to determine if the pheromone is present in bladder urine before the addition of secretions from such accessory structures as the bulbo-urethral glands, the urethral diverticulum or the preputial glands.

Female mice of either the 129/J or SJL/J strains were raised in groups of four until 70 days of age, at which time they were housed six to ten/cage (6×6×12 in., stainless steel) and exposed to urine from various sources in a male-free room. Urine was delivered to the females' cages either by the use of drip cages containing other mice (Expt. 1) or by way of syringes driven by an infusion pump (Expt. 2; Dominic, 1966). Drip cages were 6×12×6 in. galvanized steel with funnel-shaped bottoms that terminated in 5 in. stainless steel tubes. These tubes penetrated the tops of cages of experimental females, allowing urine to drip on to their bedding. Two layers of screening, one coarse and one fine, served as flooring and also prevented faeces from reaching the lower cages. Animals living in drip cages were removed and allowed to

feed for 4 hr each morning, but water was available to them at all times. The effect of urine exposure in both experiments was evaluated in terms of relative frequency of vaginal oestrus. Groups of females were tested with more than one treatment but a minimum of 3 weeks was always maintained between such tests.

Experiment 1. The presence of a male or his urine acts both to initiate oestrous cycles and to decrease their average length. An assay procedure based on vaginal smearing during the 3rd and 4th days after urine exposure begins should detect those mice in which oestrus was successfully induced while observation over a 6-day period (Days 3 to 8) should reveal an additional number of mice whose second cycles were shortened. Thus, the longer period of observation may provide a more sensitive assay but at the expense of considerably more work and a less efficient use of animals and space. In this experiment the relative efficiency of these two alternatives was evaluated as was the androgen-dependency of the urinary pheromone. The basic procedure was to house 129/J females (six or seven/cage) under drip cages containing one of four types of animals: (1) intact C57BL/6J males, (2) C57BL/6J males castrated as adults (testing took place 4 months after castration), (3) ovariectomized C57BL/6J females bearing 20-mg testosterone implants, or (4) intact

TABLE 1

DATA FROM OBSERVATIONS OF VAGINAL SMEARS OBTAINED EITHER ON DAYS 3 AND 4 OR ON DAYS 3 TO 8 (AFTER INITIATION OF URINE EXPOSURE) FROM 129/J MICE HOUSED UNDER DRIP CAGES

<i>Urine source</i>	<i>No. females tested</i>	<i>No. in oestrus on Days 3 or 4</i>	<i>% in oestrus on Days 3 or 4</i>	<i>Mean frequency of oestrus during Days 3 to 8 ($\pm$ S.E.)</i>
No urine	62	19	31	0.87 $\pm$ 0.07
Castrate C57BL/6J males	21	9	43	0.86 $\pm$ 0.14
Intact C57BL/6J males	33	26	79	1.40 $\pm$ 0.12
Androgenized C57BL/6J females	14	8	57	1.43 $\pm$ 0.17
Intact C3H/J males	27	19	70	1.48 $\pm$ 0.11

C3H/J males. Five of each type of test animal were used in each drip cage. Vaginal smears were obtained from all females on Days 3 to 8 inclusive.

The oestrus-inducing effectiveness of urine was highly dependent upon the presence of a testicular or implanted androgen source (Table 1). Data from Days 3 and 4 resulted in only two differences being significant (no urine control versus exposure to urine from intact C57BL/6J or C3H/J males; $P < 0.01$). The levels of significance with 3 to 8 days' observations, however, were increased in all cases to 0.01 except in reference to the difference between castrate males and androgenized females ($P < 0.05$).

Experiment 2. This experiment was designed to compare the pheromone content of bladder urine with that voided to the exterior. Bladder urine was collected fresh each morning from males in which the urethra had been ligated at its internal orifice during the preceding afternoon. Fresh external urine was collected by holding males over a Plexiglas plate and compressing the bladder through the abdominal wall. In the first series of tests 5 ml of urine collected

from mature C57BL/6J males was mixed with 5 ml of water containing an antioxidant (propyl gallate) and antibiotic (Achromycin, Lederle Laboratories) and delivered to the cages from an infusion pump (Dominic, 1966). A timing device allowed the mixture described above to be delivered at the rate of a single 0.2 ml aliquot every half-hour. In a second series of tests, 8 ml of urine were collected from C57BL/6J and hybrid 129/J $\times$ C57BL/6J males in a 1:1 ratio, mixed with an additional 3 ml of antioxidant-antibiotic mixture, and delivered in single aliquots of 0.46 ml every hour. SJL/J females, grouped nine to ten/cage, were exposed to urine for 3 consecutive days with vaginal smears obtained on the 3rd and 4th mornings.

Exposure to male mouse urine again resulted in a significantly higher incidence of oestrus ($P < 0.001$ in all cases; χ^2 ; Table 2). Bladder urine was as effective as externally collected urine in inducing oestrus. Control levels of 22 and 25% were raised to 48 and 50% for external and bladder urine, respectively, in the first series of tests and to 60 and 63% during the second series.

TABLE 2
FREQUENCY OF OESTRUS IN SJL/J MICE ON DAYS 3 AND 4 AFTER
INITIATION OF EXPOSURE TO MALE URINE COLLECTED EITHER EX-
TERNALLY OR FROM THE BLADDER

Urine source, amount/24 hr	No. females in oestrus/No. females exposed to:		
	Nothing	External urine	Bladder urine
C57BL/6J males, 5 ml	22/100	48/99	25/50
Mixed males, 8 ml*	17/67	30/50	19/30

* Urine from C57BL/6J and hybrid (C57BL/6J $\times$ 129/J) males mixed 1:1.

As an additional control, thirty SJL females were exposed to human male urine under these conditions; five of these females (17%) came into oestrus during the 2-day period, comparing well with frequencies obtained from control (no urine) treatment. This result supports the conclusion reached by Marsden & Bronson (1965) that these pheromones are probably species specific.

Urine from males is known to function in two pheromonal capacities among laboratory mice: blocking of implantation because of an inhibition of prolactin release and in the induction and acceleration of oestrous cycles (reviewed by Bruce, 1966; Whitten, 1966). Whether these two functions are due to the same or different urinary factors is still unclear (Bronson, 1967). The two phenomena are markedly similar in their androgen-dependency and in that urine from gonadectomized females bearing androgen implants is effective in both cases (Dominic, 1965, 1966, present data). The present experiments also demonstrate that, at least for the oestrus-accelerating factor, bladder urine is as potent as externally collected urine, thereby ruling out the necessity of addition of factor(s) to urine after it leaves the bladder.

The study of urinary fractions for the purpose of progressive isolation of the oestrus-accelerating factor appears to be amenable to a 2-day assay using SJL/J females. 129/J females responded well to urine delivered by the use of

drip cages in a 6-day assay but less well for 2 days. Unreported pilot studies similar to Expt. 2 showed the SJL/J female to be more responsive than the 129/J for this type of assay.

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