THE RELATIONSHIP OF THE MALE PREPUTIAL GLAND TO THE ACCELERATION OF OESTRUS IN THE LABORATORY MOUSE

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Summary. The effect of the male preputial gland on the oestrous cycle in mice was investigated. Urine from preputialectomized (but otherwise intact) males was slightly but not significantly more effective in stimulating oestrus than urine from castrates, but it was significantly less effective than urine from intact males. Preputialectomized males inseminated fewer females with a less pronounced Day-3 peak of inseminations than intact males. It is concluded that the preputial secretion is at least partly responsible for the acceleration or synchronization of oestrus (Whitten effect).

INTRODUCTION

The oestrous cycle of the laboratory mouse is closely related to the caging conditions. Grouped female mice have been reported to develop spontaneous pseudopregnancies (van der Lee & Boot, 1955) or to remain in dioestrus for extended periods of time (Whitten, 1959). When grouped females are exposed to a male, the majority are stimulated into oestrus with a peak occurring on the third night (Whitten, 1956). The mechanism for both the inhibition and the acceleration of oestrus is presumed to occur through olfactory pathways (van der Lee & Boot, 1956; Whitten, 1966).

The pheromone (or olfactory stimulant) responsible for the acceleration of oestrus (the Whitten effect) appears to be under gonadal control since castrated males are ineffective in accelerating oestrus (Bruce, 1965). Male urine alone is capable of eliciting oestrus in grouped female mice (Marsden & Bronson, 1964; Bronson & Whitten, 1968). Recently, male preputial fluid has been shown to be an attractant to female mice (Bronson, 1966; Bronson & Caroom, 1971) and rats (Orsulak & Gawienowski, 1972). The preputial gland also serves as a source of aggression-promoting odour in the albino mouse (Mugford & Nowell, 1970, 1971). The present study investigates the relationship of the preputial gland to the stimulation of oestrus in the laboratory mouse.

MATERIALS AND METHODS

Female mice (ICR-Swiss) from an outbred colony maintained in the authors' laboratory were employed in the experiments described below. Standard laboratory maintenance procedures were employed throughout.

In Exp. 1, 60-day-old females were grouped six per cage, the dimensions of which were $13 \times 14 \times 56$ cm, for 2 weeks to cause inhibition of oestrus. These females were then treated locally three times daily in the area of the external nares with approximately 0.05 ml urine obtained from: (1) intact males; (2) castrated males; or (3) preputialectomized males. The fourth and fifth series of animals served as controls, the former being treated with 0.05 ml saline and the latter receiving no treatment. Preputialectomies were performed under ether anaesthesia when the males were 45 to 60 days of age. Castrations were performed when the males were weaned (*c.* 21 days of age). The males were used experimentally between 75 and 200 days of age. Urine was collected daily from six or more males housed individually in stainless steel metabolism cages. The urine was pooled each morning and used only on the day of collection. A sixth series of females was exposed to male preputial fluid obtained by first removing the gland and then allowing the fluid to be absorbed by a small piece of filter paper. As the females would eat those portions of the filter paper containing the fluid, the paper was placed between two layers of aluminium screening and suspended within the cage of the females.

Mice were treated for a period of 5 days. Oestrus was determined from vaginal smears (pipette method) which were examined each morning for a period of 5 days beginning on the 2nd day of treatment.

In Exp. 2, 75-day-old females were held in groups of fifteen per stock cage, the dimensions of which were $25 \times 38 \times 51$ cm, for 2 weeks or longer to inhibit the onset of oestrus. The females were then individually paired with an adult male. Twenty preputialectomized and twenty intact males were employed as stud males. The number of days between pairing and insemination was determined by examination for the copulatory plug. The female was removed after 5 days if insemination had not taken place.

All data were subjected to χ^2 analyses.

RESULTS

The results of Exp. 1 are presented in Table 1. Significantly more of the females ($P < 0.001$) treated with urine from intact males attained oestrus than those treated with urine from castrated or preputialectomized males. Saline solution and the lack of treatment were also ineffective. A Day-3 peak of oestrus ($P < 0.001$) followed the initiation of treatment with intact male urine. No such peak occurred for the other four treatments.

The percentage of females stimulated into oestrus from exposure to the preputial fluid alone appeared to be somewhat less than that stimulated by urine from intact males, but this value was still significantly higher ($P < 0.05$) than the values for preputialectomized males or the other groups. A non-random distribution of the day on which oestrus occurred ($P < 0.001$) for this

Table 1. The results of Exp. 1 giving the percentage of female mice in oestrus and daily distributions resulting from several treatments

Treatment	No. of animals	% Oestrus	Day on which the first oestrous smear was detected					Probability of non-random distribution
			1	2	3	4	5	
Intact male urine	210	77	20	23	67	39	13	<0.001
Castrated male urine	252	53	26	24	32	35	17	N.S.
Preputialectomized male urine	220	61	27	23	36	32	16	N.S.
Saline solution	198	56	31	17	25	27	12	N.S.
Untreated	232	52	27	25	25	31	13	N.S.
Male preputial fluid	230	71	24	40	50	33	18	<0.001

N.S., not significant.

treatment supports the hypothesis that the appearance of the Day-3 peak is influenced by the secretion of the preputial gland.

The results of Exp. 2 are presented in Table 2. Significantly more females ($P<0.001$) were inseminated within 5 days by intact males than by preputialectomized males. There was a modest Day-3 peak (35% of total inseminations) and non-random distribution of the data ($P<0.05$) for those females inseminated by preputialectomized males. The Day-3 peak (53% of total inseminations) caused by intact males was numerically twice that for preputialectomized males ($P<0.05$) and highly skewed ($P<0.001$).

Table 2. Distribution of days of insemination of female mice paired with intact or preputialectomized males

Stud male	No. of animals	% Inseminated	Day of insemination				
			1	2	3	4	5
Intact	102	75	15	11	40	6	5
Preputialectomized	103	55	15	13	20	6	2

DISCUSSION

The fluid of the male preputial gland has been shown to be attractive to females (Bronson, 1966; Bronson & Caroom, 1971; Caroom & Bronson, 1971); therefore the ability of this gland to stimulate or aid in stimulating oestrus seems logical. The androgen-dependency of the preputial gland is well known (Burdick & Gamon, 1941; Ebling, Ebling & Skinner, 1969) and the castrated males had an inactive, if not undeveloped, preputial gland, since all castrations in the present study were performed at weaning. The female mouse is known to prefer the urine of intact compared to that of castrated males (Scott & Pfaff, 1970).

Urine from preputialectomized males has been shown to be significantly ($P<0.01$) less effective in stimulating oestrus than urine from intact males, but urine from preputialectomized males does appear ($0.05<P<0.10$) to have

some stimulatory effect when compared to treatment with castrated male urine. Certainly the preputialectomized male continues to produce androgens. We suggest that a second component may exist in male urine which is capable of stimulating oestrus when the preputial gland component is absent. The source and nature of the second component and the means by which it acts independently or synergistically can only be speculative at this time. It is reasonable to suggest that a preputial substance becomes incorporated in male urine and enhances the stimulation of oestrus.

Direct exposure of the female to preputial fluid alone is effective in eliciting oestrus, but less so than exposure to urine from intact males. This observation also supports the suggestion that the stimulation of oestrus is due to two components in intact male urine. Further evidence supporting this hypothesis is presented in Table 2. Preputialectomized males were less effective in inseminating females than were intact males.

The similarity of acceleration of oestrus due to male urine or the presence of the male is seen by comparing Tables 1 and 2. Of the females paired with intact males, 75% were inseminated, and of the females exposed to intact male urine, 77% came into oestrus. Conversely, only 55% of the females paired with preputialectomized males were inseminated and only 61% of the females exposed to the urine of preputialectomized males came into oestrus. Moreover, the slight Day-3 peak resulting from pairing with a preputialectomized male may only represent a resumption of oestrus due to the release from crowding (Marsden & Bronson, 1965a) and not a stimulation by the male.

The possibility exists that the preputialectomy may have interfered with the normal penile response of the male during copulation and thus have resulted in the reduced success of mating. If this were so, the data for the distribution of day of insemination by preputialectomized males should still favour the third night as for intact males, but this was not found to be the case. The difference in the frequency of insemination by intact versus preputialectomized males was due to a virtual lack of a Day-3 peak in the data for the latter.

The results and the interpretations reported here are not in complete agreement with those of Bronson & Whitten (1968) who demonstrated the androgen-dependency of the oestrus-accelerating pheromone and its presence in bladder urine as well as in excreted urine. In their studies, the preputial secretion would not appear to have caused acceleration of oestrus, or else the level of the bladder component of the pheromone was sufficient to overcome the lack of the preputial secretion. In a recent review (Bronson, 1971), it was suggested that the male preputial gland does not serve both as an attractant (signalling pheromone) and an oestrus-inducing primer. Several explanations for the disparate results may be suggested. In storing the male urine, these authors used an antioxidant and an antibiotic which might have altered the qualities of the preputial secretion. The urine samples were supplied within the females' cage on a continuous basis, whereas our samples were applied topically three times each day. Collection of excreted urine in the earlier study was made by compressing the urinary bladder of the animal through the abdominal wall. Finally, these data may represent differences in the ability of the female to perceive the male or the male to produce the pheromone. For example, certain strains of mice

lose their sensitivity to the pregnancy-blocking pheromone or the ability to produce the blocking pheromone, or both (Marsden & Bronson, 1965b; Chipman & Bronson, 1968).

The response of females to the preputial fluid deserves some comment. As noted the females would chew out those portions of the filter paper containing the fluid, and presumably ingest them, since no portions of the filter paper that had been chewed out were ever discovered in the bedding (shavings). Once the treated portions of the paper were removed, the mice appeared to show little interest in the remains, other than occasionally as nesting material. In no instance did the females attempt to chew or lick the aluminium screening although most would display a protracted interest in the screening and paper combined. The limited data for the first tests where the paper was ingested are not included but they fail to suggest any difference from the subsequent data where ingestion of the paper did not occur. It is not known if females would eat portions of filter paper impregnated with male urine.

The study presents evidence that the oestrus-accelerating pheromone is closely related to and/or augmented by the preputial gland secretion. Whether the preputial secretion is the sole stimulatory factor or functions in conjunction with a urinary androgen-dependent component remains unclear.

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