PHEROMONAL FACILITATION OF OVULATION IN THE IMMATURE MOUSE

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Summary. Facilitation of PMSG- or HCG-induced ovulation occurs in the immature female mouse exposed to the presence of, but not in contact with, an adult male. The effect is not seen if an immature or castrated adult male is used. Removal of the olfactory bulbs in the female also prevents facilitation due to the presence of the male. It is suggested that a number of different effects seen in the female due to a pheromone released by the male may be mediated through a single environmental stimulus.

Pheromones from the male mouse are known to influence reproductive activities in both the adult and immature female mouse (Lee & Boot, 1955; Whitten, 1956a; Bruce, 1959; Parkes & Bruce, 1961; Eleftheriou, Bronson & Zarrow, 1962; Vandenberg, 1967). Whitten (1966) has suggested that the male pheromone stimulates FSH secretion. The present study reports a pheromonal facilitation of gonadotrophin-induced ovulation in the immature mouse and is interpreted as indicating the release of both gonadotrophins in the female by the action of a male pheromone.

Immature female mice of the Rockland Swiss strain were weaned at 20 days of age and housed six or less to a cage in a room which only contained similar animals. Food and water were supplied freely and a light-dark cycle of 13–11 hr was used with the lights turned on at 07.00 hours. At 09.00 hours, when they were 24 days old, the females received a subcutaneous injection of either 7 or 10 i.u. PMSG dissolved in 0.1 ml of 0.85% saline. At 50 hr after the PMSG injection, each of the experimental females was placed in a cage with, but separated by a double wire partition from, one mature male mouse. The male had previously been placed in the adjoining compartment for at least 1 day to permeate the area with his odour. When the female was introduced, the male was placed on the unsoiled side and the female was placed on the freshly soiled side. Control animals were not moved. All animals were killed 72 to 77 hr after PMSG injection and ova were counted under a dissecting microscope (Rowlands, 1942; Zarrow,

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Caldwell, Hafez & Pincus, 1958). Uterine weights were also obtained for a group of control and anosmic mice. Statistical analysis involved Fisher's exact probability test and the chi-square test with Yates' correction for evaluation of percentages, and the *t* test for the mean number of eggs recovered. All probability values are based on two-tailed tests of significance.

Groups I to IV in Table 1 summarize the findings. Fifty-five per cent of the animals kept in the male-free environment and given 7 i.u. PMSG ovulated, whereas 81% of the animals exposed to the male ovulated ($P < 0.20$). The mean number of eggs recovered was 6.8 for the control group and 12.5 for the mice

TABLE 1

FACILITATION OF PMSG-INDUCED OVULATION IN THE IMMATURE MOUSE ON EXPOSURE TO AN ADULT MALE

Group	Treatment	N	% Ovulating	Mean no. of ova recovered	Group comparison	P % Ovulating	P Mean no. of ova recovered
I	7 i.u. PMSG Controls	20	55	6.8	I versus II	0.20	0.05
II	7 i.u. PMSG Exposed to adult male	16	81	12.5			
III	10 i.u. PMSG Controls	48	23	1.6	III versus IV	0.02	0.001
IV	10 i.u. PMSG Exposed to adult male	35	52	9.8			
V	10 i.u. PMSG Sham anosmic exposed to adult male	15	53	10.6	V versus VI	0.20	0.05
VI	10 i.u. PMSG Anosmic, exposed to adult male	15	20	1.8			

N = No. of female mice.

exposed to the male environment ($P < 0.05$). The data obtained with 10 i.u. PMSG showed a drop in the percentage of animals ovulating and the mean egg recovery count when compared with the group treated with 7 i.u. PMSG. However, a significant increase in both the percentage of mice ovulating ($P < 0.02$) and the average number of ova released ($P < 0.001$) was noted following exposure to the male.

The decreased response to 10 i.u. as compared to 7 i.u. has been confirmed by us with other strains of mice. While we have no data on this discrepancy at the present time, we have suggested that the decreased response in the mouse with higher doses of PMSG may be due to preluteinization (Wilson & Zarrow, 1962).

The effect of the male's presence on hCG-induced ovulation was investigated in a second experiment. Female mice, 24 days old, which had been in a male-free environment for 4 days, were separately exposed to a mature male for 55 hr. The exposure procedure was identical to the one previously described. Control

animals were individually housed in cages in the male-free environment. At 16.00 hours, when they were 26 days old, all animals received 5 i.u. HCG in 0.1 ml of 0.85% saline. Between 09.00 and 11.00 hours the following day, all animals were killed and examined for the presence of ova. These results are summarized in Table 2. Only 25% of the control animals ovulated, whereas 89% of the animals that were exposed to males ovulated ($P < 0.001$). When females were exposed to a male castrated before puberty or to an immature male (less than 30 days old), 30% and 40% of the animals ovulated ($P < 0.004$ and < 0.02 , respectively, when compared to the group exposed to an adult male). Thus, a mature testis appears to be necessary for the male to affect a significant facilitation of ovulation in the HCG-treated immature female.

TABLE 2
EFFECT OF IMMATURE MALES, CASTRATE MALES AND ANOSMIA ON OVULATION IN THE MOUSE INDUCED BY 5 I.U. OF HCG

Group	Treatment	N	% Ovulating	Mean no. of ova recovered	Group comparison	P % Ovulating	P Mean no. of ova recovered
I	Controls	16	25	2.3	I versus II	0.001	0.001
II	Exposed to adult male	19	89	12.5			
III	Exposed to castrated adult male	10	30	3.5	II versus III	0.004	0.01
IV	Exposed to immature male	10	40	5.7	II versus IV	0.02	0.05
V	Anosmic female exposed to adult male	20	35	2.8	II versus V	0.01	0.01
VI	Sham anosmic female exposed to adult male	10	90	11.3	V versus VI	0.02	0.02

N = No. of female mice.

The need for an intact olfactory system in this phenomenon was shown by the use of anosmic mice. Animals were made anosmic when they were 20 days old by removal of the olfactory bulbs (Whitten, 1956b). Sham controls were prepared by simply exposing the olfactory bulbs but not removing them. When they were 24 days old, both types of animals were then exposed to intact adult males as described above. Five i.u. of HCG were injected 55 hr later and animals were killed within 24 hr. Thirty-five per cent of the anosmic animals ovulated as compared to 90% of the sham controls (Groups V and VI, Table 2) demonstrating that anosmia in the female significantly blocks any facilitation of ovulation by the male ($P < 0.02$). As reported by many others, anosmia also significantly reduced the uterine weight.

The uteri of the control mice weighed 30.8 g while the uteri of the anosmic mice weighed 23.2 g. The values for the anosmic animals were significantly decreased ($P < 0.05$).

The experiment with anosmic mice was also carried out with PMSG-induced ovulation and these data are shown in Table 1 (Groups V and VI). The dif-

ference was not significant although more than twice as many of the sham anosmic mice ovulated. However, the mean number of ova recovered was 1.8 for the sham anosmic mice and 10.6 for the anosmic mice ($P < 0.05$).

The need for the presence of a pituitary gland in the induction of ovulation in the immature animal has been established (Quinn & Zarrow, 1964). Whether or not the male's presence specifically stimulates FSH and LH release cannot be determined at this time. However, these findings agree with those of Bronson & Desjardins (1969) and may be interpreted as indicating that exposure to the male induces both an LH release (Table 1) and an FSH release (Table 2). Recent reports now indicate that there is a surge of both LH and FSH near the time of ovulation (Caligaris, Astrada & Taleisnik, 1967; Goldman & Mahesh, 1968), but it is uncertain whether either of these hormones acts alone or whether both act in conjunction to trigger ovulation. It may be concluded that the adult male mouse provides a stimulus that promotes gonadotrophin secretion in the immature female and, as such, these findings present further evidence for a pheromonal effect on gonadotrophin release. They also indicate that a number of different effects may result from a single environmental stimulus acting through a pheromonal substance or substances.

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