References and Notes

1. T. W. Goodwin, in *Physiology of Crustacea*, T. H. Waterman, Ed. (Academic Press, New York, 1960), vol. 1, p. 101.
2. B. M. Gilchrist and J. Green, *Proc. Roy. Soc. London, Ser. B* **152**, 118 (1960).
3. R. Lenel, thesis, University of Nancy, France (1960).
4. G. Wald, *Federation Proc.* **21**, 344 (1962); *ibid.* **22**, 519 (1963).
5. L. R. Fisher and S. K. Kon, *Biol. Rev. Cambridge Phil. Soc.* **34**, 1 (1959).
6. E. Baldwin and R. A. Beatty, *J. Exptl. Biol.* **18**, 136 (1941).
7. R. A. Beatty, *ibid.* **26**, 125 (1949).
8. ———, *ibid.* **18**, 144 (1941).
9. B. Maguire, Jr., *Texas J. Sci.* **13**, 363 (1961).
10. W. R. Eberly, *Systematic Zool.* **9**, 29 (1960).
11. R. Rhoades, *Am. Midland Naturalist* **31**, 111 (1944).
12. Beckman DK2 spectrophotometer.
13. L. R. Fisher, S. K. Kon, S. Y. Thompson, *J. Marine Biol. Assoc. U.K.* **31**, 229 (1952).
14. R. Kuhn, J. Stene, N. A. Sorenson, *Chem. Ber.* **72**, 1688 (1939).
15. M. de Nicola, *Biochem. J.* **56**, 555 (1954); *Exptl. Cell Res.* **10**, 441 (1956).
16. A. T. Diplock, J. Green, J. Bunyan, *Brit. J. Nutr.* **17**, 199 (1963).
17. D. L. Fox, *Animal Biochromes and Structural Colours*, (Cambridge University Press, Cambridge, 1953), pp. 117–131.

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Estrous Synchrony in Mice: Alteration by Exposure to Male Urine

Abstract. *Exposure of grouped, virgin female laboratory mice to urine from male mice for 2 days prior to pairing significantly altered the expected pattern of estrous synchrony. A higher proportion of mice exposed to male urine attained estrus during the 2 days after pairing than did mice exposed to female urine or control mice.*

Female laboratory mice that have been housed in groups show a nonrandom distribution of copulations after individual pairing with a male. Specifically, a higher proportion than expected achieve estrus on the 3rd night after pairing, and a lower proportion on nights 1, 2, and 4. This synchrony of estrus is apparently mediated by olfactory stimuli derived from the male since (i) exposure of the female to a male for 2 days without physical contact results in a higher proportion of copulations on the first night after pairing (that is, the 3rd night after individual exposure to a male); (ii) females which have had their olfactory bulbs removed do not show estrous synchrony; and (iii) exposure of females to cages recently soiled by males slightly alters the synchrony pattern (*1*). The work reported herein shows that a high degree of estrous synchrony can be attained by exposing grouped female mice to male urine for 2 days prior to pairing.

A total of 350 virgin female C57BL/6J mice, 70 to 85 days of age, were used in this experiment. The animals were weaned at 21 to 28 days of age, and placed in groups of 4 to 6 in steel cages (15 by 30 by 15 cm) until 40 to 50 days of age. Between 3 and 5 weeks prior to the experiments, new groups of 10 per cage (30 by 46 by 15 cm) were established.

Females were delegated to one of three experimental groups. Group 1 consisted of 140 females exposed to male urine for 2 days prior to pairing; group 2, 100 females similarly exposed to female urine; and group 3, 110 females constituting a control group which were neither handled nor exposed to urine. Exposure to urine consisted of placing one drop (about 0.05 ml) on the general area of the external nares 4 times daily during the 2 days prior to pairing. This treatment was given at 2-hour intervals beginning at 9 a.m. Urine was collected twice daily from mature studs in breeding colonies or from grouped virgin females of at least 70 days of age. A different set of animals provided the urine for each of the 2 days of treatment. All females from each group of ten were assigned to the same treatment.

At 9 a.m. on the day of pairing each female was placed in a clean 15 by 30 by 15-cm steel cage with one of 60 experienced stud males. On each of the following 4 mornings, females were examined for the presence of vaginal plugs. Females not showing a plug within 4 days were not used in comparing frequency distributions among the three groups.

The females in group 1 (exposed to male urine), had the highest plug frequencies on days 1 and 2, while those in group 2 (exposed to female urine) and in the control group 3 were highest on day 3 (Fig. 1). The difference between groups 1 and 2 was tested by the chi-square method and was significant ($p < .001$). There was also a significant difference between groups 1 and 3 ($p < .02$). There was not a significant difference between group 2 and group 3 ($p < .10$), although there was a trend toward delayed estrus in group 2. Seventeen (12 percent) of the females in group 1 did not show a plug within 4 days, 15 (15 percent) in group 2, and 22 (20 percent) in group 3. These differences were not significant.

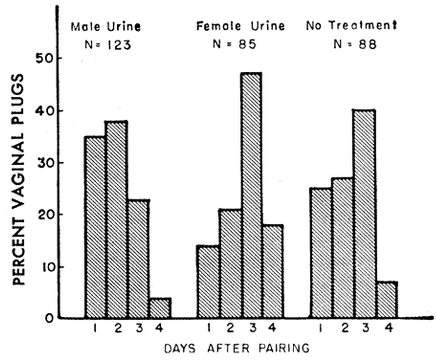

Fig. 1. Frequency distributions of vaginal plugs by days after pairing following 2 days of exposure prior to pairing, to male urine, female urine, or nothing. Values are expressed as percentages of total vaginal plugs occurring within 4 days of pairing. Sample sizes are indicated above each histogram.

It can be concluded that exposure of grouped female mice to male urine can significantly alter the pattern of estrous synchrony obtained after individual pairing with a male. A significant proportion of mice so treated achieve estrus 1 or 2 days earlier than do mice treated with female urine or untreated controls.

The role of olfaction in mammalian reproduction was reviewed by Parkes and Bruce (*2*). Pregnancy block, spontaneous pseudopregnancy, and estrous synchrony were discussed as related phenomena in that all may be the result of social-olfactory stimulation. A specific source of the stimuli has not been reported for any of these phenomena. In estrous synchrony it appears that male urine is a definite stimulus source. It remains to be determined exactly what component of urine is acting as a pheromone in the induction of estrus.

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References and Notes

1. W. K. Whitten, *J. Endocrinol.* **13**, 399 (1956); *Nature* **180**, 1436 (1957); *J. Endocrinol.* **17**, 307 (1958); *ibid.* **18**, 102 (1959); D. R. Lamond, *ibid.* **18**, 343 (1959).
2. A. S. Parkes and H. M. Bruce, *Science* **134**, 1049 (1961).

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