EFFECT OF PHOTOPERIOD ON OVARIAN FUNCTION IN THE VOLE, *MICROTUS AGRISTIS*

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It has been shown that photoperiod affects the reproductive development of the vole, *Microtus agrestis*, the ovaries and uteri weighing more in laboratory-simulated summer than in laboratory-simulated winter (Clarke & Kennedy, 1967). Photoperiod has a similar effect on ovarian function in *M. arvalis* (Lecyk, 1962; Thibault, Courot, Martinet, Mauléon, du Mesnil du Buisson, Ortavant, Pelletier & Signoret, 1966). In order to define more accurately the influence of light on the sexual development of *M. agrestis*, the effect of six different photoperiods on the onset of puberty and on ovarian activity in virgin voles after 8 weeks of treatment was investigated.

The experimental animals were the progeny of a breeding colony kept in the Department of Agricultural Science at Oxford. Females, 18 days old, were placed in light-proof cupboards (see Clarke & Kennedy, 1967) and subjected to 8, 10, 12, 14, 16 or 24 hr light/24 hr for 56 days. Animals were inspected daily for vaginal perforation and smears were taken if this had occurred. The relative abundance of leucocytes, cornified and nucleated epithelial cells was recorded. At the end of the experiment, the animals were killed, uteri removed and weighed fresh, and ovaries weighed after fixation in Bouin's fluid. Ovaries were serially sectioned at 7 μ , stained with Ehrlich's haematoxylin and eosin, and Graafian follicles were measured and counted as described by Breed (1969). Data were subjected to analysis of variance and treatment means were compared by Duncan's multiple range test.

The age at which the vagina opened varied significantly between the different groups ($F = 8.10$, $P < 0.001$; Table 1); animals subjected to 14 or more hr light/day (long photoperiods) became perforate earlier than those on 12 or less hr light/day (short photoperiods). There were, however, no significant differences between the means within either the long or short photoperiods.

None of the vaginal smears indicated regular vaginal oestrous cycles, nor were there any clear-cut differences between treatments in the vaginal smear patterns. About half the females in each group had continuously cornified smears, whereas the remaining animals had mixtures of cornified and nucleated epithelial cells, with or without leucocytes.

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TABLE 1
EFFECT OF DIFFERENT PHOTOPERIODS ON TIME OF VAGINAL PERFORATION AFTER THE BEGINNING OF THE EXPERIMENT,
AND ON OVARIAN AND UTERINE WEIGHTS, SIZE AND NUMBERS OF GRAAFIAN FOLLICLES AT AUTOPSY

Photoperiod		No. of animals	Time (days) to vaginal opening	Ovarian wt (mg)	Uterine wt (mg)	No. of Graafian follicles/female	Maximum Graafian follicle diameter (μ)
Hr light	Hr dark						
8	16	8	$37.1 \pm 1.7^*$	2.92 ± 0.59	20.62 ± 4.18	2.9 ± 0.6	493 ± 28
10	14	9	31.4 ± 1.5	3.24 ± 0.56	23.63 ± 3.94	2.2 ± 0.5	530 ± 26
12	12	9	42.0 ± 1.5	2.70 ± 0.56	19.21 ± 3.94	2.1 ± 0.5	503 ± 26
14	10	8	16.6 ± 1.7	4.17 ± 0.59	40.75 ± 4.18	4.7 ± 0.6	599 ± 28
16	8	8	12.2 ± 1.7	5.90 ± 0.59	47.61 ± 4.18	7.4 ± 0.6	632 ± 28
24	0	9	10.7 ± 1.5	5.43 ± 0.56	42.56 ± 3.94	6.6 ± 0.5	619 ± 26

Treatment length: 56 days.

* Mean $\pm$ S.E. calculated from the within-group variance.

Figures within the same brace do not differ significantly.

Treatments also had a significant effect on ovarian ($F = 5.61$, $P < 0.001$) and uterine weights ($F = 9.78$, $P < 0.001$); mean body weights were, however, unaffected and ranged from 28.3 ± 1.6 to 31.5 ± 1.5 g. Mean ovarian and uterine weights for animals subjected to the long photoperiods were all significantly greater than those for animals subjected to the short photoperiods (Table 1), but within both the long and the short photoperiods, no significant differences between the means occurred. There were no corpora lutea in any ovaries but there was a significant effect of treatments on both the number ($F = 16.81$, $P < 0.001$) and maximum size ($F = 5.07$, $P < 0.01$) of the Graafian follicles (Table 1). In the 16- and 24-hr photoperiods, females had significantly more Graafian follicles than in all other groups. The number for the 14-hr photoperiod was also significantly greater than the number for the females subjected to the short photoperiods. The maximum sizes of Graafian follicles did not differ significantly within either the long or short photoperiods but, whereas the figure for the 16-hr photoperiod was significantly greater than those for all the short photoperiods, that for the 24-hr only differed significantly from the 12- and 8-hr and that for the 14-hr only from the 8-hr photoperiods.

These results indicate that the onset of puberty in female voles, as indicated by vaginal perforation, depends on the length of the photoperiod, 14 or more hr light inducing earlier maturation. Puberty was not, however, further accelerated by light regimens greater than 14 hr light, nor was it retarded more by the two light regimens of less than 12 hr light.

Similarly, it was found that, within the limits of this experiment, there was a threshold photoperiod greater than 12 hr and less than 14 hr light at and above which the reproductive organs were more activated. Clarke & Kennedy (1967) exposed voles either to 6 or 16 hr light for 84 days but there were no significant differences between treatments in the maximum follicular sizes. The greater duration of this earlier experiment may have permitted follicles in females on short days to develop, albeit more slowly, to the same size as those of animals on long days. Thus, short photoperiods appear to delay but do not prevent ovarian development.

The occurrence of larger ovaries with both more and larger follicles suggests that animals on long photoperiods had higher circulating levels of gonadotrophin. Ovariectomy in the vole results in a marked decrease in uterine weight and closure of the vagina, but daily subcutaneous injections of $0.01 \mu\text{g}$ of oestrogen reverses these effects (Breed, 1968). The results obtained indicate that animals on 14 or more hr light/day secreted sufficient oestrogen to induce earlier vaginal perforation and the heavier uteri indicate higher levels of oestrogen at termination of the experiment. The lack of corpora lutea in all animals is consistent with other results showing that the vole is an induced ovulator (Austin, 1957; Breed, 1967).

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