Influence of a skeleton photoperiod on reproductive organ atrophy in the male golden hamster

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Summary. The timing of reproductive events is critically related to the exposure of a photosensitive animal to environmental light. Male hamsters were maintained in long photoperiods, short photoperiods or in darkness interrupted by brief periods (15 min) of light at 6 h intervals for 11 weeks. Gonadal atrophy did not occur in hamsters maintained in long photoperiods or in those maintained in the interrupted photoperiodic cycle, although hamsters maintained in a photoperiod of 2L:22D showed severe involution of the gonads and accessory sex glands (seminal vesicles and coagulating glands). The results indicate that the time of occurrence of environmental light during the photoperiodic cycle in which an animal is maintained is more important in determining the reproductive status of an animal than is the total amount of darkness or the dark to light ratio.

Introduction

Testicular atrophy is induced in hamsters by exposure of the animals to short daily photoperiods (Hoffman & Reiter, 1965a) which are defined as anything less than 12.5 h light/24 h (Gaston & Menaker, 1967). Gonadal atrophy has also been shown to be a pineal-mediated response because there is no atrophy of the reproductive organs in pinealectomized hamsters exposed to restricted photoperiods (Hoffman & Reiter, 1965a). However, the effects of the pineal gland upon reproduction are inextricably linked with those of the photoperiod on reproduction.

The exact mechanism by which the pineal mediates the effects of darkness is not known. It is generally accepted that darkness allows the pineal gland to synthesize and to secrete an 'antigonadotrophin' which either directly or indirectly regulates reproductive function. The effect of the photoperiod on the reproductive status is thought to depend on the ability of the animal to distinguish the length of one photoperiod from that of another. Bunning (1964) suggested that an organism possesses an endogenous circadian rhythm of sensitivity to light, i.e. a photoinducible phase. According to this theory, photoperiodic induction by long days occurs when light extends into the photoinducible phase of the cycle and, in contrast, induction would fail in short days, presumably because light is restricted to the non-sensitive phase of the rhythm.

Rudeen & Reiter (1979) examined photoperiodic induction by monitoring pineal enzyme activity in hamsters maintained in various photoperiods. In hamsters maintained in a 'skeleton' photoperiodic cycle, light extended into a loosely defined sensitive phase of the endogenous rhythm of sensitivity and resulted in obliteration of the nocturnal increase in pineal enzyme activity.

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The purpose of the present study was to determine if reproductive competency is sustained when animals are maintained in a 'skeleton' photoperiodic cycle despite the fact that hamsters are maintained in an excess of 22 h of darkness daily.

Materials and Methods

The male hamsters purchased from Lakeview Hamster Colony (Newfield, New Jersey) were adults and weighed 80–100 g at the onset of experiments. All 98 animals were caged in polycarbonate cages with 6–8 animals per cage. Food (Wayne Lab Blox) and water were always available. Animals maintained in a 14 h light (L):10 h dark (D) lighting regimen were housed in a windowless room with a fluorescent light source. The lighting was regulated by an automatic timer (lights on at 06:00 h). Humidity and temperature were automatically controlled (ambient temperature 22 ± 2°C).

Animals kept in photoperiods other than the standard 14 L:10 D were placed in environmental chambers which had independent lighting and ventilation systems; conditions other than the light were therefore similar to those in the main room. Light cycles in the chambers were controlled by automatic timers enabling any combination of light and darkness. Light was provided by ‘cool-white’ fluorescent bulbs.

Surgical procedures consisted of pinealectomy or blinding. The animals were anaesthetized with anhydrous ether and maintained in an anaesthetized state throughout the surgical procedure. Pinealectomy was performed by the technique described by Hoffman & Reiter (1965b). Blinding was effected by transection of the optic nerve followed by removal of the eye. Pinealectomized animals were examined at necropsy for completeness of the operation. Control animals were subjected to sham-operations involving incisions but no tissue removal.

At the end of an 11-week period, animals were killed by decapitation and the paired testes, seminal vesicles and coagulating glands were removed. The fluids in the accessory sex glands (seminal vesicles and coagulating glands) were expressed, and the paired testes and accessory sex glands were weighed separately. One testis, one epididymis and the paired accessory sex glands were fixed in Bouin's fluid for subsequent morphological examination. After fixation for 48 h, the tissues were dehydrated through a series of ethyl alcohols, cleared with amyl acetate, embedded in paraffin wax, sectioned at 8 µm and stained with Harris' haematoxylin and eosin.

Experiment 1


Experiment 2

Groups of 10 hamsters were treated as follows. Group 7: intact hamsters kept in 14L:10D. Group 8: blinded and sham-pinealectomized hamsters kept in 14L:10D. Group 9: blinded and pinealectomized hamsters kept in 14L:10D. Group 10: sham-pinealectomized hamsters were placed in darkness which was interrupted at 6-h intervals with light for 15 min (0.25L:5.75D × 4). Group 11: pinealectomized hamsters kept in darkness with intermittent light as in Group 10.
### Statistical analyses of data

The data were compared by an analysis of variance (ANOVA). When this test indicated significance, the data were further analysed by a t test for differences between the means (Bruning & Kintz, 1977). Significant difference was accepted at the 95% level of confidence.

### Results

#### Experiment 1

The absolute and relative weights of testes and accessory sex glands are shown in Text-fig. 1. Hamsters in Groups 3 and 5 had very low organ weights but values in Groups 1, 2, 4 and 6 were similar.

Normal spermatogenesis and spermiogenesis were observed histologically in hamsters in Groups 1, 2, 4 and 6. Most tubules contained free spermatozoa in the lumina and the heads of developing spermatozoa were seen attached to Sertoli cells. The epididymis also contained spermatozoa. In Groups 3 and 5 the seminiferous epithelium contained Sertoli cells, spermatogonia and some primary spermatocytes. There were no spermatozoa present in the

![Figure 1](https://via.placeholder.com/150)

**Text-fig. 1.** Absolute (open bars) and relative (hatched bars) weights of paired testes and accessory sex glands of hamsters maintained in various photoperiodic cycles (see text for details of treatments). Values are mean ± s.e.m. for the numbers of animals indicated in parentheses. In Exp. 1, compared with equivalent values for Group 1, *P < 0·001. In Exp. 2, compared with equivalent values for Group 7, *P < 0·001; **P < 0·01; ***P < 0·05.
lumina which were often obscured by large numbers of sloughed cells. Only spermatocytes or spermatids could be identified in the cellular debris. The epididymides appeared atrophic; the lumina contained cellular debris, presumably from the germinal epithelium, and included large cells resembling spermatids and eosinophilic masses.

Experiment 2

The hamsters in Group 8 had significantly reduced accessory sex gland weights (Text-fig. 1). Testicular weights in Groups 7, 9, 10 and 11 were similar, but accessory sex gland weights in Groups 9 and 10 were higher than those in Group 7. The seminiferous tubules and germinal epithelium of hamsters in Groups 9, 10 and 11 were histologically similar to those of hamsters maintained in Group 7. All stages of spermatogenesis and spermiogenesis were apparent and free spermatozoa were present in the lumina in many of the seminiferous tubules of animals in Groups 7, 9, 10 and 11. The epididymides of these animals also contained spermatozoa. The histological changes of the reproductive organs observed in animals maintained in Group 8 were similar to those described for Groups 3 and 5.

Discussion

The present results confirm that blinded hamsters maintained for 11 weeks in a long photoperiod experience reproductive organ involution (Groups 3 and 8). This is a pineal-mediated response since blind animals do not undergo gonadal atrophy if the animals are first pinealectomized (Groups 4 and 9). Furthermore, short light periods (2L:22D) are as effective as total light deprivation in inducing reproductive organ atrophy when hamsters are maintained for 11 weeks in the respective photoperiodic cycle.

Short periods of light given throughout the photoperiodic cycle were capable of completely inhibiting the nocturnal increase in hamster pineal N-acetyldtransferase activity (Rudeen & Reiter, 1979). It was suggested that, since the pineal enzyme activity was suppressed throughout the photoperiodic cycle, no antagonadotrophic factors were elaborated and animals maintained in this photoperiodic cycle of > 22 h darkness would not suffer from reproductive organ involution. The present results show that hamsters maintained in darkness interrupted with brief periods of light at 6-h intervals for 11 weeks did not undergo gonadal involution (Group 10) although hamsters maintained in a shortened photoperiod (2L:22D) for the same duration suffered severe involution of the gonads and accessory organs (Group 5).

This observation may be explained according to Bunning's theory (1964), whereby photoperiodic maintenance of reproduction would occur when light extends into the photoinducible phase of the cycle. On the contrary, if light is absent during the photoinducible phase, the pineal is activated (Rudeen & Reiter, 1979) and the gonads atrophy. The present findings (Group 10) indicate that even small amounts of light presented during the photoinducible phase of the cycle maintain the gonads, possibly by inhibiting the antagonadotrophic influence of the pineal gland.

The ability of a short period of light to maintain normal reproductive capability has been examined by several investigators. Elliot, Stetson & Menaker (1972) exposed hamsters to “resonance” photoperiods by coupling light periods with dark periods of 18, 30, 42 or 54 h. The cycle lengths of 24 (6L:18D) and 48 h (6:42D) resulted in testicular regression, but those of 36 h (6L:30D) and 60 h (6L:54D) maintained testicular weights. Elliot et al. (1972) suggested that the light phases which occurred during the presumptive photoinducible phase in the 36 h and 60 h cycle were capable of maintaining the reproductive organs, despite the prolonged periods of darkness intervening between light periods.

Hoffman & Melvin (1974) attempted to determine whether the dark-induced gonadal
atrophy was due to a ratio of light to dark or whether it was due to the length of darkness occurring at any one time by maintaining hamsters in either a short photoperiod or in darkness interrupted with brief periods of light. The short photoperiodic cycle led to the expected gonadal collapse whereas the gonads of hamsters maintained in the ‘skeleton’ photoperiod developed but not to the adult condition. Hoffman & Melvin (1974) concluded that the duration of the dark period determined the reproductive function rather than the dark : light ratio, and further suggested that the effects of a photoperiodic cycle upon the reproductive organs may be mediated by changes in the activity of the pineal gland.

Eskes & Zucker (1978) examined testicular involution in response to short photoperiods in hamsters that were given 7.5% deuterium oxide. The authors suggested that the deuterium oxide treatment changed the phase relationships between the light : dark cycle and the circadian rhythm of sensitivity to light. In doing so, the hamster’s photoinducible phase was stimulated with light during short days and the testes remained large.

The results of these experiments, taken collectively, indicate that the response by an organism to a long day or a short day is determined by the presentation of the light phase during the photoinducible phase of the endogenous rhythm of sensitivity. Photoperiodic control of reproduction is probably not related to the actual length of the daily dark period or to the dark : light ratio. The present data suggest that the pineal is critically involved in inducing gonadal involution and that the effects of light may act either upon circadian timers within the central nervous system or the pineal gland to alter its activity relative to a time when an increase in pineal function is capable of inducing reproductive organ atrophy. Whatever the mechanism, these results indicate that the time of occurrence of environmental light during the photoperiod in which an animal is maintained is more important in determining the animal’s reproductive status than is the total amount of darkness or the dark to light ratio.

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References


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