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## Photoperiodic Effects in the Djungarian Hamster

Rate of Testicular Regression and Extension of Pineal Melatonin Pattern Depend on the Way of Change from Long to Short Photoperiods

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**Key Words.** Photoperiod · Pineal · Melatonin · Djungarian hamster · Testis

**Abstract.** In the Djungarian hamster, short photoperiods induce regression of testes and accessory glands. There is evidence that the length of time melatonin levels are elevated is the signal conveying the photoperiodic effects to the neuroendocrine axis. When the temporal course of decompression of the pattern of pineal melatonin content was followed after a change from long to short photoperiods by symmetrical extension of the dark time, extension proceeded first into the morning hours. The present study investigated whether, after transition from long to short photoperiods by either extending the dark period into the morning hours, or starting the darktime at noon, differences in the gonadal reaction and in the rate of extension of the pineal pattern ensue. Three and 7 weeks after transition from long to short photoperiods highly significant differences were found in the rate of involution of testes and accessory glands. Regression was more advanced after extension of the dark period into the morning hours. The length of time that pineal melatonin content was elevated also differed markedly between the two groups. After 3 weeks in short photoperiods, the length of the melatonin peak was more than 9 h when the dark period was extended into the morning, but only about 7 h if it was extended into the afternoon. It is suggested that the different rate of gonadal regression after different ways of transition into the same photoperiod is due to the different rate of decompression of the melatonin pattern.

The Djungarian hamster *Phodopus sungorus* is a photoperiodic species. Under natural illumination it shows a marked annual cycle in gonadal size and activity, as well as in body weight and pelage color [3, 6]. In male hamsters that are transferred from long to short photoperiods, regression of testes and accessory glands occurs after some time. Long photoperiods reverse this process and accelerate gonadal recrudescence [4, 5]. The pineal gland participates in conveying the photoperiodic information; this holds for the inhibitory effect of short photoperiods as well as for the stimulatory effect of long photoperiods [5, 11]. There is strong evidence in this species that the daily temporal pattern of synthesis and release of the pineal hormone melatonin, mainly the length of the period of elevated melatonin levels, may be involved in transferring photoperiodic information [1, 2, 10].

Pineal melatonin content and serum levels of melatonin run parallel, as has been shown in other species [13, 18]. The rhythm of pineal melatonin content in the Djungarian ham-

ster and the rhythm of pineal N-acetyltransferase activity which defines the period of high nocturnal melatonin production also run parallel [8, 16]. The pattern of both rhythms correlates well with the length of the effective dark-time in a photoperiodic regimen [6, 10, 19]. However, full adjustment of these rhythms after a change from long to short photoperiods takes several weeks when the dark-time is lengthened symmetrically to midnight [14], and extension of the pattern was found to proceed, first into the morning hours and only later into the evening hours [14]. On the other hand, after transition from short to long photoperiods, adjustment to the new light conditions seems to be rapid [9].

In most experiments on the photoperiodic reaction in *Phodopus*, changes in the length of illumination were performed symmetrically to noon and midnight. Little work has been done in any photoperiodic species to examine whether the mode of change from long to short photoperiods has an influence on the gonadal reaction. Some observations in the Djungarian hamster suggested such an influence. Accordingly, a systematic study was performed to determine the rate of the photoperiodic reaction when the

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change from long to short photoperiods was achieved by extension of the dark period either into the morning or into the evening hours. In addition, the pattern of pineal melatonin content which reflects the pattern of pineal melatonin production and of serum levels of melatonin was determined under these conditions.

## Material and Methods

Male Djungarian hamsters derived from our breeding colony were used. They had been maintained in long photoperiods (LD 16:8, light from 4 to 20 h) from birth until the start of the experiments. At this time they were between 3 and 6 months old. Light intensity in the experimental chambers directly above the cages was between 60 and 600 lx depending on the position of the cage in the room. No influence of intensity was observed within this range. Temperature was kept at  $20 \pm 2^\circ\text{C}$ . Food and water were provided ad libitum.

In the first experiment, two groups of 20 hamsters each were transferred to short photoperiods (LD 8:16). In group A this was attained by prolongation of the dark period by 8 h into the evening hours (light from 4 to 12 h), in group B by extension of the dark period by 8 h into the morning (light from 12 to 20 h, compare fig. 1a). After 7 weeks in these conditions the hamsters were sacrificed and fresh weight of testes and accessory glands (seminal vesicles, coagulating glands, ampullary glands) as well as body weight were determined. In addition, 10 hamsters were sacrificed at the beginning of the experiment to serve as initial controls (IC). As we have never observed any change in testis and accessory gland weight in Djungarian hamsters of similar age that were maintained in LD 16:8 for a longer time [7, 12], another control group killed at the end of the experiment was not included.

In a second experiment, two groups of 91 males each were transferred from LD 16:8 to the same two short-day regimes. After 3 weeks, 7 animals each were killed by decapitation at the times indicated in figure 3. If this occurred during the dark period, they were exposed to dim red light for less than 1 min prior to decapitation. Pineals were removed rapidly and stored in Petri dishes on solid  $\text{CO}_2$  and weight of testes and accessory glands were determined. In addition, 46 comparable hamsters were sacrificed at the beginning of the experiment to serve as initial controls, and weight of testes and accessory glands was determined.

Within 4 days following decapitation, individual pineal glands were homogenized in 100  $\mu\text{l}$  of 0.05 M phosphate buffer, pH 7. The homogenates were diluted with 500  $\mu\text{l}$  of the same buffer and stored at  $-25^\circ\text{C}$ . Within 2 weeks each homogenate was extracted with 1 ml of methylene chloride. The organic phase was washed twice with 0.2 ml of 0.1 M NaOH and evaporated to dryness. Melatonin was estimated in the dry residue in duplicate by a radioimmunoassay as described previously [14]. Melatonin concentration was expressed as ng/pineal gland. The limit of detection of the assay was 6 pg/tube. The intra- and interassay coefficients of variation were 8.1% (for 50 pg,  $n = 5$ ) and 11.3% (for 50 pg,  $n = 4$ ), respectively. Kits for the melatonin assay were purchased from WHB, Bromma, Sweden.

The data on testis and accessory gland weight were analyzed by the Kruskal and Wallis test, an analysis of variance by rank. Sam-

ples were then compared by Mann-Whitney's U test or by t test. The data on the melatonin rhythm were analyzed using one-way analysis of variance. t test with Bonferroni probabilities (BMDP Statistical Software, University of California, Los Angeles) was employed for the post hoc comparison, with  $\alpha = 0.05$  required for significance. Heterogeneity of variance was reduced by log transformation of the data.

## Results

### Experiment 1

After 7 weeks in LD 8:16, regression of testes and accessory glands was more progressed in those animals in which the dark period had been extended into the morning hours (fig. 1). The difference was significant (overall significance for the 3 groups  $p < 0.001$  for testis weight and  $p < 0.005$  for accessory gland weight; difference between the two groups in short photoperiods  $p < 0.01$  for testis weight and  $p < 0.05$  for accessory glands, two-tailed U test). Compared to initial controls there was significant regression in group A ( $p < 0.05$  for testis weight and  $p < 0.01$  for accessory glands, one-tailed U test). Due to the photoperiodic response, body weight in group A declined from the initial value of  $47.7 \pm 0.7$  g by  $2.6 \pm 0.7$  g ( $= 5.5\%$ ) during the 7 weeks in short days; in group B, body weight declined from  $47.8 \pm 0.7$  g by  $5.2 \pm 0.7$  g during this time. The difference in the decrease of body weight between the two groups was significant ( $p < 0.02$ , two-tailed U test).

### Experiment 2

In the second experiment, qualitatively the same result was obtained though exposure to the short photoperiods had only lasted for 3 weeks (fig. 2). After an extension of the dark period into the morning hours (B) there was again more regression of testes and accessory glands than when the dark period was extended into the evening hours (A). The difference was highly significant ( $p < 0.001$  for both testis and accessory gland weight, two-tailed t test). Compared to initial controls, some regression was also observed in group A ( $p < 0.01$  for testes and  $p < 0.02$  for accessory glands, one-tailed t test). Initial weight was not determined in these animals but final body weight was lower in group B ( $45.5 \pm 0.5$  g versus  $47.2 \pm 0.5$  g in group A,  $p < 0.025$ , two-tailed t test). Weight of initial controls was  $47.6 \pm 0.6$  g.

A marked difference in the temporal pattern of pineal melatonin content between the two groups could also be discerned after the 3 weeks in short photoperiods (fig. 3). In group B, in which the dark period was extended into the morning hours, the time of decline of the high nocturnal melatonin values was delayed for about 4.5 h as compared with the previous decline under LD 16:8, while the evening rise was only slightly delayed. In group A, in which the dark-time was extended into the afternoon, there was an

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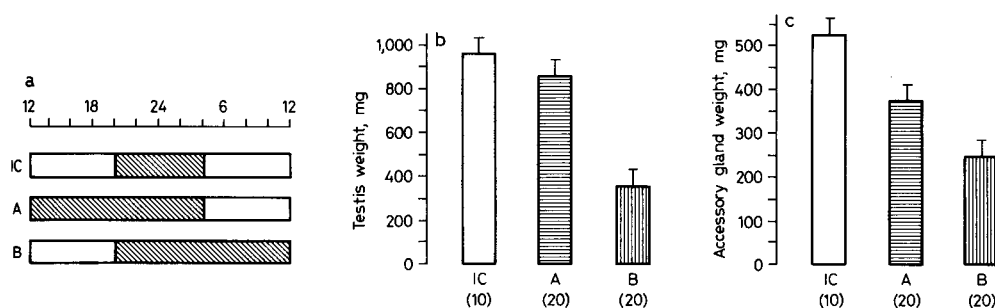


Fig. 1. Weight of testes and accessory glands after 7 weeks in short photoperiods. **a** Schedule of the light-dark cycle before (IC) and after (A, B) transition from LD 16:8 to LD 8:16. **b** Weight of testes (both testes combined). **c** Weight of accessory glands (seminal vesicles, coagulating glands, ampullary glands) before and after transition in short photoperiods by beginning the dark period early (A) or late (B). Means + SEM. Numbers of animals are given in parentheses. For statistics see text.

Fig. 2. Weight of testes (left) and accessory glands (right) before (IC) and after (A, B) 3 weeks in short photoperiods. The photoperiodic schedules are the same as in figure 1. Means  $\pm$  SEM. Number of hamsters in parentheses. For statistics see text.

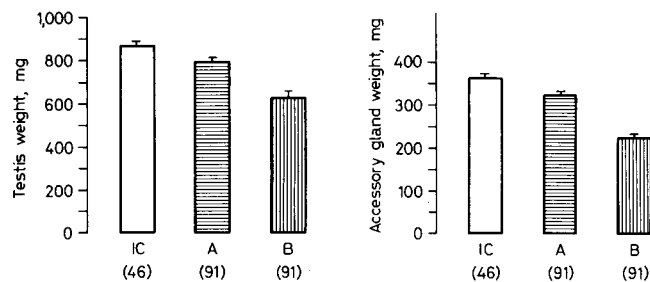
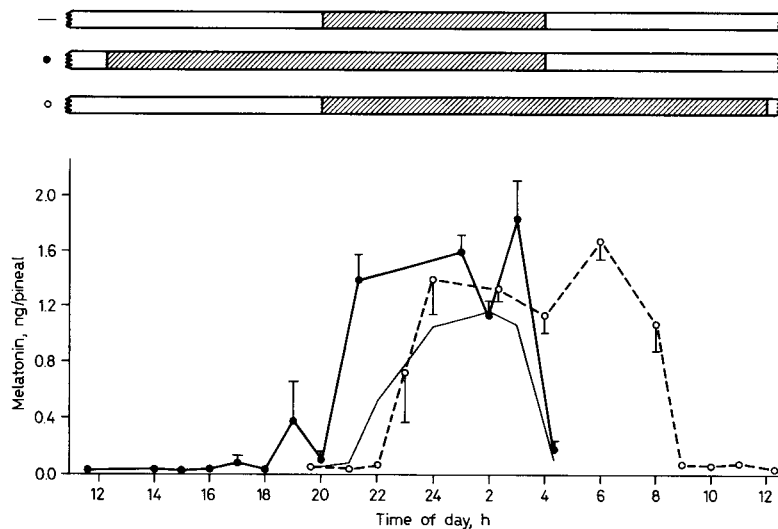


Fig. 3. Pineal melatonin concentration after 3 weeks in the photoperiodic schedules indicated. Symbols are given next to the photoperiodic schedules. Means + SEM of 7 determinations for each point. Curve for LD 16:8 (IC) is taken from (14). Note that the time of elevated values is longer after extension of dark into the morning hours.



advance of the evening rise for about 2 h while the morning decline was practically unchanged as compared to data from LD 16:8. There was an indication, however, that, in individual animals, the evening rise was more advanced (at 19 h 2 of 7 animals showed values higher than 0.6 ng/pineal).

When the dark period was extended into the evening hours (A) the melatonin concentration was significantly increased for the first time above the basal daytime value at 21.20 h ( $p < 0.001$ ). Hence, the rise occurred between 20.00 and 21.20 h. The morning decline occurred between 3.00 and 4.20 h, when the concentration was already signifi-

cantly lower than the high nocturnal levels ( $p < 0.001$ ). Under these conditions, the period of elevated melatonin levels lasted for about 7 h. When the dark period was extended into the morning hours (B) the melatonin level was significantly increased above the basal daytime value at 23.00 h ( $p < 0.05$ ). The rise apparently occurred between 22.00 and 23.00 h. The morning decline occurred between 8.00 and 9.00 h, as the concentration at 9.00 h was already significantly lower than the preceding high nighttime value ( $p < 0.01$ ). Hence the period of elevated melatonin levels lasted for more than 9 h. Though in both groups the period extended as compared to the period of about 5 h under LD 16:8 [14], the extension was greater by more than 2 h when the dark period was prolonged into the morning than when it was prolonged into the evening.

### Discussion

A significant difference in the rate of regression of testes and accessory glands was found after transition into the same short photoperiod, depending on the way in which the light regimen was changed relative to the former long-day schedule. To our knowledge, this is the first demonstration of such an effect in any mammal. Similar differences were found in the decrease of body weight. Previous experiments have shown that, in this species, regression of testes and accessory glands in short photoperiods is always accompanied by a fall in body weight [3, 6, 7].

An influence of photoperiodic pretreatment was reported for the golden hamster by *Morin* [17]. This investigator phase-shifted the light schedule in long photoperiods several days prior to bilateral enucleation and found differences in the rate of regression depending on whether the phase-shift advanced or delayed the cycle. It is difficult to compare these results with those reported here, however, since, in the golden hamster, onset and end of the light-time were both shifted in the same direction and, only several days later, by blinding, were conditions applied that induced gonadal regression.

In most of our former experiments, the photoperiodic schedule was changed symmetrically to midnight to be closer to the natural events. In many laboratories, however, onset or end of the light-time are preserved. As shown here, this may lead to drastic differences depending on which procedure is followed.

A marked difference was also found in the length of time pineal melatonin content was elevated following the two schedules for transition from long to short photoperiods. Three weeks after the dark period was lengthened into the morning hours, melatonin levels were high for more than 9 h, but only for about 7 h if the change in photoperiodic schedule was achieved by starting the dark-time at noon. It is suggested that the difference in decompression of the

melatonin pattern is causally related to the difference in the rate of gonadal regression. In *Phodopus*, the length of time pineal melatonin values are elevated, or serotonin-N-acetyltransferase activity is high, reflects the photoperiodically effective length of the dark-time in a photoperiodic schedule [8, 10, 19]. This holds also if the animals are exposed to skeleton photoperiods [10]. The final length of the time of high melatonin concentrations after transition from long to short photoperiods, however, is only achieved after considerable time in this species [14].

Pineal melatonin content and serum levels of melatonin run parallel, as has been shown in other species [3, 8]. In a series of elegant experiments *Carter and Goldman* [1] have shown that in pinealectomized young *Phodopus* under long photoperiods, infusion of melatonin for 8–12 h daily induced gonadal regression similar to that observed in intact animals maintained in short photoperiods. Infusion for only 7 h induced regression at a slower rate, while infusion for 4 or 6 h did not cause regression but stimulated gonadal development [2]. In the present study, the melatonin pattern had extended to about 7 h after 3 weeks in LD 8:16 in the group in which there was little regression, but to more than 9 h in the group that showed a considerable amount of testicular involution. This is in good agreement with the findings of *Carter and Goldman* [1] and suggests that the length of time melatonin was high was the factor instrumental in bringing about the different rate of regression, i.e. that the threshold of melatonin effectiveness was reached earlier in the group that showed advanced regression. In general, the results reported here are in agreement and support the view that the timespan melatonin values are elevated above the low daytime values is the factor responsible for conveying the photoperiodic message to the neuroendocrine axis. The observations of *Morin* [17] mentioned earlier might also be due to an influence of the different phase-shifts on the melatonin pattern after blinding.

The values for the melatonin pattern reported here are in line with previous observations on the pattern after symmetrical extension of the dark-time [14]. In these experiments there was first an extension into the morning hours. After 2 weeks in LD 8:16, high melatonin values lasted for about 8 h, which is 1 h more than found here after 3 weeks when the dark-time started at noon, but 1 h less than when dark was extended until noon. Four weeks after symmetrical extension of the dark-time, high melatonin levels lasted for about 9 h which corresponds to the value found here after 3 weeks when dark was extended into the morning.

When we recently examined the pineal rhythm of N-acetyltransferase activity in rats after similar changes of the photoperiodic schedule, we observed a similar difference in the pattern [15]. Decompression proceeded more rapidly into the morning than into the evening hours, and the length of time of high values showed differences qualitatively similar to those observed here in *Phodopus*. However,

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lengthening of the pattern was much faster than in *Phodopus*, and seemed to be nearly complete after 6 days. In *Phodopus*, on the other hand, full decompression of the pattern of pineal melatonin content and NAT activity seems to be not yet complete after 6 weeks in short photoperiods [14]. This indicates that there are marked species differences in the rate in which the pineal pattern adjusts to short photoperiods. Hence, it seems possible that species differences in the gonadal reaction to different ways of change into short photoperiods can be expected, depending on the rate at which the pineal pattern adjusts to new light schedules.

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