from active to inactive life is in agreement with the clearly defined seasonal cyclic changes in the adrenocortical tissue observed in other snakes by histological and histochemical methods; in the winter phase only little signs of activity are shown[5]. Besides, in winter the lowered temperature induces a torpid state in *Vipera aspis* with a complete absence of food uptake. Consequently the potassium level in the extracellular fluids decreases. At least in mammals studies in vivo suggest that following potassium deficiency aldosterone secretion is decreased and after potassium loading it is increased[6-8]. The low plasma potassium seen in inactive adult snakes could therefore be a cause of the low plasma aldosterone and the low plasma sodium concentration could be a consequence of the low aldosterone level.

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## Maturation of the pineal melatonin rhythm in long- and short-day reared Djungarian hamsters[1]

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*Summary.* Male Djungarian hamsters, reared under long (16L/8D) or short (10L/14D) days, were sacrificed at various ages during the day or night, or at night following a 30-min light pulse. The pineal melatonin rhythm matured similarly under long and short days by 20 days of age. The results are discussed in context of the hypothesis that melatonin mediates the photoperiod effects which forestall puberty in short-day reared hamsters.
*Key words.* Hamster, Djungarian; melatonin rhythm, pineal; long-day rearing; short-day rearing.

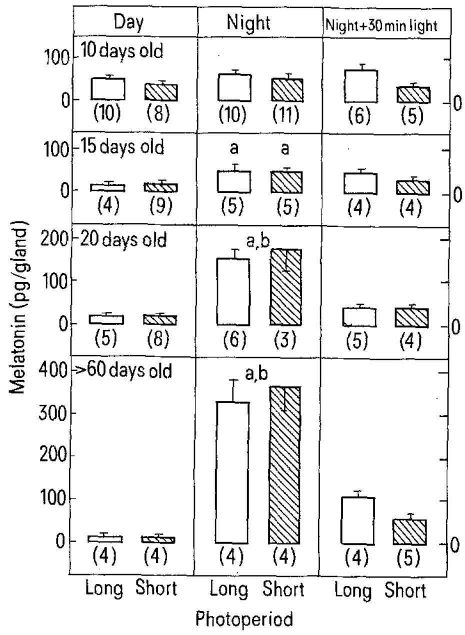
The pineal gland is known to mediate the photoperiodic regulation of adult reproductive function in a variety of seasonal breeding mammals[4-6]. The putative pineal hormone, melatonin, appears to be involved in the mediation of these photoperiod influences. Increased pineal melatonin production is restricted to and appears proportional to the hours of darkness[7]. Moreover, timed melatonin treatments in pinealectomized hamsters have been employed to mimic the inductive and suppressive effects of both long and short days on reproductive function[8-10]. In the Djungarian hamster, the mature adult rhythm in pineal melatonin content is characterized by low daytime and high nighttime concentrations[4,11]; exposure to light suppresses nocturnal production of melatonin and its rate-limiting enzyme, N-acetyl transferase, to low daytime values[12,13]. Previous studies have implicated the daily melatonin rhythm in the photoperiodic mechanism controlling sexual maturation in the Djungarian hamster[5,14]. In hamsters raised under long days, a day/night rhythm in pineal melatonin content develops by the end of the 2nd week of life[12]. By 3 weeks of age, the mechanism which allows light to suppress the nocturnal increase in pineal melatonin is also functional. However, in contrast with the normal reproductive development that occurs under long days, exposure to short days from soon after birth forestalls gonadal maturation within 20 days of age. Discrimination of long from short days may be hypothesized to depend upon the presence of a mature melatonin rhythm. The paucity of information on short-day reared hamsters led to the present study which determined when maturation of the pineal melatonin rhythm occurred in Djungarian hamsters reared under long or short photoperiods, respectively.

*Materials and methods.* Djungarian hamsters *(Phodopus sungorus)* were born in a long-day breeding colony (16L:8D, light on 02.00–18.00 h; original stock provided by Dr Klaus Hoffmann, Max-Planck-Institut für Verhaltensphysiologie, Erlings-Andechs, FRG). Litters were either maintained under long days or transferred to short days (10L:14D, lights on 05.00–15.00 h) on the day of birth. Weaning occurred at 18 days of age and males were group housed (4–5 per cage) for later use. Food and water were available ad libitum with a once a week sunflower seed supplement as previously described[15]. All nighttime sacrifices were performed under dim red illumination which does not interfere with pineal production of melatonin[15]. Males were sacrificed at various ages (fig.) by decapitation during the day (2–4 h before lights off) or night (5 h after lights off) or at night immediately following exposure to a 30-min pulse of light (daytime fluorescent lights, about 500 lux, 4:5–5 h after lights off). In addition, two groups of 12 adults were born and reared in under either long a short days and sacrificed in the same protocol at 60–80 days of age. After decapitation, pineal glands were rapidly removed, stored frozen (− 60 °C) in individual vials and then shipped frozen to Dr Tamarkin (NIH, NICHHD, Bethesda, MD) for radioimmunoassay of melatonin[16,17]. The limit of assay sensitivity was 2 pg/tube; intra- and interassay variation was 2% and 10%, respectively. Data was analyzed by a two-way ANOVA followed by selected t-tests comparing age versus photoperiod treatments. Significant differences between comparisons were noted at $p < 0.05$.

*Results and discussion.* At 60–80 days of age a mature pineal melatonin rhythm was present (fig., lower panel); high nighttime levels were suppressed to low daytime concentrations by a brief exposure to light. Differences among the three photoperiod treatments (day, night and night + 30 min light) were statistically significant. A similar melatonin pattern occurred in 20-day-old males; however, the nocturnal increase 5 h after lights off was significantly less in both long and short days as compared with adult hamsters. This result suggests that the pineal melatonin rhythm had matured within three weeks of birth. This confirms previous results for long-day reared hamsters[12]. Amplitude differences in pineal melatonin content between 20- and 60–80-day-old hamsters are difficult to assess with only a single nighttime sample. In long-day reared Syrian hamsters, though, lower amplitude rhythms in pineal melatonin content were observed prior to puberty than in the adult[18]. Also, in Djungarian hamsters, 18-day-old animals had a lower amplitude pineal melatonin rhythm as compared to 30-day-old hamsters[19].

Complete maturation of the pineal melatonin rhythm was not observed in 10- or 15-day-old hamsters. At 10 days of age, neither long or short-day reared males exhibited a day/night rhythm nor did exposure to light affect melatonin levels. By 15 days of age, a nighttime rise was observed. This may be attributed to lower pineal melatonin values in the day group under both long and short photoperiods; the nocturnal increase was still low, by comparison to hamsters at later ages. This result, in conjunction with the inability of light to reduce nighttime pineal melatonin content suggests an immature rhythm was present prior to 15 days of age. Development of a small but significant day/night difference may indicate that entrainment of the circadian pineal melatonin ryhthm had occurred by the 2nd week after birth. Thus, based upon the ability of light to alter pineal melatonin production, it may be possible to distinguish between the entraining properties of light on the circadian system and the acute inductive effects of light at this early stage of development[20].

These results suggest that maturation of the pineal melatonin rhythm occurs similarly in long and short-day reared hamsters between 15 and 20 days of age. Presumably the pattern of melatonin production is neurally mediated because innervation of the pineal body is completed by 15 days of age[21]. During this period, males acquire the ability to distinguish stimulatory from nonstimulatory photoperiods as evidenced by rapid testicular growth under long days while in short days the testes remain immature[22]. Short days also block the increases in serum FSH, LH, and prolactin which normally would occur in long days during this period. Changes in testes weight, serum gonadotropins and prolactin that occur in long days are blocked by timed melatonin injections, suggesting that the target for melatonin action is competent before 20 days of age[23]. However, maturation of the pineal melatonin rhythm is not the sole determining factor for puberty. In this species, neonatal pinealectomy[24] or pineal denervation (bilateral superior cervical ganglionectomy)[25] resulted in substantial testicular growth during the prepubertal period. Although gonadal growth may occur in the absence of adult pineal melatonin production, the melatonin rhythm appears to be an essential component in the mechanism by which a short-day photoperiod can delay puberty in the Djungarian hamster.




Pineal melatonin content (pg/gland) in long (open bars, 16L/8D; lights on at 02.00 h) or short (hatched bars, 10L/14D; lights on at 05.00 h) photoperiod reared hamsters which were sacrificed at 10, 15, 20 or 60–80 days of age during the day (2–4 h before lights off) or night (5 h after lights off) or night + 30 min light (5 h after lights off prior to which exposure to 30 min of light had occurred). Night pineal melatonin content was significantly elevated compared to day values at 15, 20 and 60–80 days of age in both long and short-day reared males (a: $p < 0.05$). Among the night + 30 min light groups, pineal melatonin was significantly suppressed from night levels at 20 and 60–80 days of age. (b: $p < 0.05$).

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