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Pineal Melatonin Content in Male Hamsters throughout the Seasonal Reproductive Cycle (40981)[1]

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Abstract. The daily rhythm in pineal melatonin content has been characterized at 10-week intervals in male hamsters sequentially exposed to long photoperiods (14 hr light:10 hr dark) for 10 weeks, short photoperiods (8 hr light:16 hr dark) for 30 weeks, and then long photoperiods once again for 10 weeks. These photoperiodic manipulations induced the gonadal changes associated with the seasonal reproductive cycle experienced by this species. At the end of the initial long photoperiod, mean melatonin concentrations of 79–114 pg/gland were found throughout the day and the first 6 hr of darkness and a peak concentration of 949 pg/gland was found 8 hr after the onset of dark. Hamsters exposed to short photoperiods for either 10, 20, or 30 weeks had pineal melatonin concentrations ranging from 50 to 140 pg/gland throughout the day and the first 7 hr of darkness and peak concentrations ranging from 764 to 1025 pg/gland 13 hr after the light to dark transition. The last group of hamsters, which had been in long photoperiods for 10 weeks after exposure to 30 weeks of short photoperiods, had a melatonin concentration profile similar to that of the first group. If, in fact, pineal melatonin levels are related to the amount of indole secreted then the present results do not support the hypotheses which require dramatic alterations in the quantity of melatonin produced by the pineal gland to explain seasonal reproductive changes in hamsters. In particular, photorefractoriness in this species cannot be explained by exhaustion of the capacity of the pineal gland to produce melatonin.

Adult Syrian hamsters exhibit a seasonal cycle in reproductive function which is regulated by photoperiodic cues (1). Upon exposure to short photoperiods, male hamsters experience a dramatic drop in testes weight within 6 to 12 weeks (2). Twenty to twenty-five weeks after initiation of short photoperiodic conditions, testicular function returns even though there has been no change in environmental lighting. At this time hamsters are refractory toward photoperiodic effects and remain refractory until exposed to long photoperiods for at least 10 weeks (3). Since the response of hamsters to short photoperiods is dependent upon an intact pineal gland (2) and can be mimicked by daily melatonin injections (4), it is tempting to postulate that short photoperiods result in increased melatonin production and release by the pineal gland, that this increased production results in gonadal regression and maintenance of the regressed state, that the pineal gland's capacity to produce melatonin eventually becomes exhausted resulting in gonadal recrudescence, and that an interval of long photoperiodic stimulation must intervene so that the pineal gland may rest, this period being the refractory period. To examine the validity of this postulate, we have quantified pineal melatonin content throughout the 24-hr photoperiodic cycle at 10-week intervals in hamsters which have been exposed to 10 weeks of long photoperiods (14 hr light:10 hr dark), followed by 30 weeks of short photoperiods (8 hr light:16 hr dark), and then returned to long photoperiodic conditions. Hamsters exposed to this photoperiodic regimen experience gonadal changes normally associated with the changing seasons (2).

Materials and methods. Seven groups of young adult male Syrian hamsters, 80 to 100 g, were obtained from ARS/Sprague–Dawley (Madison, Wisc.) at 10-week in-

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tervals. Directly after arrival, each group of hamsters was housed in a room with overhead fluorescent lighting (Sylvania Super Saver 35, F40CW/RS/SS) in which lights were automatically turned on at 0600 hr and off at 2000 hr (14 hr light:10 hr dark). Ten weeks later the hamsters were transferred to a cabinet with lights (Sylvania F20T12-CW) turned on at 0700 hr and off at 1500 hr (8 hr light:16 hr dark). They remained in this photoperiod for 30 weeks and then were returned to long photoperiods for a final 10 weeks (Fig. 1). Representative hamsters from each group were decapitated at 2-hr intervals throughout a 24-hr period on Weeks 50, 60, and 70; two hamsters from each group were killed every 2 hr on Weeks 50 and 70, and one hamster from each group was killed every 2 hr on Week 60. Directly following decapitation, pineal glands were collected and placed into individual 1-ml aliquots of 0.1 *M* phosphate buffered saline, pH 6.8, homogenized with a Kontes microultrasonic cell disrupter (Vineland, N.J.), and stored at −20°. Testes were removed from the remaining carcasses and weighed. After pineal samples from all hamsters had been collected, melatonin contents were quantified in duplicate 200-μl aliquots from each 1 ml pineal homogenate by radioimmunoassay (5). Mean pineal melatonin contents were computed from the five replicates representing those hamsters which were killed at the same time of day and which had the same photoperiodic history. Mean testicular weights were computed from the 60 replicates representing hamsters which had the same photoperiodic history.

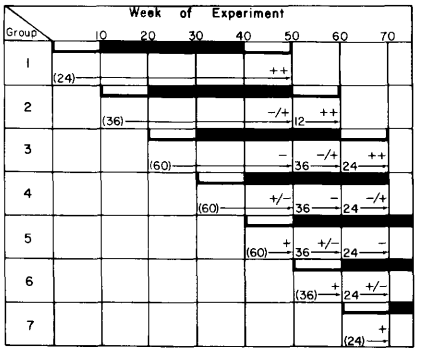

FIG. 1. Summary of photoperiodic manipulations. Values in parentheses represent the number of hamsters purchased and introduced into the colony during the indicated week. The number of hamsters remaining in each group after sacrifices on Weeks 50 and 60 are indicated. Closed bars designate the interval when hamsters of each group were exposed to short photoperiods (8 hr light:16 hr dark). Open bars designate exposure to long photoperiods (14 hr light:10 hr dark). Hamsters which had functional gonads and were sensitive to short photoperiods are designated by +; those which were experiencing a transition between gonadal function and atrophy are designated by +/−; those which had atrophic gonads are designated by −; those which were experiencing gonadal recrudescence are designated by −/+; and those which had functional gonads but were refractory towards short photoperiods are designated by ++.

Results. Paired testes weights for adult male hamsters exposed to long photoperiods (14 hr light:10 hr dark) for 10 weeks, transferred to short photoperiods (8 hr light:16 hr dark) for 30 weeks, then returned to long photoperiods for an additional 10 weeks are depicted in Fig. 2. Values represent the mean ± SEM for 60 hamsters. Testicular weights were significantly depressed 10 weeks after exposure to short photoperiods ($P < 0.001$), remained depressed for an additional 10 weeks, then returned to initial values even though the hamsters had remained in short photoperiods. Subsequent return to long photoperiods resulted in testicular weights slightly higher ($P < 0.001$) than those found

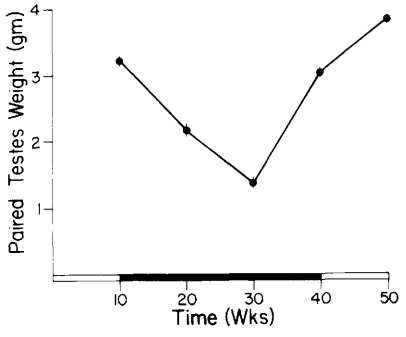

FIG. 2. Testicular weights as a function of photoperiodic history. Values represent the mean ± SEM for 60 hamsters. The solid bar on the time axis represents those weeks when hamsters were exposed to short photoperiods (8 hr light:16 hr dark). The open bar represents those weeks when hamsters were exposed to long photoperiods (14 hr light:10 hr dark).

in hamsters which had not had a previous exposure to short photoperiods.

Pineal melatonin content throughout a 24-hr cycle in adult male hamsters sampled at 10-week intervals throughout the above photoperiodic regimen are depicted in Figs. 3–7. There were no detectable differences between the pineal melatonin contents of hamsters purchased at different times of the year for each of the experimental groups. In those radioimmunoassays utilized to obtain these values, control samples estimated to contain 772, 1282, and 2564 pg/ml, had respective intraassay coefficients of variation averaging 14.9, 14.6, and 23.3%. The respective interassay coefficients of variation were 20.8, 21.7, and 23.5%. The least detectable concentration of melatonin ranged between 9 and 46 pg/gland. Values in Figs. 3–7 represent the mean ± SEM for five hamster pineal glands.

The quantities of melatonin in hamster pineal glands throughout one cycle of the initial 14 hr light:10 hr dark photoperiod are depicted in Fig. 3. These hamsters had been maintained in long photoperiods for 10 weeks. There is a distinct rhythm in pineal melatonin content with mean basal concentrations of 79 to 114 pg/gland found throughout the day and the first 6 hr of darkness and a mean peak concentration of 949 pg/gland found 8 hr after the onset of darkness. The 0600-hr sample was collected in the dark indicating that the drop in pineal melatonin content anticipated the dark to light transition. Elevated melatonin concentrations were found at two sample times indicative of a 2- to 6-hr duration of peak values.

The three sets of hamsters exposed to short photoperiods (8 hr light:16 hr dark) for 10, 20, and 30 weeks, respectively, had pineal melatonin concentration profiles similar to each other (Figs. 4–6). In each case the rhythm in pineal melatonin content persisted. Mean basal concentrations ranging from 50 to 140 pg/gland were found throughout the day and the first 7 hr of darkness. Mean peak concentrations ranging from 764 to 1025 pg/gland occurred 13 hr after the onset of darkness. The last sample collected in darkness was collected 1 hr prior to lights on. Elevated melatonin concentrations were found at four sample times indicating that concentrations were elevated for a period of 6 to 10 hr.

Pineal melatonin content in the last group of hamsters is depicted in Fig. 7. This group of hamsters had experienced one complete gonadal cycle and had been returned to a 14-hr-light:10-hr-dark photoperiod 10 weeks earlier. The circadian rhythm in pineal melatonin content was similar to that found in the first group of hamsters which had been maintained in the same photoperiod. Maximal concentrations of melatonin, 620 pg/gland, were found 8 hr after the

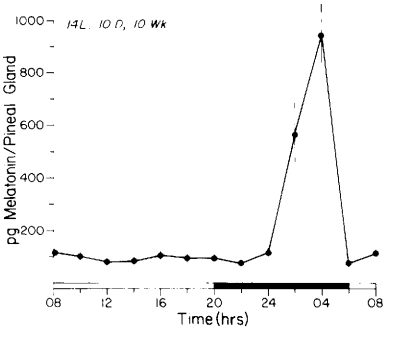

FIG. 3. Pineal melatonin content throughout a 24-hr cycle in hamsters exposed to long days (14 hr light:10 hr dark) for 10 weeks. Values represent the mean ± SEM for five hamster pineal glands. The solid bar on the time axis represents times when hamsters were in the dark.

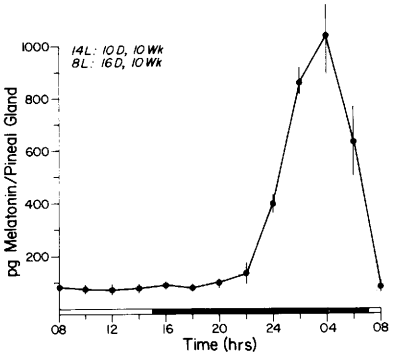

FIG. 4. Pineal melatonin content throughout a 24-hr cycle in hamsters exposed to long days (14 hr light:10 hr dark) for 10 weeks followed by short days (8 hr light:16 hr dark) for 10 weeks. Values represent the mean ± SEM for five hamster pineal glands. The solid bar on the time axis represents times when hamsters were in the dark.

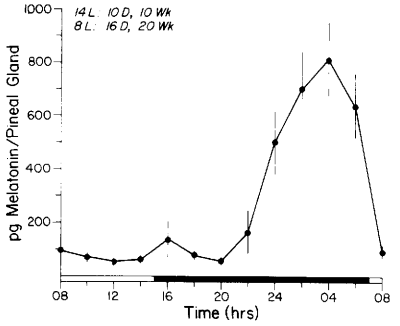

FIG. 5. Pineal melatonin content throughout a 24-hr cycle in hamsters exposed to long days (14 hr light:10 hr dark) for 10 weeks followed by short days (8 hr light:16 hr dark) for 20 weeks. Values represent the mean ± SEM for five hamster pineal glands. The solid bar on the time axis represents times when hamsters were in the dark.

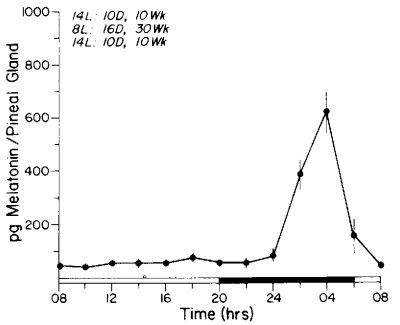

FIG. 7. Pineal melatonin content throughout a 24-hr cycle in hamsters exposed to long days (14 hr light:10 hr dark) for 10 weeks, short days (8 hr light:16 hr dark) for 30 weeks, and then returned to long days for an additional 10 weeks. Values represent the mean ± SEM for five hamster pineal glands. The solid bar on the time axis represents times when hamsters were in the dark.

onset of darkness. Elevated concentrations were present at two sample times indicating a peak duration of 2 to 6 hr.

Discussion. Following 10 weeks exposure of male hamsters to an 8-hr-light:16-hr-dark photoperiod there is a marked reduction in testicular weight. Thirty weeks after initiation of short photoperiods the testes regrow even though no change in environmental photoperiod has occurred. This sequence of events has been well documented in the past (2, 3) and it has been shown (6, 7) that in male hamsters

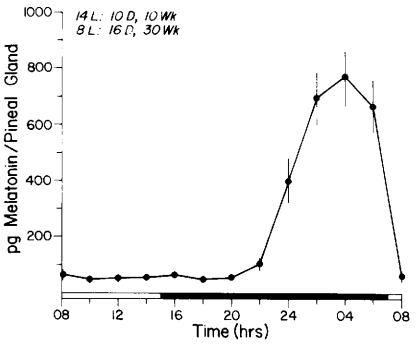

FIG. 6. Pineal melatonin content throughout a 24-hr cycle in hamsters exposed to long days (14 hr light:10 hr dark) for 10 weeks followed by short days (8 hr light:16 hr dark) for 30 weeks. Values represent the mean ± SEM for five hamster pineal glands. The solid bar on the time axis represents times when hamsters were in the dark.

there is a corresponding decrease in serum concentrations of the gonadotropins LH, FSH, and prolactin. The decrease in gonadotropin output is followed by a spontaneous return to normal values at the time of testicular recrudescence (6, 8). It occurs despite the fact that when the gonads are atrophic serum concentrations of gonadal steroids are very low (8, 9). The lack of a pituitary response to removal of negative steroid feedback has been attributed to an alteration in hypothalamic sensitivity (8, 10). An analogous decrease in serum gonadotropin concentrations can be induced by daily melatonin injections (4), a response that supports the contention that melatonin mediates photoperiodic effects upon the hamster reproductive system.

The results reported in this communication demonstrate that seasonal alterations in gonadal function are not associated with dramatic changes in the magnitude of either basal or peak pineal melatonin concentrations. A small increase in the duration of elevated pineal melatonin concentrations found in the populations of hamsters exposed to short photoperiods may reflect increased lability in the timing of peak melatonin output rather than an actual increase in the duration of an individual's productive period. Such an increase in lability is evidenced by the increased variance in timing of activity onset reported

for hamsters maintained in short photoperiods (11).

The lack of an alteration in the quantity of melatonin produced by the pineal gland during different photoperiods fails to support the initial hypothesis, i.e., that seasonal gonadal changes in the Syrian hamster are the consequence of changes in the amount of melatonin produced by the pineal gland. Pineal exhaustion cannot be evoked to explain the refractory period, that time in the seasonal reproductive cycle when hamster gonads are fully functional despite continual exposure to short photoperiods. This conclusion is consistent with data (12) demonstrating that daily melatonin injections are incapable of inducing gonadal regression in pineal-intact refractory hamsters or of preventing development of the refractory condition. The data in this communication, however, do not force rejection of pineal melatonin involvement in the regulation of seasonal gonadal changes altogether. There is an apparent phase shift in peak melatonin concentrations relative to lights out in the different photoperiods. This phase shift may be significant if melatonin acts via a coincidence mechanism, whereby, the proper phase relationship must exist between peak melatonin production and some other cyclic phenomenon (13).

It should be pointed out that the reproductive organs of hamsters respond in a similar manner regardless of the season during which they are initially exposed to reduced daylengths. The response is similar in terms of the timing of the changes and the degree of atrophy (14).

1. Elliott, J. A., Fed. Proc. **35**, 2339 (1976).
2. Reiter, R. J., Anat. Rec. **173**, 365 (1972).
3. Stetson, M. H., Watson-Whitmyre, M., and Matt, K. S., J. Exp. Zool. **202**, 81 (1977).
4. Tamarkin, L., Westrom, W. K., Hamill, A. I., and Goldman, B. D., Endocrinology **99**, 1534 (1976).
5. Rollag, M. D., and Niswender, G. D., Endocrinology **98**, 482 (1976).
6. Turek, F. W., Elliott, J. A., Alvis, J. D., and Menaker, M., Biol. Reprod. **13**, 475 (1975).
7. Bex, F., Bartke, A., Goldman, B. D., and Dalterio, S., Endocrinology **103**, 1069 (1979).
8. Matt, K. S., and Stetson, M. H., Biol. Reprod. **20**, 739 (1979).
9. Berndtson, W. E., and Desjardins, C., Endocrinology **95**, 195 (1974).
10. Turek, F. W., Endocrinology **101**, 1210 (1977).
11. Ellis, G. B., and Turek, F. W., J. Comp. Physiol. **132**, 277 (1979).
12. Bittman, E. L., Science **202**, 648 (1978).
13. Pittendrigh, C. S., and Daan, S., J. Comp. Physiol. **106**, 333 (1976).
14. Reiter, R. J., "The Pineal Gland: A Report on Some Recent Physiological Studies," Edgewood Arsenal Tech. Rep. No. 4110, p. 116. Edgewood, Md. (1967).

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