

Relative organ weights

| Treatment | Testes | Preputial | Epididymides | Seminal vesicles Full | Expressed |
|-----------|--------------|-------------|--------------|--------------------------|---------------|
| Sighted | | | | | |
| Intact | 7.04 ± 0.61* | 2.15 ± 0.26 | 2.31 ± 0.28 | 154.3 ± 14.4 | 78.80 ± 9.52 |
| SPX + SBX | 7.36 ± 1.74 | 2.42 ± 0.37 | 2.43 ± 0.09 | 172.5 ± 16.9 | 88.72 ± 8.84 |
| PX + SBX | 5.11 ± 1.74 | 2.94 ± 0.16 | 2.12 ± 0.38 | 161.9 ± 4.2 | 85.80 ± 14.20 |
| SPX + BX | 6.14 ± 0.74 | 2.23 ± 0.33 | 2.36 ± 0.33 | 148.9 ± 12.8 | 80.87 ± 2.28 |
| PX + BX | 6.28 ± 0.91 | 1.08 ± 0.67 | 2.03 ± 0.37 | 150.5 ± 10.1 | 52.80 ± 3.40 |
| SCGX | 6.87 ± 0.33 | 2.87 ± 0.28 | 2.41 ± 0.35 | 155.7 ± 11.0 | 69.40 ± 5.60 |
| SCGX + BX | 6.06 ± 0.87 | 2.57 ± 0.36 | 2.19 ± 0.15 | 163.4 ± 7.3 | 71.30 ± 6.40 |
| Blinded | | | | | |
| SPX + SBX | 7.18 ± 0.40 | 3.21 ± 0.60 | 2.86 ± 0.15 | 135.5 ± 19.5 | 72.80 ± 10.80 |
| PX + SBX | 6.97 ± 0.34 | 2.92 ± 0.82 | 2.29 ± 0.14 | 135.6 ± 22.1 | 82.60 ± 11.40 |
| SPX + BX | 6.50 ± 0.55 | 2.27 ± 0.52 | 2.37 ± 0.18 | 142.0 ± 17.3 | 85.50 ± 12.32 |
| PX + BX | 7.93 ± 1.30 | 2.46 ± 0.63 | 2.85 ± 0.23 | 132.1 ± 16.4 | 62.80 ± 14.10 |
| SCGX | 6.87 ± 0.73 | 2.16 ± 0.81 | 2.73 ± 0.44 | 139.3 ± 15.6 | 69.70 ± 9.80 |
| SCGX + BX | 6.90 ± 0.91 | 2.20 ± 0.75 | 2.55 ± 0.52 | 141.0 ± 13.7 | 76.70 ± 13.20 |

* The relative organ weights are expressed in mg/g b.wt ± SEM.

does not undergo a pineal-mediated regression in response to shortened photoperiod. It is thought reproduction in this specie may be modulated by the pituitary-adrenal-gonadal axis in response to influences such as population size and/or increased social interactions^{15,16}.

1 Supported by NICHD grant No. HD0 7119-092.

2 Supported by NICHD grant No. 5P5 OHDO 8338.

3 Supported by NSF grant No. SPI 7922 372.

4 R.J. Reiter, *Chronobiology* 1, 365 (1974).

5 R.J. Reiter, *Prog. Reprod. Biol.* 4, 169 (1978).

6 O.K. Ronnekliev and S.M. McCann, *Neuroendocrinology* 19, 97 (1975).

7 S. Bloch, *Revue suisse Zool.* 71, 687 (1964).

8 F.W. Turek, C. Desjardins and M. Menaker, *Biol. Reprod.* 15, 94 (1976).

9 A.S. Berkowitz, J.A. Lloyd and Mridula Chowdhury, *J. Endocr.* 83, 61 (1979).

10 R.A. Hoffman and R.J. Reiter, *Anat. Rec.* 153, 19 (1965).

11 R.J. Reiter and R.J. Hester, *Endocrinology* 79, 1168 (1966).

12 K. Hoffman, *J. comp. Physiol.* 95, 267 (1973).

13 M.K. Vaughan, G.M. Vaughan and R.J. Reiter, *J. Reprod. Fert.* 32, 9 (1973).

14 J.R. Baker and R.M. Ranson, *Proc. Roy. Soc. B.* 110, 313 (1932).

15 J.J. Christian, *J. Endocr.* 74, 669 (1964).

16 J.J. Christian, *J. Endocr.* 75, 653 (1964).

Daily melatonin injections inhibit short-day-induced testicular regression in hamsters¹

F.W. Turek and P. Pappas

Department of Biological Sciences, Northwestern University, Evanston (Illinois 60201, USA), 19 March 1980

Summary. Daily injections of melatonin were found to retard testicular regression in hamsters exposed to LD 10:14, if the injections occurred in the morning (i.e., 0.5 h after lights on), but not if they occurred in the afternoon (i.e., 6 h after lights on). These results indicate that appropriately timed injections of melatonin can at least partially block the inhibitory effects of short days on gonadal activity in the photoperiodic hamster.

In attempts to determine if melatonin is the pineal substance which is involved in the photoperiodic control of neuroendocrine-gonadal activity in the golden hamster, 2 different methods of administering melatonin have been utilized. 1 approach has been to administer melatonin continuously via s.c. depots, such as melatonin-filled beeswax pellets or Silastic capsules^{2,3}. A 2nd approach has been to administer melatonin via single or multiple daily injections^{4,5}. The continuous administration of melatonin by melatonin-filled Silastic capsules inhibits testicular function in golden hamsters exposed to long days (e.g., LD 14:10, 14 h of light per 24 h)³. In addition to this inhibitory effect, a stimulatory effect has also been demonstrated: melatonin implants prevent testicular regression in hamsters transferred from long to short days (e.g., LD6:18)⁶. Daily melatonin injections have been found to inhibit gonadal function in hamsters exposed to long days^{4,5}.

However, unlike s.c. melatonin implants, daily melatonin injections have not previously been shown to stimulate gonadal function. In the present study, we sought to determine if daily melatonin injections could maintain testicular function in hamsters transferred from stimulatory long days to nonstimulatory short days.

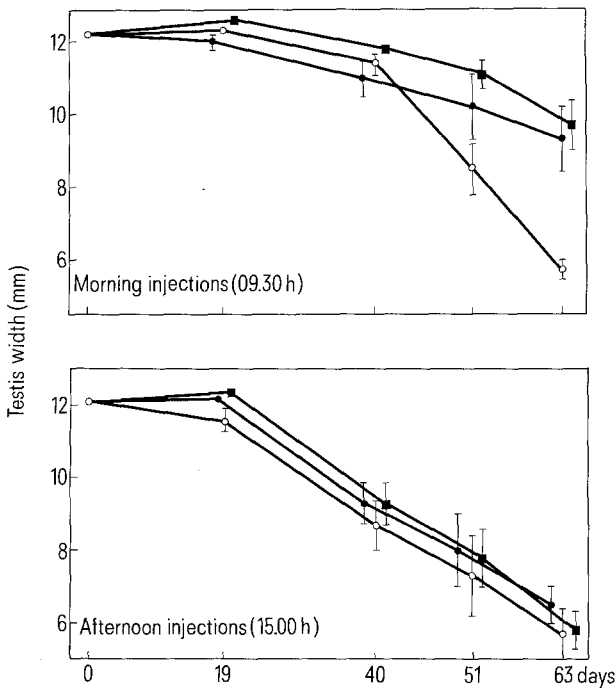
9-week-old sexually mature male golden hamsters (*Mesocricetus auratus* Lak: LVG (SYR)) were purchased from Lakeview hamster colony, Newfield, N.J. Animals were housed 3–4 per cage and food (Teklad hamster diet) and water were supplied ad libitum. The animals were maintained under an LD 14:10 light:dark cycle (lights on 07.30–21.30 h) for 2 weeks before being transferred to an LD10:14 photoperiod (lights on 09.00–19.00 h). Over the next 63 days the animals received daily s.c. injections of either 0.1 ml sesame oil or melatonin (25 or 50 µg) dissolved in 0.1 ml sesame oil. 3 groups of animals (7–8 per

group) were injected at 09.30 h (morning injection groups) while 3 other groups were injected at 15.00 h (afternoon injection groups). The melatonin was obtained from the Sigma Chemical Company. On the first day of LD 10:14 (day 0), and on days 19, 40, 51 and 63 of the study, the width of the right testis was determined by measurement through the scrotum, as de-

Mean weight of the testes and seminal vesicles of hamsters after 63 daily injections of either oil or melatonin

| Time of injection | Injected daily | Testis wt (mg) | Seminal vesicle wt (mg) |
|---------------------|------------------|----------------|-------------------------|
| Morning (09.30 h) | Oil | 386 ± 36 | 111 ± 8 |
| | Melatonin, 25 µg | 1378 ± 400* | 139 ± 12 |
| | Melatonin, 50 µg | 1799 ± 293* | 158 ± 7* |
| Afternoon (15.00 h) | Oil | 530 ± 224 | 102 ± 8 |
| | Melatonin, 25 µg | 526 ± 105 | 107 ± 12 |
| | Melatonin, 50 µg | 454 ± 197 | 110 ± 11 |

* Significantly greater ($p < 0.02$) than oil-injected animals.



Mean width of the right testis in male hamsters that were exposed to LD 10:14 for 63 days. The animals were injected daily either with oil (○—○) or 25 (●—●) or 50 (■—■) µg of melatonin. The injections occurred either in the morning (0.5 h after lights on: top panel) or in the afternoon (6 h after lights on: bottom panel). The standard error is represented by the vertical lines, unless it falls within the point symbol.

scribed previously⁶. On day 63 the animals were sacrificed, and the testes and seminal vesicles were removed and weighed. A Student t-test was used to compare the organ weights of the melatonin-injected groups with the vehicle-injected groups.

Exposure to LD 10:14 induced testicular regression in all the hamsters that received morning (i.e. 09.30 h) or afternoon (i.e. 15.00 h) injections of oil (figure). Daily afternoon injections of melatonin (25 or 50 µg/day) did not alter the inhibitory effects of short days, as evidenced by the observation that testicular regression in these animals was similar to that of the oil-injected animals. In contrast, testicular regression was retarded in those animals that received daily injections of melatonin in the morning. After 63 days of morning injections, the testes of the animals injected with either 25 or 50 µg of melatonin per day weighed significantly more ($p < 0.02$) than the testes of oil-injected animals (table). In addition, the seminal vesicles of the animals injected with 50 µg of melatonin per day at 09.30 h were significantly heavier ($p < 0.02$) than the seminal vesicles of oil-injected animals.

These results demonstrate that appropriately timed daily melatonin injections can at least partially block the inhibitory effects of short days on testicular function in the male hamster. Whether or not a higher dose of melatonin or a different injection time would totally prevent short-day-induced testicular regression is not known. It is interesting to note that the same dose of melatonin (25 µg/day) that in the present study was found to inhibit testicular regression in hamsters exposed to a nonstimulatory LD10:14 light cycle, has been shown to induce testicular regression in hamsters maintained on a photostimulatory LD14:10 light cycle^{4,7}. This inhibitory effect of melatonin during exposure to LD14:10 was observed in hamsters injected late during the light period (e.g., 6–13 h after lights on) but not in hamsters injected early in the light period (e.g., 2–3 h after lights on)^{4,7}. In contrast, the stimulatory effect of melatonin during exposure to LD10:14 (figure, table) was observed in hamsters injected early in the light period (i.e., 0.5 h after lights on), but not in animals injected late in the light period (i.e., 6 h after lights on).

Both daily melatonin injections and the continuous administration of melatonin via subdermal implants have now been shown to inhibit short-day-induced testicular regression in hamsters (table)^{2,6}. In addition, both daily melatonin injections and the continuous administration of melatonin via subdermal implants have been shown to inhibit gonadal function in hamsters exposed to photostimulatory long days^{5,8}. Taken together, these results indicate that there is a complex relationship between the action of melatonin and signals from the photic environment. Further support for the hypothesis that the effect of melatonin depends upon the photoperiodic conditions is the finding that neither daily melatonin injections nor the continuous administration of melatonin is able to interfere with an event which is independent of photic input. Spontaneous testicular recrudescence, which occurs in the hamster after a prolonged exposure to short days, does not appear to be affected by either mode of melatonin treatment^{6,9}.

1 We wish to thank Susan Losee and Susan Stice for excellent technical assistance and Gary Ellis for his comments. This investigation was supported by NIH grants HD-09885, HD 12622 and Research Career Development Award HD-00249 (F.W.T.) from the National Institute of Child Health and Human Development.
2 R.J. Reiter, M.K. Vaughan and P.J. Waring, *Acta endocr.* 84, 410 (1977).
3 F.W. Turek, *Biol. Reprod.* 20, 1119 (1979).
4 R.J. Reiter, D.E. Blask, L.Y. Johnson, P.K. Rudeen, M.K. Vaughan and P.J. Waring, *Neuroendocrinology* 22, 107 (1976).
5 B. Goldman, V. Hall, C. Hollister, P. Roychoudhury, L. Tamarkin and W. Westrom, *Endocrinology* 104, 82 (1979).
6 F.W. Turek and S.H. Losee, *Biol. Reprod.* 18, 299 (1978).
7 L. Tamarkin, W.K. Westrom, A.I. Hamill and B.D. Goldman, *Endocrinology* 99, 1534 (1976).
8 F.W. Turek, *Proc. Soc. exp. Biol. Med.* 155, 31 (1977).
9 E.L. Bittman, *Science* 202, 648 (1978).