increase intracellular sodium levels. Normal physiological concentrations of prolactin remain in the range of 5-20 ng/ ml. Higher levels are encountered during lactation, stress, surgery, pituitary adenoma, galactorrhea, amenorrhea from varied causes, and also on prolonged treatment with drugs such as phenothiazines, alphamethyl DOPA, reserpine and combined contraceptive pills. The significant increase in red cell sodium observed only at concentrations of 50 ng and above, suggests that the high intracellular concentration of sodium observed in hyperprolactinemic states may be a reflection of prolactin action on cellular ATPase by selectively interacting with NaK dependent ATPase activi-

Acknowledgments. Ovine prolactin was generously supplied by Ferring (Malmö, Sweden) and NPA, NIH (Maryland, USA). Thanks are also due to Prof. S. Dutta, Wayne State University, Detroit, for the gift of ouabain octahydrate and Dr

- M. Ramachandran and Prof. S. Ramakrishnan, Department of Biochemistry, JIPMER, Pondicherry, for their encouragement and keen interest in this study.
- D.F. Horrobin, I.J. Lloyd, A. Lipton, P.G. Burstyn, N. Durkin and K.L. Muriuki, Lancet 2, 352 (1971)
- L. Parke and D. F. Horrobin, Br. med. J. 1, 262 (1976).
- R.A. Karmali, D.F. Horrobin, M.S. Manku and B.A. Nassar, in: Prolactin, p.20. A. Res. Reviews, MTP Publications, Lon-
- G.Y.N. Iyer, Br. J. Haemat. 15, 561 (1968).
- T.J. Gill and A.K. Solomon, Nature 183, 1127 (1959).
- S.S. Kaplay, Am. J. clin. Nutr. 31, 579 (1978).
- C.H. Fiske and Y. Subba Row, J. biol. Chem. 66, 375 (1925). O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. 193, 265 (1951).
- I.R. Falconer and J.M. Rowe, Nature 256, 327 (1975).
- I. J. Lloyd, I.R.C.S. 1, 11 (1973).
- R.P.S. Edmondson, R.D. Thomas, P.J. Hilton, J. Patrick and N.F. Jones, Lancet 1, 12 (1974).

Influence of the pineal gland on the reproductive system of the male house mouse¹

R. Philo, A.S. Berkowitz², F.L. Jackson³, J.A. Lloyd and J.P. Preslock

Department of Reproductive Medicine and Biology, The University of Texas Medical School at Houston, P.O. Box 20708, Houston (Texas 77025, USA), 3 March 1980

Summary. Procedures designed to express pineal-mediated antigonadotropic activity were performed upon male house mice. Neither blinding nor blinding plus olfactory bulbectomy of house mice resulted in testicular involution within 12 weeks. The pineal gland appears to be of little significance to reproduction in the house mouse.

The pineal gland is important for transduction of photoperiod duration into humoral factors for control of reproductive cycles in some mammalian species⁴. Photoperiods less than 12.5 h of light per day result in increased pineal activity and consequent regression of testes and sex accessory organs in the golden hamster^{4,5}. In the rat, olfactory bulbectomy is required in addition to blinding for sensitization to actions of the pineal gland⁶. Sympathectomy denervates the pineal gland and renders the gland incapable of mediation of photoperiod-induced testicular regression⁵.

However, the pineal gland and photoperiod may not be involved in regulation of reproduction in the house mouse, Mus musculus, 7,8. The studies contained herein were undertaken to determine the possible existence and nature of pineal-gonadal interactions in the house mouse.

Materials and methods. 60 mature male JC-1 house mice were utilized in this study. The animals were inbred and maintained at this institution9, and were housed with 12 h of light daily (L/D 12/12, lights on 07.00 h). Food and water were available ad libitum. To simulate darkness, 30 adult male mice were anesthetized (methoxyflurane) and blinded via orbital enucleation. Another 30 remained intact. In each of the blinded and sighted groups, subgroups of 5 animals each were:

a) sham pinealectomized (SPX) and sham olfactory bulbectomized (SBX), b) pinealectomized (PX)¹⁰ and SBX, c) SPX and olfactory bulbectomized (BX)6, d) PX and BX, e) superior cervical ganglionectomized (SCGX)11 and f) SCGX and BX. 1 group of 5 naive animals served as intact controls. In SPX groups, the superior sagittal sinus was punctured through the interval between bone disc and calvarium to stimulate bleeding, but the disc was not removed. Holes were drilled in the frontal bones for sham olfactory bulbectomy, but the bulbs were not aspirated.

12 weeks after the surgical procedures, the mice were decapitated. Body weights and weights of preputial gland, epididymides, testes and seminal vesicles (full and expressed of fluid) were measured. Testicular material was

prepared for light microscopic examination (Bouin's fixative, hematoxylin and eosin stain). Data was analyzed by means of a 1-way analysis of variance performed on a Hewlett-Packard 9830-A Computer.

Results. Surgical manipulations designed to increase or decrease pineal activity did not produce significant changes in relative organ weights of the testes or accessory sex organs (table). Examination of histological material indicated testicular regression did not occur in any group. All testicular sections displayed normal spermatids and mature

Discussion. In addition to the hamster and the rat, photoperiodic influences acting through the pineal gland have also been implicated in annual reproductive rhythms in the Djungarian hamster Phodopus sungorus¹², and in the darkinduced sexual regression of the vole Microtus montanus¹³. Seasonal rhythms of reproduction in Microtus agrestis14 may result from combinations of effects of the pineal gland and environmental influences.

Although reproductive capabilities of several rodent species are apparently modulated by photoperiod acting via the pineal gland, results of the present study substantiate prior work suggesting a non-involvement of the pineal gland and/or photoperiod in reproductive function in the male house mouse^{7,8}. Turek et al.⁸ have speculated the pineal gland is more important for the control of testicular function in species responsive to photoperiod such as the golden hamster, and may have no effect in non-photoperiodic species such as the house mouse.

Reiter⁴ has suggested inbreeding of rodents for laboratory purposes may have abolished reproductive responses to changes in photoperiod. The JC-1 strain of house mouse studied herein has been inbred for about 25 years. However, the long standing commensalism between the house mouse and man may have resulted, by natural means, in a similar attenuation of photoperiodic responses noted by Reiter⁴ in laboratory species.

The data presented here suggest the male house mouse

Relative organ weights

Treatment	Testes	Preputial	Epididymides	Seminal vesicles Full	Expressed
Intact	$7.04 \pm 0.61*$	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}.

- Supported by NICHHD grant No. HDO 7119-092.
- Supported by NICHHD grant No.5P5 OHDO 8338. Supported by NSF grant No.SPI 7922 372.
- R.J. Reiter, Chronobiology 1, 365 (1974).
- R.J. Reiter, Prog. Reprod. Biol. 4, 169 (1978).
- O.K. Ronnekliev and S.M. McCann, Neuroendocrinology 19, 97 (1975).

- S. Bloch, Revue suisse Zool. 71, 687 (1964).
- F.W. Turek, C. Desjardins and M. Menaker, Biol. Reprod. 15, 94 (1976).
- A.S. Berkowitz, J.A. Lloyd and Mridula Chowdhury, J. Endocr. 83, 61 (1979)
- R.A. Hoffman and R.J. Reiter, Anat. Rec. 153, 19 (1965).
- R.J. Reiter and R.J. Hester, Endocrinology 79, 1168 (1966).
- K. Hoffman, J. comp. Physiol. 95, 267 (1973).
- M.K. Vaughan, G.M. Vaughan and R.J. Reiter, J. Reprod. Fert. 32, 9 (1973). 13
- 14 J.R. Baker and R.M. Ranson, Proc. Roy. Soc. B.110, 313 (1932).
- J.J. Christian, J. Endocr. 74, 669 (1964).
- 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. I 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 days4,5. 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