Luteal activity of LH-induced[a] postovulatory corpora lutea or luteinized unruptured follicles (LUF) and of spontaneous[b] postovulatory corpora lutea (POCL) in 4-day cyclic female rats

Groups		Number of animals	Total number of POCL and LUF	Number of POCL	Number of LUF	Ovulation coefficient	Blood progesterone concentration ng/ml
LH treatment on dioestrus 2[c]	Without indomethacin	11	11.9 ± 0.9	9.0 ± 0.9*	2.9 ± 0.6*	0.77 ± 0.04*	16.2 ± 1.5*
	With indomethacin[d]	11	12.6 ± 0.8	3.5 ± 0.5**	9.2 ± 0.9**	0.28 ± 0.04**	11.6 ± 2.2**
Natural cycle		12	12.9 ± 0.5	12.7 ± 0.5***	0.3 ± 0.1***	0.98 ± 0.01***	24.3 ± 1.2***

Values are the mean ± SE. [a] Sacrifice on the expected day of oestrus. [b] Sacrifice on the day of dioestrus 1. [c] 7.1 µg/100 g b.wt s.c. LH-M4 in equivalent of NIH-LH-S3 (LH-M4 = 2.25 X NIH-LH-S3) was injected at 10.30 h of an artificial light cycle with lights on 23.00–13.00 h. [d] 5 mg/100 g b.wt i.v. were given by 6 h 30 min after LH. ** $p < 0.05$ vs * and ***; *** $p < 0.05$ vs *.

1 This investigation was partially financed by the 'Centre National de la Recherche Scientifique (E.R.A. No. 566).
2 Acknowledgments. We wish to express our gratitude to Dr M. Justisz for providing the LH-M4 preparation and to Merck Sharp and Dohme-Chibret Laboratories for indomethacin.
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0014-4754/84/050500-02$1.50 + 0.20/0

Season-dependent effects of melatonin on testes and fur color in mountain hares (*Lepus timidus* L.)[1]

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Summary. Melatonin was administered in 3 seasons to adult male mountain hares (*Lepus timidus* L.) kept in long or short photoperiods. Melatonin and short photoperiods induced testis regression and fur whitening in summer and autumn but not in winter. Both treatments combined seemed to provoke an advanced onset of the refractory period.

A large number of studies, mainly on rodents, have shown that the pineal hormone melatonin plays an important role in the regulation of annual rhythms that are controlled by the photoperiod, such as reproductive activity and change of fur color[3–5]. Findings in golden and djungarian hamsters indicated that the effect of administered melatonin depends on the photoperiodic conditions and on the phase of the annual cycle of the animals at the beginning of the experiment[6,7]. In order to test whether reactions to melatonin treatment similar to those observed in rodents could also be seen in lagomorphs, we carried out experiments with adult male mountain hares. This species shows marked seasonal rhythms in gonadal activity and fur color (brown in summer, white in winter) that are controlled by the photoperiod[8] and therefore possibly by melatonin released from the pineal gland.

Materials and methods. All hares, descendants of wild animals captured in Scandinavia, were purchased from the breeder Urogallo, Asiago, Italy (45.7° N, 1000 m). They had been kept outdoors for at least 1 year before the beginning of the experiments. During the experiments they were individually housed in special cages (for details see Spagnesi[9]). They were fed a standard diet (C2 EX, Palatamangimi, Bologna, Italy) and water ad libitum. The temperature was kept at 20 ± 4 °C; the humidity was about 70%. The only light was provided by fluorescent bulbs; the light intensity in the cages varied between 100 and 400 lx. Three experiments were carried out during different seasons; the first used animals in summer condition with large testes and brown fur, the second used animals in autumn condition with partly regressed testes and partial molt to white winter fur, the third was carried out with hares in complete winter condition with fully regressed testes and white fur. On July 6th, October 12th and January 8th the hares received either empty or melatonin-filled silastic tubes (i.d. 1.47 mm, 30 cm/animal, daily release about 150 µg/animal). The s.c. implantations were performed with the animals under anesthesia (Ketalar 50 mg/ml, Parke Davis). From the day of implantation half of each treatment group was kept in long (16 h light/day) while the remainder were kept in short (8 h light/day) photoperiods for 7 weeks. Changes in testis size were estimated once a week by palpation. Fur color was estimated from the regrowing fur on a plucked area on the back of the hare. At the end of each experiment the animals were killed and the testes removed, weighed and prepared for histological examination. As the number of animals per group was very limited (2–4)

Experientia 40 (1984), Birkhäuser Verlag, CH-5010 Basel/Switzerland

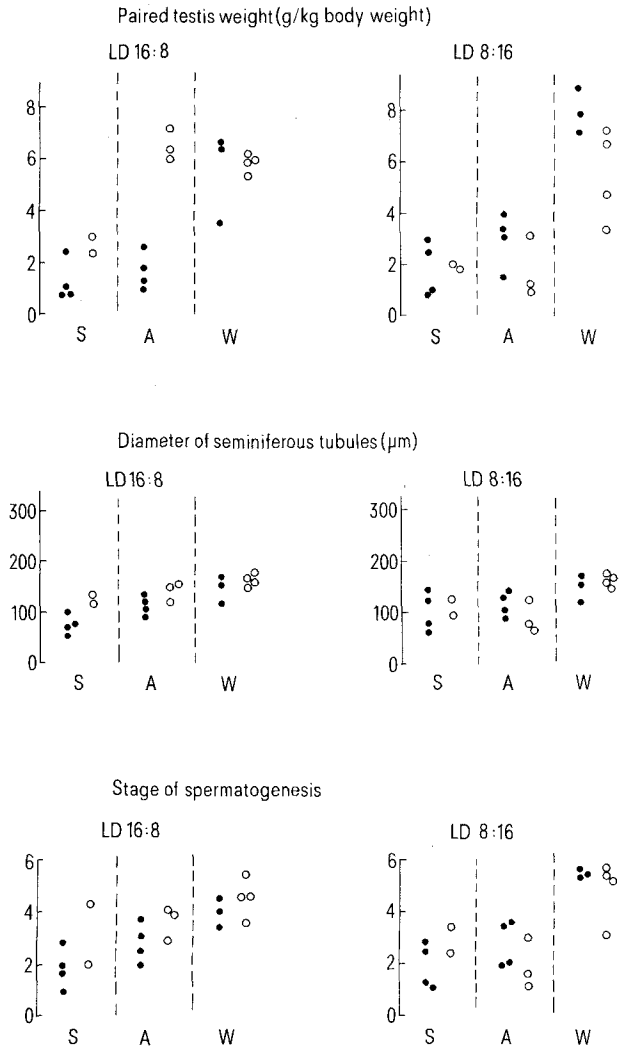

Figure 1. Weight and histological appearance of both testes of mountain hares kept for 7 weeks in long (LD 16:8) or short (LD 8:16) photoperiods. ●, Melatonin-treated animals; ○, control animals; S, summer; A, autumn; W, winter. Dots and circles represent the values obtained from individual animals. Stages of spermatogenesis: 1 = Sertoli cells, spermatogonia, very few primary spermatocytes; 2 = many primary spermatocytes, a few secondary spermatocytes; 3 = many secondary spermatocytes, a few spermatids; 4 = many spermatids, a few immature spermatozoa; 5 = many immature spermatozoa, mature spermatozoa in a few tubuli; 6 = tubuli full of mature spermatozoa.

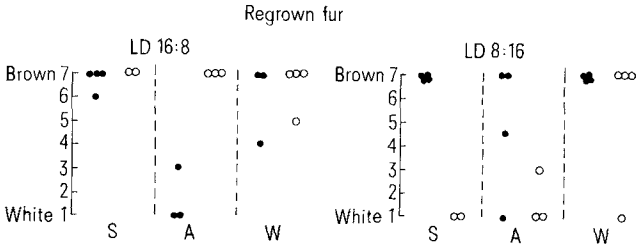

Figure 2. Color of regrown fur of mountain hares kept for 7 weeks in long or short photoperiods. Symbols as in figure 1. Graduation of color of regrown fur: 1 = regrown hairs completely white; 2 = a few hairs light brown at the proximal part, most hairs completely white; 3 = most hairs light brown at the proximal part, a few completely white; 4 = all regrown hairs light brown at the proximal part, white at the distal part; 5 = a few hairs completely brown, most like graduation 4; 6 = most hairs completely brown, a few like graduation 4; 7 = all regrown hairs completely brown.

because of the restricted availability of specimens of this rare species, no statistical evaluation was made.

Results and discussion. Long photoperiods induced gonadal activity in the control animals, in winter more than in summer and autumn. Melatonin treatment inhibited the stimulatory effect in summer and autumn but not in winter (fig. 1, left). Long photoperiods also stimulated the regrowth of brown fur in all 3 seasons, while the indoleamine was effective in inducing growth of white fur only in autumn (fig. 2, left). Short photoperiods caused or furthered gonadal quiescence in the control groups in summer and autumn, while in winter they did not suppress spontaneous testicular recrudescence. Melatonin did not alter the antigonadotropic influence of the light regimen in summer whereas in autumn it seemed to reduce the inhibitory influence of short days (fig. 1, right). In winter no influence of melatonin on gonadal redevelopment was observed at the histological level. Similarly, short photoperiods induced the regrowth of white fur in the control animals in summer and autumn, while in winter 3 out of 4 hares regrew brown fur. Melatonin counteracted this effect of short days in summer and autumn, i.e. most of the treated hares regrew brown fur (fig. 2, right). In winter melatonin exerted no apparent effect on the color of the regrowing fur. These observations show that mountain hares respond to long or short photoperiods and melatonin similarly to other mammals studied in this respect[6,7]. They also show that the effect of exogenous melatonin in this species depends on the photic environment and thus the physiological state of the animals at the time of exposure to a particular treatment. Another observation, which needs more detailed investigation, should be mentioned: in autumn, when the melatonin-treated hares in short photoperiods had almost involuted testes, regression went on for about 4 weeks. The testes then redeveloped within the following 3 weeks, this being about 2 months earlier than natural recrudescence would occur. Since we did not observe this phenomenon in the animals treated with either melatonin or short photoperiods we feel that the double treatment induced an advanced refractoriness which points to a zeitgeber-transducing function of melatonin rather than to a direct gonadotropic action.

1 Acknowledgments. The authors thank Dr Russel J. Reiter, University of Texas, for critically reading the manuscript.

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