Effect of constant-release implants of melatonin on seasonal cycles in reproduction, prolactin secretion and moulting in rams

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Summary. Seasonal cycles in the size of the testes, blood plasma concentration of testosterone, FSH and prolactin, intensity of the sexual skin flush, timing of rutting behaviour and moulting of the body coat were recorded in Soay rams after s.c. implantation of melatonin contained in a Silastic envelope which increased the circulating blood levels of melatonin to 200–600 pg/ml for many months. Two groups of 8 adult rams were held under alternating periods of short days (8L:16D) and long days (16L:8D) to drive the seasonal cycles and the treatments with melatonin were initiated during the long or short days, and one group of 8 ram lambs was kept out of doors and given implants during the long days of summer (4 melatonin-implanted and 4 control (empty implants) rams per group).

The treatments demonstrated that (1) melatonin implants during exposure to long days resulted in a rapid 'switch on' of reproductive redevelopment similar to that produced by exposure to short days; (2) melatonin implants prevented the rams from showing the normal responses to changes in the prevailing photoperiod rendering them non-photoperiodic; and (3) long-term cyclic changes in testicular activity, prolactin secretion and other characteristics occurred in the melatonin-implanted rams; the pattern was similar to that previously observed in rams exposed to prolonged periods of short days.

The overall results are consistent with the view that melatonin is the physiological hormone that relays the effects of changing photoperiod on reproduction and other seasonal features, and that continuous exogenous melatonin from an implant interferes with the normal 'signal' and produces an over-riding short-day response.

Introduction

Recent studies with sheep have shown that the secretion of melatonin from the pineal gland plays a key role in mediating the effects of changing photoperiod on reproduction and other seasonal responses. In ewes and rams, the secretion of melatonin is closely linked to the daily light–dark cycle with a marked increase in the peripheral blood levels of melatonin at night (Rollag, O’Callaghan & Niswender, 1978; Lincoln, Almeida, Klandorf & Cunningham, 1982; Almeida & Lincoln, 1982, 1984; Bittman, Dempsey & Karsch, 1983). The changes in the daily pattern of secretion of melatonin are related to changes in daylength and there is convincing evidence for ewes that it is the duration of daily exposure to melatonin which provides the index of night length and therefore daylength; a long duration of melatonin secretion signals a short day and vice versa (Bittman et al., 1983; Karsch et al., 1984). Consistent with this view are observations that daily treatment of ewes with melatonin during anoestrus will induce short-day responses, including suppression of prolactin secretion and early onset of oestrous cyclicity (Nett & Niswender, 1982; Kennaway, Gilmore & Seamark, 1982a; Arendt, Symons, Laud & Pryde, 1983).
The aim of the present study was to extend these observations on the role of melatonin as a time-cue by recording the effect of constant-release implants of melatonin on the photoperiodic responses in rams. The prediction based on the duration hypothesis is that a melatonin implant would act like a short day. Three experiments were performed to test the following questions:

(1) will a melatonin implant given to adult rams during exposure to long days (16L : 8D) result in the induction of testicular activity in the same way as occurs in response to a switch to short days (8L : 16D), and will these implanted rams be able to respond to subsequent changes in photoperiod?

(2) will a melatonin implant given to adult rams during exposure to short days (8L : 16D) prevent regression of the testes and increase in prolactin secretion induced by long days (16L : 8D), and how quickly do the rams return to normal after removal of the implant? and

(3) can a melatonin implant hasten the onset of puberty in ram lambs living under summer daylengths out-of-doors?

**Materials and Methods**

Rams of the Soay breed were used because they have pronounced seasonal cycles in testicular activity, prolactin secretion and moulting of the body wool, and these cycles are responsive to artificial changes in photoperiod (Lincoln & Short, 1980). The animals were kept at the Dryden Field Station of the Animal Breeding Research Organisation near Edinburgh (56°N). The melatonin implants were made from Silastic sheeting (500–1 Dow Corning, Midland, MI, U.S.A.) sealed into an envelope with a total surface area of 32–42 cm², containing 1–4 g melatonin (Sigma Chemicals, Poole, Dorset, U.K.). The implants were placed beneath the skin above the rib cage while the animals were briefly anaesthetized with fluothane.

**Experiment 1**

Eight adult Soay rams were housed in a light-proof shed and exposed to an artificial lighting schedule consisting of alternating 10–30-week periods of long days (16 h light : 8 h darkness, 16L : 8D) and short days (8 h light : 16 h darkness, 8L : 16D) for 68 weeks (see Text-fig. 1). At Week 14 during long days, 4 of the rams received a melatonin implant (surface area 42 cm² containing 4 g melatonin) and the other 4 rams received empty implants to act as sham-operated controls. The implants remained in place throughout the remainder of the experiment during which the photoperiod was alternated between long days and short days.

**Experiment 2**

Eight adult Soay rams were housed in a light-proof shed and exposed to alternating 12–16-week periods of long days (16L : 8D) and short days (8L : 16D) for 72 weeks (Text-fig. 3). At Week 12 during a period of short days, 4 of the animals were given a melatonin implant (surface area 42 cm² containing 4 g melatonin), and the other 4 rams were given empty implants to act as controls. The implants were removed at Week 40.

**Experiment 3**

Eight Soay ram lambs born 12–25 April were kept with their mothers in a grass paddock out-of-doors until 6 months old. Four of the lambs received melatonin implants (surface area 32 cm² containing 1 g melatonin) in mid-May when about 1 month old, and the other 4 were given empty implants. The implants were removed in mid-October after 22 weeks because the experimental lambs were stunted and in poor condition at this stage; all the animals were moved indoors and exposed to long days (16L : 8D). One control lamb was killed by a dog in September while out-of-doors, and one experimental lamb died of pneumonia in October (Text-fig. 5).
In the 3 experiments, the changes in the diameter of the testes, the intensity of the sexual skin flush in the inguinal region, and moulting of the wool from the scrotum were recorded every 2 weeks (Lincoln & Davidson, 1977). The period of rutting behaviour was indicated by the overt aggressive behaviour of the rams including threats, horn banging, vocalizations, pawing the bedding into a mound and the increased incidence of Flehmen. The rams were weighed every 1–2 months (Exp. 1) or every 2 weeks (Exp. 3). A blood sample was collected from all animals from the jugular vein every week, and on 3 occasions (once during Exp. 1 and twice during Exp. 2) blood samples were collected hourly for 24 h using a jugular cannula inserted the day before. All blood samples were heparinized, and the plasma separated within 30 min of collection and frozen at \(-20°C\) until required for the hormone assays.

Radioimmunoassays

The concentrations of follicle-stimulating hormone (FSH), prolactin, testosterone and melatonin in the blood plasma were measured by radioimmunoassay using the methods described by McNeilly, McNeilly, Walton & Cunningham (1976) for FSH, McNeilly & Andrews (1974) for prolactin, Corker & Davidson (1978) for testosterone, and Rollag & Niswender (1976) for melatonin. The limit of detection was 10 ng/ml for FSH using NIH-FSH-S18 as standard, 2·0 ng/ml for prolactin using NIH-P-S9 as standard, 0·1 ng/ml for testosterone and 10 pg/ml for melatonin. The intra- and inter-assay coefficients of variation for all assays were <12·0%.

Statistical analysis

The changes in the size of the testes and the concentration of the various hormones throughout each experiment were tested for a significant time effect and time/group interaction using analysis of variance and co-variance with repeated measures (ANOVAR). The nature of the short-term responses to a change in photoperiod or the implantation of melatonin were compared for the control and treated rams by calculating the incremental change from Week 0 to the time of the maximum response based on previous studies following an abrupt switch between long days and short days (Lincoln & Davidson, 1977; Lincoln, McNeilly & Cameron, 1978). The times of maximum increment were taken as Weeks 12–15 for testicular diameter and plasma levels of testosterone, Weeks 8–11 for plasma levels of FSH, Weeks 6–9 for the sexual skin flush and Weeks 2–5 for plasma levels of prolactin. The incremental change for each animal was calculated (mean for 4-week period of expected maximum minus mean for 4-week period before experimental test) and the group means were compared by Student’s \(t\) test (Tables 1–3). In addition, the mean ± s.e.m. plasma concentrations of melatonin in the control and melatonin-treated rams based on the mean of a series of daily blood samples or hourly samples collected for 24 h were compared by Student’s \(t\) test.

Results

Experiment 1: adult rams given melatonin implants during long days

The rams were implanted with melatonin at Week 14 during exposure to long days, and the results are summarized in Text-fig. 1. Plasma concentrations of melatonin were significantly \((P < 0·01)\) increased in the melatonin-implanted rams in daytime samples collected at 6-week intervals (overall daytime mean ± s.e.m. values 332 ± 30 and 34 ± 14 pg melatonin/ml) and in hourly samples collected for 24 h at Week 52 (24 h mean ± s.e.m. 461 ± 94 and 128 ± 23 pg melatonin/ml). There was a diurnal change in the plasma concentrations of melatonin at Week 52 in both groups of rams, with the highest levels at night; melatonin concentrations in the experimental rams were higher throughout the 24 h than the nocturnal maximum for the control rams (Text-fig. 2).
Text-fig. 1. Changes in diameter of the testes (mean ± s.e.m.), plasma concentrations of testosterone, FSH and prolactin (mean ± s.e.m.), intensity of the sexual skin flush (individual values), the period of intense rutting behaviour (individual values, small open bars) and the timing of the moult of wool from the scrotum (individual values) in 4 melatonin-implanted and 4 control adult Soay rams in Exp. 1, exposed to alternating 10–30-week periods of short days (SD, 8L:16D) and long days (LD, 16L:8D) for 68 weeks. The implants were introduced at Week 14 during long days, and left in place throughout the remainder of the study.
Text-fig. 2. Changes in the concentration (mean ± s.e.m.) of melatonin and prolactin in the blood plasma in 4 melatonin-implanted and 4 control adult Soay rams in Exp. 1, based on samples collected hourly for 25 h under long days at Week 52. The times of daylight (open bar) and darkness (closed bar) are shown.

The size of the testes and the blood plasma levels of testosterone, FSH and prolactin for the experimental and control rams changed significantly ($P < 0.001$) with time and there was a significant ($P < 0.001$) time/group effect, indicating that the long-term changes in all the values differed in the two groups (see Text-fig. 1). The differences between the experimental and control rams were evident within 14 weeks of the implantation since there was a significantly ($P < 0.01$) more rapid increase in the size of the testes and the plasma concentrations of testosterone and FSH, and a decrease in the plasma levels of prolactin in the melatonin-implanted animals (Table 1). In addition these treated animals were precocious in developing their sexual skin flush and rutting behaviour (Table 1; Text-fig. 1).

After the phase of full reproductive activity induced by the implantation of melatonin, there was a rapid decline in the size of the testes and the plasma concentrations of FSH and testosterone. These regressive changes in the treated rams occurred over the change from long days to short days which appeared to stimulate testicular activity in the controls (Text-fig. 1). For the melatonin-implanted rams a sexual nadir occurred at about 30 weeks after implantation followed by a slow recovery. The long-term reproductive changes were not correlated with the alterations in photoperiod as was obvious in the control rams, and the reciprocal relationship between the changes in the plasma levels of FSH and prolactin was no longer apparent (Text-fig. 1). During this period the plasma levels of prolactin gradually increased associated with an earlier and gradual onset of the moulting of the body wool (Text-fig. 1). The difference in prolactin secretion between the experimental and control rams was apparent at Week 52 when blood samples were collected hourly for 24 h; the control rams were clearly hyperprolactinaemic as expected during exposure to long days, but the overall levels in the experimental rams were lower and there was no obvious diurnal variation (Text-fig. 2). The difference between the two groups in the responses to photoperiod was also evident at Week 56 when the lighting was switched from long days to short days; reproductive devel-
Experiment 1: summary of the reproductive changes in control (N = 4) and melatonin-implanted (N = 4) adult Soay rams in response to the initial implantation of melatonin or sham-operation during long days (16L:8D) at Week 14 or a switch in photoperiod from long days (16L:8D) to short days (8L:16D) at Week 56

<table>
<thead>
<tr>
<th>Reproductive changes after:</th>
<th>Implantation at Week 14</th>
<th>Short days at Week 56</th>
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<tr>
<td></td>
<td>Control</td>
<td>Melatonin-implanted</td>
</tr>
<tr>
<td>Testis diameter (mm)</td>
<td>3.9 ± 1.9</td>
<td>*14.3 ± 1.4</td>
</tr>
<tr>
<td>Plasma testosterone (ng/ml)</td>
<td>4.9 ± 2.3</td>
<td>*14.9 ± 1.2</td>
</tr>
<tr>
<td>Sexual flush (scale 0–5)</td>
<td>0</td>
<td>*4.5 ± 0.4</td>
</tr>
<tr>
<td>Plasma FSH (ng/ml)</td>
<td>37.5 ± 16.6</td>
<td>*685.6 ± 100.9</td>
</tr>
<tr>
<td>Plasma prolactin (ng/ml)</td>
<td>−29.5 ± 7.4</td>
<td>−65.9 ± 9.2</td>
</tr>
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Values represent the mean ± s.e.m. incremental change (see text for time period for each measurement).
* Significantly different from corresponding control value, *P < 0.05 (Student's *t* test).

opment was stimulated in the control rams but not in the melatonin-implanted rams (Table 1). The changes in the control rams in response to short days at Week 56 were similar in timing and extent to those in the melatonin-implanted rams in response to the initial implantation of melatonin at Week 14, confirming that melatonin given during long days induces 'short day' responses.

Experiment 2: adult rams given melatonin during short days

Melatonin implants were inserted at Week 12 during exposure to short days, and the results are summarized in Text-fig. 3. There was a significant (*P < 0.01) increase in the blood plasma concentration of melatonin in the experimental rams based on daytime blood samples collected every 6 weeks (overall daytime mean ± s.e.m. values 224 ± 14 and 10 ± 1 pg melatonin/ml) and hourly samples collected for 24 h on one day during long days and one day during short days (24 h mean ± s.e.m. melatonin values 385 ± 37 and 339 ± 40 pg/ml in experimental rams and 55 ± 11 and 68 ± 21 pg/ml in control rams at Weeks 28 and 37, respectively). The highest concentrations of melatonin occurred at night in both groups in long and short days, but the values in the experimental rams were always higher than the nocturnal maximum for the controls (Text-fig. 4).

All the reproductive characteristics changed significantly (*P < 0.001) in relation to time in the experimental and control rams, and there was a significant (*P < 0.001) time versus group effect, indicating that the long-term changes were different in the two groups. These differences were not apparent, however, during the initial period of short days (up to Week 16) or during the subsequent period of long days (Week 16–28) as judged by the size of the testes and the plasma concentrations of FSH and testosterone; a decline occurred in both groups after full testicular activity (Table 2; Text-fig. 3). However, the exposure to long days resulted in a significantly (*P < 0.05) reduced prolactin response (Table 2) in the experimental rams, and there was no clear reproductive response to the switch from long days to short days at Week 28 unlike the marked changes in the control group (Table 2). The difference in prolactin secretion in the two groups over this period was also apparent at Weeks 28 (long days) and 37 (short days), when blood samples were collected hourly for 24 h (Text-fig. 4). On both occasions the plasma levels of prolactin were in the intermediate range in the experimental rams compared to the high (long days) and low (short days) values for the controls.
Text-fig. 3. Changes in the diameter of the testes (mean ± s.e.m.), plasma concentrations of testosterone, FSH and prolactin (mean ± s.e.m.), intensity of the sexual skin flush (individual values), the period of intense rutting behaviour (individual values, small open bars) and the timing of the moult of wool from the scrotum (individual values, * indicates delayed regrowth) in 4 melatonin-implanted and 4 control adult Soay rams in Exp. 2, exposed to alternating 12–16-week periods of short days (SD, 8L:16D) and long days (LD, 16L:8D) for 72 weeks. The implants were introduced at Week 12 during short days and removed at Week 40 during short days.
Text-fig. 4. Changes in the concentrations (mean ± s.e.m.) of melatonin and prolactin in the blood plasma of 4 melatonin-implanted and 4 control adult Soay rams in Exp. 2 sampled at hourly intervals for 25 h once during long days (16L:8D; Week 28) and once during short days (8L:16D; Week 37). The times of daylight (open bar) and darkness (closed bar) are shown.

Table 2. Experiment 2: summary of the reproductive changes in control (N = 4) and melatonin-implanted (N = 4) adult Soay rams in response to a switch from short days (8L:16D) to long days (16L:8D) at Week 16 and a switch from long days (16L:8D) to short days (8L:16D) at Week 28

<table>
<thead>
<tr>
<th>Reproductive changes after:</th>
<th>Long days at Week 16</th>
<th>Short days at Week 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Melatonin-implanted</td>
<td>Control</td>
</tr>
<tr>
<td>Testis diameter (mm)</td>
<td>−10.3 ± 1.6</td>
<td>−9.3 ± 1.4</td>
</tr>
<tr>
<td>Plasma testosterone (ng/ml)</td>
<td>−8.4 ± 3.6</td>
<td>−7.2 ± 2.7</td>
</tr>
<tr>
<td>Sexual skin flush (scale 0-5)</td>
<td>−0.8 ± 0.3</td>
<td>−0.4 ± 0.4</td>
</tr>
<tr>
<td>Plasma FSH (ng/ml)</td>
<td>−39.3 ± 21.9</td>
<td>−71.7 ± 22.8</td>
</tr>
<tr>
<td>Plasma prolactin (ng/ml)</td>
<td>22.7 ± 5.2</td>
<td>*4.4 ± 2.1</td>
</tr>
</tbody>
</table>

Values represent the mean ± s.e.m. incremental change (see text for time period for each measurement).
* Significantly different from corresponding control value, P < 0.05 (Student's t test).

The implants were removed at Week 40 during exposure to short days, and within a few weeks the rams that had been previously treated with melatonin began to show normal responses to the changing photoperiod. For example, at Week 44 (4 weeks after implant removal) there was a marked increase in the blood plasma level of prolactin in all the rams (Text-fig. 3) although the testicular cycle took longer to readjust to the normal pattern (Text-fig. 3).
Table 3. Experiment 3: summary of the reproductive changes in control (N = 4) and melatonin-implanted (N = 4) young Soay ram lambs in response to implantation or sham-operation performed in May while the animals were living out-of-doors under natural lighting

<table>
<thead>
<tr>
<th>Reproductive changes after:</th>
<th>Sham-operation</th>
<th>Implantation with melatonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis diameter (mm)</td>
<td>27.4 ± 0.9</td>
<td>29.4 ± 0.7</td>
</tr>
<tr>
<td>Plasma testosterone (ng/ml)</td>
<td>1.1 ± 0.5</td>
<td>*4.8 ± 0.6</td>
</tr>
<tr>
<td>Sexual skin flush (scale 0–5)</td>
<td>0</td>
<td>*1.3 ± 0.5</td>
</tr>
<tr>
<td>Plasma FSH (ng/ml)</td>
<td>−1.1 ± 3.6</td>
<td>*60.0 ± 13.6</td>
</tr>
<tr>
<td>Plasma prolactin (ng/ml)</td>
<td>10.5 ± 28.9</td>
<td>−41.8 ± 20.0</td>
</tr>
</tbody>
</table>

Values are the mean ± s.e.m. incremental change (see text for time period for each measurement).

* Significantly different from corresponding control value, P < 0.05 (Student's t test).

**MELATONIN-IMPLANTED RAMS**

**CONTROL RAMS**

Text-fig. 5. Changes in the diameter of the testes (mean ± s.e.m.), plasma concentrations of testosterone, FSH and prolactin (mean ± s.e.m.), intensity of the sexual skin flush (individual values) and the timing of the moult of wool from the scrotum (range) in 4 melatonin-implanted and 4 control Soay ram lambs in Exp. 3, living under natural lighting from birth in April until October when they were moved indoors under long days (16L:8D). The implants were introduced in May (Week 0) when the lambs were about 1 month old and removed in October (Week 22).

**Experiment 3: young rams given melatonin implants during long days out-of-doors**

Melatonin implants were given to 1-month-old ram lambs living out-of-doors in May, and the results are summarized in Text-fig. 5. The experimental lambs had a significantly (P < 0.001) increased blood plasma level of melatonin based on daytime samples collected every 6 weeks overall.
daytime mean ± s.e.m. 447 ± 16 compared with 10 ± 1 pg melatonin/ml for the controls). During the 30-week study, both groups of lambs became sexually mature as judged by the increase in the size of the testis, although there were significant \( P < 0.05 \) differences between the two groups. Plasma FSH and testosterone concentrations increased more rapidly in the melatonin-implanted lambs and these animals developed the sexual skin flush prematurely (Table 1; Text-fig. 5). After this initial stimulatory response the testes of the melatonin-implanted rams began to regress earlier than in the controls, associated with a decline in testosterone concentration and disappearance of the sexual skin flush. At this stage the melatonin-implanted lambs had stopped growing and were thin compared to the controls (body weight at Week 16: 14 ± 0.5 kg for experimental lambs, 16.3 ± 0.9 kg for controls) and this effect continued until Week 22 (12.6 ± 0.8 and 16.8 ± 0.7 kg) when one of the treated lambs died in poor condition and the experiment was terminated.

**Discussion**

The results presented here illustrate that constant administration of melatonin from a s.c. implant induces changes in the cycles in testicular activity in rams, and interferes with the normal photoperiodic regulation of reproduction. The effects can be summarized as follows.

1. Implantation with melatonin during exposure to long days induces all the reproductive changes normally associated with an exposure to short days.

2. Implantation with melatonin blocks the effects of long days (e.g. stimulation of prolactin secretion) and in the long term renders rams unresponsive to changes between long days and short days, i.e. the animals become non-photoperiodic.

3. Implantation with melatonin does not lead to a stable reproductive condition, instead the treated rams show long-term cyclic changes in their physiology.

Each of these effects is consistent with the view that melatonin is the hormone that functions physiologically to relay effects of changing photoperiod, and that exogenous melatonin induces its responses by interfering with this process. In particular, the constant supply of exogenous melatonin appears to be registered as a short day and to over-ride any effects of endogenous melatonin secretion. In the short term, this results in stimulation of testicular activity if the rams are already photoreceptive due to prior exposure to long days (e.g. Exp. 1 & 3). In the long term, this results in cyclic changes in testicular activity similar to those previously recorded in rams exposed to prolonged periods of short days (8L:16D) (Almeida & Lincoln, 1984). The long-term response to implantation of melatonin (Exp. 1) is compared to previously published results on the long-term response to short days in Text-fig. 6. It is evident that both treatments result in an initial phase of rapid testicular growth followed by involution and then a phase of slow testicular growth. The in-volution of the testes during exposure to short days has been referred to as short-day refractoriness, and it is clear this condition also occurs in the presence of constant melatonin and might equally be referred to as melatonin refractoriness.

The only previous studies in which sheep have been given s.c. implants of melatonin similar to those used here were on Merino × Border Leicester ewes and ewe lambs treated in the summer (Kennaway, Dunstan, Gilmore & Seamark, 1983; Kennaway & Gilmore, 1984). These treatments led to a decline in the blood plasma levels of prolactin as in the present studies, but there were no clear effects on reproduction in the ewes and the onset of oestrus cyclicity was delayed in the ewe lambs. In the experiment of Kennaway & Gilmore (1984) the lambs were treated at a very early age and may not have been exposed to long days for long enough to render them responsive to short days or the effect of constant melatonin. In addition, Merino crossbreed sheep are likely to be less responsive to photoperiod or melatonin manipulations compared to a semi-domesticated northern breed of sheep like the Soay. Melatonin implants have been used frequently in studies on laboratory species, and the effects vary according to the species and the size of the implant. For example, a melatonin implant induces regression of the testes in the Turkish hamster, *Mesocricetus brandti*,

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Melatonin and reproduction in rams

Text-fig. 6. Changes in the diameter of the testes (mean ± s.e.m.) in adult Soay rams (a) implanted with melatonin (Exp. 1) and (b) exposed to short days for a prolonged period (Almeida & Lincoln, 1984). Rapid testicular development occurred after implantation with melatonin during long days, or the switch from long days (LD) to short days (SD); this was followed by regression of the testes and slow redevelopment. The lines indicating the rates of testicular growth were fitted by eye.

exposed to long days; in this species this is a short-day response. However, identical treatment of the Syrian hamster, Mesocricetus auratus, has no effect on animals exposed to long days and actually blocks the reproductive response to a switch to short days (Goldman, Carter, Hall, Roychoudhury & Yellon, 1982).

The measurement of melatonin in the blood of the rams used in the present study illustrated that the s.c. melatonin implants were capable of maintaining the plasma levels of melatonin at 200–600 pg/ml for many months. However, the 24-h profiles revealed that the levels of melatonin were higher at night than during the day in the experimental and in the control rams, indicating that the constant supply of melatonin from the implants had not blocked the endogenous secretion of the hormone which still occurred largely during the daily dark phase. This lack of an inhibitory-feedback effect of exogenous melatonin has been described previously (Kennaway et al., 1982b). While the experimental rams continued to secrete melatonin, this did not apparently result in the normal photoperiodic responses, and the continual presence of >200 pg melatonin/ml in the blood from the implants must negate the normal effects of the endogenous melatonin. Whether this periodic release of endogenous melatonin modified the effects of the implants is questionable. However, the advantage of using rams with a functional pineal gland was that it was possible to treat animals which were known to be photosensitive and to observe the effects of melatonin on the normal photoperiodically induced changes in reproduction. It is unlikely that the endogenous secretion of melatonin greatly complicated the results since short-day effects induced by implantation of melatonin similar to those reported here have been observed in superior cervical ganglionectomized rams and red deer stags which had minimal endogenous melatonin secretion at the time of treatment (G. A. Lincoln, unpublished results; Lincoln, Fraser & Fletcher, 1984).

Although treatments with melatonin clearly affect reproduction they also influence other aspects of the animals’ seasonal physiology which are responsive to changes in daylength. In the
present study, implantation with melatonin influenced prolactin secretion, moulting of the wool and overall body weight. The effect of a melatonin implant on the moult cycle has been described for many seasonal species including the weasel (Rust & Meyer, 1969), Djungarian hamster (Hoffmann, 1973), white-footed mouse (Lynch & Epstein, 1976) and mink (Allain, Martinet & Rougeot, 1981). The studies on the mink have illustrated that the effects on the coat are probably mediated through changes in prolactin secretion, because the decrease in prolactin secretion that occurs after a melatonin implant or exposure to short days is associated with the growth of the winter coat, while administration of prolactin to these animals can lead to moulting and development of the summer coat. In the current studies, the increase in the blood levels of prolactin was associated with moulting and regrowth of the wool. The change in the timing of the moult after melatonin treatments was consistent with the effect being mediated through a change in prolactin secretion (e.g. Text-figs 1 & 3). Changes in body weight may also be dictated by the prolactin concentration as judged by studies on sheep and other ungulates in which prolactin has been shown to stimulate appetite and body growth (Meier, 1977; Ryg & Jacobsen, 1982; Brinklow & Forbes, 1983). The effect of the melatonin treatment was to induce suppression of prolactin secretion and this was associated with anorexia and weight loss which is a response normally seen in winter during short days. In the adult rams, weight loss began 12–16 weeks after implantation with melatonin (results not presented) and the animals reached a nadir of condition at about 30 weeks before spontaneously regaining weight again. In the lambs, the effect of melatonin was to reduce growth rate; these animals were in poor condition by 22 weeks after treatment and had to be moved indoors since they were vulnerable to the colder weather in autumn.

In conclusion, the constant administration of melatonin to rams by means of a s.c. Silastic implant induces effects on reproduction, moulting, body weight and the associated endocrine changes similar to those induced by exposure to short days.

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References


Melatonin and reproduction in rams


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