How Does Melatonin Code for Day Length in the Ewe: Duration of Nocturnal Melatonin Release or Coincidence of Melatonin with a Light-Entrained Sensitive Period?¹

NANCY L. WAYNE,² BENOIT MALPAUX,³ and FRED J. KARSCH³

Consortium for Research in Developmental and Reproductive Biology
Department of Physiology
The University of Michigan
Ann Arbor, Michigan 48109

ABSTRACT

The main objective of the study was to test the hypothesis that the phase of melatonin release with respect to the light-dark cycle mediates the effects of photoperiod on the reproductive response of the ewe. To test the phase hypothesis, we eliminated endogenous melatonin secretion by pinealectomy and then restored physiological levels of serum melatonin with rises of the same duration but at different phases of the light-dark cycle (either at night or in the middle of the day). Serum melatonin patterns were determined by radioimmunoassay in samples taken hourly for 24 h. The reproductive state was monitored by measuring serum luteinizing hormone (LH) in ovariectomized ewes treated with constant-release estradiol implants. Infusion of a long-day pattern of melatonin was equally effective in maintaining reproductive suppression when given during the night or the middle of the day. LH remained low for approximately 150 days and then rose as ewes became refractory to the inhibitory melatonin signal. These results do not support the phase hypothesis. Rather, they are consistent with the hypothesis that the duration of the nocturnal secretion of melatonin codes for day length.

INTRODUCTION

For many seasonal breeders, photoperiod is the primary environmental factor that synchronizes reproductive activity to a time of year that promotes survival of the young. In a wide range of mammals, including both long- and short-day breeders, the reproductive response to photoperiod is mediated by the pineal gland (long-day breeders: hamsters, Hoffman and Reiter, 1965; voles, Farrar and Clarke, 1976; ferrets, Herbert et al., 1978; white-footed mice, Glass and Lynch, 1981; short-day breeders: sheep, Lincoln, 1979; Bittman et al., 1983a; deer, Plotka et al., 1984). Two major hypotheses have been advanced to explain how melatonin codes for day length: the duration of nocturnal melatonin secretion and the phase of melatonin release with respect to the light-dark cycle (Rollag et al., 1978; Tamarkin et al., 1979; Goldman et al., 1982). Inclusive in the phase hypothesis is the concept of a rhythm of sensitivity to melatonin that is entrained by the light-dark cycle: when the periods of melatonin release and sensitivity coincide, a photoperiodic response occurs. There is experimental support for both hypotheses, stemming largely from work on rodents (phase: Stetson and Tay, 1983; Watson-Whitmyre and Stetson, 1983; Stetson et al., 1986; duration: Carter and Goldman, 1983a,b; Goldman et al., 1984; Dowell and Lynch, 1987). Which hypothesis most accurately describes how melatonin mediates the photoperiodic response remains an issue of considerable controversy.

The primary objective of the present study was to test the phase hypothesis in the female sheep. In this species, a model has been developed in which circulating melatonin patterns can be artificially controlled and closely monitored for their physiological accuracy (Bittman et al., 1983b). Although definitive studies have not yet been performed in the sheep, earlier observations have been interpreted as being consistent with both hypotheses (phase: Rollag et al., 1978; Almeida and Lincoln, 1982; duration: Bittman 66
growing body of evidence that seasonal reproductive transitions in the ewe are not directly driven by endogenous rhythm. This rhythm is entrained by photoperiod and, under natural conditions, reproductive (or refractory) to the prevailing photoperiod, these large swings in serum LH (>30-fold) provide a highly robust and photoperiodically sensitive marker of the seasonal reproductive state (Legan et al., 1977).

All ewes were maintained in light-controlled rooms under artificial fluorescent illumination (350 lux at sheep head level, as measured at beginning of study). Dim red light (<3 lux) was supplied continuously to facilitate nighttime blood collection. Lights-on was at 0600 h EST during both long (16L:8D) and short (8L:16D) day lengths. Rams were excluded from the buildings to minimize social and olfactory influences from the male on reproductive activity. Temperature was not controlled in this study.

Melatonin Treatment

Pinealectomized ewes were treated with melatonin using a modification of the procedure developed by Bittman and Karsch (1984). Each ewe was chronically implanted with a jugular-cardiac cannula that was attached to a portable, battery-operated infusion pump that could be programmed to turn on and off to the nearest minute (AS-6MP, Auto-Syringe, Hooksett, NH). Pumps were contained in backpacks that consisted of a stainless steel box attached to a harness. This arrangement allowed complete mobility and normal behavior during melatonin treatment, which continued without interruption for the 1¼ yr of this study. Aliquots from a stock solution of melatonin (40 mg/ml of 95% ethanol) were diluted 300-fold in physiological saline containing sodium heparin (100 U/ml). This solution was delivered at a rate (38.5 µg melatonin/h) calculated to achieve physiological nighttime concentrations of serum melatonin. On 2 occasions during the study, the...
concentrations of serum melatonin were spot-checked in blood samples taken intermittently during a 24-h period. At the end of the study, hourly blood samples were taken from each ewe for 24 h to characterize the circulating melatonin pattern. All infusion lines were flushed with heparinized saline each week to verify that they were patent and had not become dislodged.

Assays

Serum was obtained after blood was allowed to clot overnight at 4°C and stored at −20°C until assayed. LH was determined in duplicate 25- to 200-μl aliquots of serum and is expressed as ng NIH-LH-S12 per ml, as previously described (Niswender et al., 1968, 1969; Hauger et al., 1977). The intraassay coefficient of variation (CV) for standard sera containing 0.97 ± 0.04 (mean ± SEM), 2.28 ± 0.05 and 25.48 ± 0.93 ng/ml averaged 7.9%. The interassay CV for the 3 serum pools averaged 15.2%. The limit of detection (2 standard deviations from the buffer controls) for the 21 assays in this study averaged 0.28 ± 0.02 ng/ml (ranging from 0.13 to 0.48 ng/ml), for 200 μl of serum. To standardize the baseline for statistical purposes, LH data below 0.48 ng/ml (upper value from the range of limit of detections in this study) were assigned this value.

To verify the completeness of pinealectomy, melatonin was initially determined in duplicate 500-μl extracts of serum (5 day- and 5 nighttime blood samples from each ewe) using the method of Rollag and Niswender (1976), modified as described by Bittman et al. (1983a). By the time the experiment was completed (about 3 yr after pinealectomy), a different radioimmunoassay for serum melatonin had been set up in the laboratory (English et al., 1986; modified as described by Malpaux et al., 1987, 1988a). This assay was used to characterize endogenous melatonin secretion in pineal-intact ewes and the infused melatonin patterns in pinealectomized ewes. For this purpose, melatonin was assayed in duplicate 200-μl aliquots of unextracted serum. Intraassay CV for standard sera containing 81 and 402 pg/ml averaged 7% (26 assays). The interassay CV for the 2 serum pools averaged 21%. The limit of detection was 16 pg/ml (for 200 μl serum).

Experimental Design

Our basic assumption was that, if a light-entrained sensitive period exists, it would coincide with at least a portion of the night (when melatonin is normally secreted). In this case, melatonin administered during the middle of the day would miss any sensitive period and ewes should respond as if they did not receive melatonin. Therefore, we treated pinealectomized ewes with the same duration of melatonin but at different times of the light-dark cycle. Our present approach was to test the ability of ewes treated with a suppressive, long-day pattern of melatonin during the middle of the day to maintain a previously established inhibition of serum LH.

The experimental design is illustrated in Figure 1. The study began with an 8-mo priming period that had 3 purposes: 1) to synchronize reproductive activity among sheep, 2) to demonstrate that the ewes were capable of exhibiting rises and falls in LH levels as a function of melatonin signals, and 3) to establish a state of reproductive suppression within a time frame in which ewes would still be responsive to the inhibitory melatonin signal during the experimental period. Beginning 27 November 1985, 17 pinealectomized ewes were subjected to 90 days of infusion of a long-day pattern of melatonin (Stage 1), followed by 90 days of infusion of a short-day pattern of melatonin (Stage 2), and finally 65 days of a long-day pattern of melatonin (Stage 3). During these 3 stages of the priming period, melatonin patterns matched the photoperiod. The long-day pattern of melatonin consisted of a 8-h infusion of melatonin during an 8-h night, and the short-day pattern of melatonin consisted of a 16-h infusion of melatonin during a 16-h night. All infusions were begun at the time of lights-off.
On Day 0 of the experimental period (31 July 1986), the pinealectomized ewes were allocated to 3 treatment groups (Fig. 1). In one control group, the 8-h melatonin infusion was terminated and replaced with an 8-h infusion of saline during the night (STOP MEL, \( n = 5 \); one ewe not given saline due to technical problems). This determined the response to removal of an inhibitory signal. A second control group continued receiving 8-h melatonin during the 8-h dark period to maintain reproductive suppression (NIGHT MEL; \( n = 6 \)). The experimental group had its 8-h melatonin shifted to the middle of the 16-h light period, thus testing whether the daytime infusions could maintain reproductive suppression (DAY MEL; \( n = 6 \)).

As an additional control, 6 pineal-intact ewes were treated during the priming period with the same photoperiod shifts experienced by the pinealectomized, melatonin-infused ewes (beginning 27 November 1985). On Day 0 of the experimental period, these ewes were transferred from 16L:8D to 8L:16D. The purpose of this group was to demonstrate a normal inductive response to short days and to compare this pattern to that which followed termination of the inhibitory melatonin signal in the STOP MEL group.

Analysis of Data

Rises and declines in serum LH were identified by slight modification of an algorithm that uses regression-like statistics with a moving variable-length window to detect changes in hormone levels (Malpaux et al., 1988a). The modification consisted of centering the window at each time point using windows with lengths 7, 9, and 11 points. Therefore, the resolution into phases by the algorithm applied to the original time series is similar to that obtained after reversing the series with respect to time. A variable-length window is used because there is no unique window width that is optimal. A single width would reduce the flexibility of the algorithm to smooth across data values and to fulfill the criterion for identifying phases of a cycle. This algorithm can identify 4 phases of a cycle: baseline, rise, plateau, and decline. The series can start in any phase. The mean value during the plateau phase is described as the peak level. A single width would reduce the flexibility of the algorithm to smooth across data values and to fulfill the criterion for identifying phases of a cycle. This algorithm can identify 4 phases of a cycle: baseline, rise, plateau, and decline. The series can start in any phase. The mean value during the plateau phase is described as the peak level. Times of rises and declines of LH were further analyzed by the Kruskall-Wallis test for analysis of variance and the Mann-Whitney U test (Siegal, 1956).

Since the standard deviations for the peak levels of LH were similar between groups, differences in mean peak levels were analyzed by a one-way ANOVA (Brown and Hollander, 1977). Values were considered significantly different if \( p < 0.05 \).

RESULTS

Melatonin Profiles

The melatonin patterns at the end of the experimental period are shown in Figure 2. The STOP MEL group had a low to undetectable serum melatonin level throughout the 24-h period (Fig. 2A). The infusions produced elevations in serum melatonin in the NIGHT MEL and DAY MEL groups that were 12 h out of phase, yet identical in both amplitude and duration (Fig. 2B and C). These infused patterns closely replicated the endogenous melatonin pattern from a separate group of 6 pineal-intact ewes held on the same photoperiod of 16L:8D (Fig. 2D).

![Figure 2](image-url)
LH during Priming Period

The LH patterns during the three stages of the priming period are shown in Figure 3. At the beginning of Stage 1, pinealectomized ewes were reproduc-tively asynchronous as indicated by their variable serum LH concentrations. By the end of this period, LH was suppressed in all ewes in response to the long-day melatonin pattern. All pineal-intact ewes had elevated serum LH at the start of Stage 1; LH subsequently plummeted to low levels in response to long days, reaching baseline after 30 ± 4 days (mean ± SEM). During Stage 2, serum LH began to rise in the pinealectomized ewes 49 ± 4 days after the switch to the inductive short-day melatonin pattern, reaching peak levels of 9.7 ± 1.0 ng/ml. LH also rose in the pineal-intact ewes 64 ± 3 days after the switch to short days, reaching levels of 9.4 ± 1.1 ng/ml. This rise was significantly later than that in the infused ewes (p<0.025). During Stage 3, serum LH fell in the pinealectomized ewes, reaching baseline 39 ± 4 days after the switch to the long-day melatonin pattern. LH levels fell in the intact ewes, reaching baseline 21 ± 1 days after the switch to long days. This decline was significantly earlier than that in the infused ewes (p<0.001). At the end of Stage 3, pinealectomized ewes were assigned to one of three experimental groups; there was no difference in the LH patterns of these three groups during the priming period.

LH during Experimental Period

Results are summarized in Figure 4 and Table 1. In the STOP MEL group, serum LH began to rise 92 ±
Table 1. Characteristics of luteinizing hormone (LH) response during experimental period.

<table>
<thead>
<tr>
<th></th>
<th>Onset LH rise (days)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>LH return to baseline (days)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Duration of peak (days)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Peak LH level (ng/ml)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>STOP MEL</td>
<td>92 ± 12</td>
<td>179 ± 7</td>
<td>47 ± 12</td>
<td>6.7 ± 1.1</td>
</tr>
<tr>
<td>NIGHT MEL</td>
<td>152 ± 8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>n.d.&lt;sup&gt;d&lt;/sup&gt;</td>
<td>n.d.</td>
<td>7.2 ± 1.2</td>
</tr>
<tr>
<td>DAY MEL</td>
<td>152 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>n.d.</td>
<td>n.d.</td>
<td>6.8 ± 1.1</td>
</tr>
<tr>
<td>PINEAL INTACT</td>
<td>64 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>144 ± 6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45 ± 8</td>
<td>9.4 ± 1.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± SEM.
<sup>b</sup><sup>p</sup><0.01 relative to STOP MEL group.
<sup>c</sup><sup>p</sup><0.02 relative to STOP MEL group.
<sup>d</sup>n.d., No data for determination.

12 days following termination of the inhibitory long-day melatonin pattern, remained elevated at peak levels for approximately 50 days, and then fell to baseline (Fig. 4A). The one ewe that did not receive saline showed an LH response indistinguishable from that in the saline-treated ewes; therefore, her LH values were averaged with the others. In the NIGHT MEL group, serum LH remained suppressed for 152 ± 8 days (Fig. 4B). All but one of the ewes then showed an increase in LH levels despite continued nightly infusion of the long-day melatonin pattern. This rise in LH was significantly delayed relative to that in the STOP MEL group. Ewe #4015 remained reproductively suppressed throughout the experiment (values plotted separately). The LH response in the DAY MEL group was virtually identical to that in ewes infused with melatonin during the night (Fig 4C).

The reproductive response in the STOP MEL group following termination of the long-day melatonin pattern is compared in Figure 5 to that in pineal-intact ewes following transfer to a short photoperiod. Pineal-intact ewes switched from long to short days showed a rise in LH 64 ± 3 days after transfer to the short photoperiod, remained elevated at peak levels for approximately 50 days, and then fell to baseline. The overall patterns of LH in the two groups were the same, but the timing of the LH rise and subsequent fall was significantly delayed in the STOP MEL group. In addition, these same pinealectomized ewes showed a rise in LH 46 ± 7 days after transfer from the long- to short-day pattern of melatonin during Stage 2 of the priming period (gray line in Fig. 5). This rise in LH after the transfer to the short-day melatonin pattern was significantly earlier than that following termination of the long-day pattern of melatonin (<sup>p</sup><0.01).

**DISCUSSION**

**Rationale for Experimental Design**

We assumed that if a light-entrained sensitive period exists, it would coincide with at least a portion of the night when melatonin is normally secreted. We chose to use a long-day pattern of melatonin (short duration) to test for phase so that the daytime infusion would not overlap with the night, thereby avoiding the putative sensitive period. There are two ways to demonstrate a long-day reproductive effect in our experimental model: (1) drive an inhibition of high levels of circulating LH, or (2) maintain already suppressed serum LH concentrations. For the
first approach, serum LH must be elevated in response to an inductive short-day signal prior to driving inhibition with long days. Since refractoriness to a short-day signal develops soon after the onset of this rise (Karsch et al., 1986; Malpaux et al., 1987), it is difficult to distinguish between inhibition caused by long-day melatonin and inhibition due to refractoriness to short-day melatonin. In fact, on two previous occasions we attempted to test for phase using this approach; the results did not permit a definitive conclusion because we could not differentiate between long-day inhibition and short-day refractoriness (Wayne, Bittman and Karsch, unpublished observations). On the other hand, refractoriness to an inhibitory photoperiod takes much longer, approximately 150–200 days from the onset of the long-day signal (Karsch et al., 1986; Malpaux et al., 1988b). This allows plenty of time to distinguish between an LH rise resulting from long-day refractoriness and one resulting from the termination of an inhibitory signal; this discrimination was of critical importance for definitive interpretation of our data. Therefore, we considered the maintenance of long-day inhibition to be the most appropriate way to test for phase in the ewe.

Test for Phase

If there were a sensitive period to melatonin during the night, the day infusion should have missed it, and the ewes should have responded as if melatonin infusions had stopped. This however, was clearly not the case. Rather, ewes interpreted the 8-h block of melatonin given in the middle of the day as an inhibitory signal. These results do not support a role for phase, but are consistent with the hypothesis that duration of elevated melatonin mediates the photoperiodic response. The present data corroborate our earlier work in which the duration of the melatonin elevation was systematically manipulated in the pinealectomized ewe; the reproductive response always conformed to duration (Bittman and Karsch, 1984; Yellon et al., 1985).

Although our data are in accordance with the duration hypothesis, we cannot completely rule out a role for phase. It could be argued, for example, that there is a broad band of melatonin sensitivity and that both of our infusion blocks overlapped with this period. In addition, we did not test the possibility that a period of sensitivity to melatonin might be entrained by factors other than the light-dark cycle, such as the melatonin rhythm itself, as recently postulated by Stetson and coworkers (1986). Indeed, there is evidence that the pineal gland and melatonin can influence entrainment of the circadian rhythms of wheel-running activity and drinking in rats, and it has been suggested that melatonin can affect the rhythms of melatonin secretion and body temperature in humans (Quay, 1970; Redman et al., 1983; Armstrong et al., 1986; Wright et al., 1986). Nevertheless, testing the hypothesis that melatonin can entrain its own rhythm of sensitivity is difficult, at best, since there is no direct assay for a melatonin sensitive period.

The controversy over the phase and duration hypotheses has arisen largely from work in rodents. Studies in the juvenile Djungarian hamster treated with timed infusions of melatonin have led to the conclusion that the reproductive response depends on the duration of elevated melatonin, not its temporal relationship to the light-dark cycle (Carter and Goldman, 1983a,b; Goldman et al., 1984). Recent work in the white-footed mouse, in which melatonin was implanted intracranially for varying durations at different times of day, also supports the duration hypothesis (Dowell and Lynch, 1987). These observations are at variance with the findings in Syrian and Djungarian hamsters injected with melatonin at different times of the light-dark cycle. Injections given in the late afternoon or early morning produced gonadal regression; injections at other times of the day had no effect, thereby supporting the phase hypothesis (Stetson and Tay, 1983; Watson-Whitmyre and Stetson, 1983; Stetson et al., 1986).

The inconsistencies of the findings in rodents are most likely due to differences in the mode of administration of melatonin. The dose of melatonin used in the infusion studies (low nanogram range) was calculated to approximate the rate of melatonin synthesis in the hamster pineal during the night (Carter and Goldman, 1983a), and it was similar to the infusion rate used in the present study (normalized for body weight). In marked contrast, the dose of melatonin used in the injection studies (microgram quantities) far exceeds the nanogram amounts synthesized by the pineal during the course of the night. In our view, this compromises the physiological relevance of the injection studies and weakens the experimental basis for the phase hypothesis. Further, it is important to stress that the characteristics of the melatonin signal provided in those
HOW MELATONIN CODES FOR DAY LENGTH

studies cannot be resolved because there were no descriptions of the melatonin patterns produced in hamsters treated with either infusions or bolus injections.

One of the unique strengths of using the sheep as a model lies in our ability to control and characterize infused melatonin patterns. Using infusions to produce melatonin patterns that were documented to be physiological, we have gathered strong support for the duration hypothesis in this and earlier studies. Further support for this hypothesis has recently been obtained by Arendt and coworkers (1988) who exposed ewes to photoperiodic manipulations which altered the phase of the melatonin peak, but not its duration. The reproductive response of those ewes reflected the duration of melatonin elevation. It is important to note that there is no definitive support for the phase hypothesis in the sheep.

Loss of Response to a Long-Day Melatonin Signal

Refractoriness to long days has been associated with onset of the natural breeding season in the ewe, and thus plays an important role in timing the seasonal reproductive cycle (Robinson et al., 1985; Worthy et al., 1985). Two mechanisms have been proposed to account for the development of photorefractoriness, an alteration in the generation of the melatonin signal and a spontaneous change in the post-pineal processing of that signal. There is evidence in the Soay ram and Saanen goat that photorefractoriness is associated with altered melatonin patterns, in that elevated levels were observed during the day (Almeida and Lincoln, 1984; Maeda et al., 1986).

In the course of distinguishing between phase and duration, we have gathered support for the hypothesis that the loss of response to an inhibitory photoperiod results, at least in part, from a post-pineal process. Specifically, pinealectomized ewes infused with a fixed long-day pattern of melatonin became refractory to the inhibitory signal over much the same time course a group of pineal-intact ewes that became refractory to short days (Karsch et al., 1986). In addition, we obtained no evidence for an alteration of the phase or duration of the melatonin rhythm in pineal-intact ewes during the development of refractoriness to a fixed short day (Malpaux et al., 1987). Our results suggest that refractoriness to an inductive photoperiod, like that for an inhibitory one, develops at a post-pineal level.

It is of interest that the time course observed for the development of refractoriness to the long-day pattern of melatonin in the present study was identical in ewes infused with melatonin during the night or in the middle of the day. Whatever mechanisms underlie refractoriness to a melatonin signal, therefore, it appears to make no difference what time of day the melatonin signal is present.

Termination of an Inhibitory Photoperiod Cue

The response of the STOP MEL group shows that, following the termination of a long-day pattern of melatonin, both reproductive induction and its subsequent suppression occur. This response closely resembled that of the pineal-intact ewes transferred from long to short days. Specifically, following the removal of the long-day signal by either stopping the melatonin infusion or switching to short days, LH levels rose after a certain lag, remained maximal for about 50 days, and then fell to baseline. These results lead to the hypothesis that the primary role of short days is to remove inhibition produced by long...
days. This hypothesis is further supported by recent work in the adult and immature ewe in which long-day signals were terminated by either pinealectomy or superior cervical ganglionectomy and the response was similar to that of ewes switched to short days (Wayne and Karsch, unpublished observations, Foster et al., 1987).

Other observations in our study suggest that, in addition to removing long-day inhibition, short days can actively promote reproductive induction. The pinealect-ntact ewes receiving an inductive signal showed an earlier rise in serum LH than did the pinealectomized ewes that had their inhibitory melatonin signal terminated (STOP MEL group). Conclusions drawn from direct comparisons between the pineal-intact and pinealectomized ewes, however, should be tempered because of differences observed in their responses during the priming period. Nevertheless, a role for short days in advancing reproductive induction is also suggested by our finding that pinealectomized ewes switched from a long- to short-day pattern of melatonin during the priming period showed a significantly earlier rise in LH than when these same ewes had their long-day melatonin pattern terminated (SD MEL vs. STOP MEL in Fig. 5). Additional studies are therefore required to clarify whether or not short days can stimulate an active process that accelerates reproductive induction.

Duration Hypothesis

The central focus of this study was to investigate whether the phase of the melatonin rise, with respect to the light-dark cycle, or the duration of this elevation mediates the effects of photoperiod on the reproductive response of the ewe. Using a model in which circulating melatonin patterns could be artificially controlled and closely monitored for their physiological accuracy, we could not find evidence for a role of phase. Rather, our results are consistent with the hypothesis that the duration of nocturnal melatonin secretion codes for day length. In absolute terms, however, the duration hypothesis is likely an oversimplification of the operation of the photoperiodic timekeeping system in animals exposed to gradual changes in day length, such as during the natural photoperiodic cycle. Recent reports in both the sheep and hamster show that the same duration of elevated melatonin results in opposite reproductive responses depending on the previous melatonin pattern (Hoffman et al., 1986; Robinson and Karsch, 1987; Hastings et al., 1987). Therefore, it is not the absolute duration, but a change in duration of nocturnal melatonin secretion, that conveys photoperiodic information to the reproductive system.

ACKNOWLEDGMENTS

We thank Mr. Douglas Doop and Kirk VanNatter for their assistance with the animal experimentation; Drs. Lee E. Claypool, Douglas L. Foster, Alan H. Kaynard, Jane E. Robinson, and Ms. Celia Woodfill for the design and conduct of the experiment; Drs. Josephine H. Arendt, Gordon D. Niswender, and Leo E. Reichert, Jr. for supplying assay reagents; the Sheep Research Core Facility for maintaining the animals; Dr. Morton B. Brown and the Data Analysis Core Facility for processing the data; and the Standards and Reagents Core Facility for preparing the reagents.

REFERENCES

HOW MELATONIN CODES FOR DAY LENGTH

Stetson MH, Sarafidis E, Rollag MD, 1986. Sensitivity of adult male Djungarian hamsters (Phodopus sungorus) to melatonin injections throughout the day: effects on the reproductive system and the pineal. Biol Reprod 35:618–23
Worthy K, Haresign W, 1983. Evidence that the onset of seasonal anoestrus in the ewe may be independent of increasing prolactin concentrations and daylength. J Reprod Fertil 69:61–48
Worthy K, Haresign W, Dodson S, McLeod BJ, Foxcroft GR, Haynes NB, 1985. Evidence that the onset of the breeding season in the ewe may be independent of decreasing plasma prolactin concentrations. J Reprod Fertil 75:237–46