

## Light control of prolactin secretion in sheep. Evidence for a photoinducible phase during a diurnal rhythm

par J.-P. RAVAUULT \*, R. ORTAVANT

Station de Physiologie de la Reproduction, I.N.R.A.  
Nouzilly 37380 Monnaie, France

\* Laboratoire de Physiologie Comparée, Faculté des Sciences,  
Parc Grandmont, 37200 Tours, France

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**Summary.** The secretion of prolactin comes under important seasonal variations in sheep. Thus, in the ram the highest levels are in summer and the lowest in winter. These variations of secretion are under light control because illumination of long duration (long photoperiods) produces high prolactin levels ( $220 \pm 2$  ng) ; short photoperiods produce low levels ( $12 \pm 2$  ng).

Using the hypothesis of Bunning, we study a photoinducible phase during diurnal rhythm. For this purpose, rams preconditioned either in short photoperiods or in long photoperiods, receive 8 hours of illumination in two fractions of 7 and 1-hour durations. The beginning of the 7-hour period is considered to be a subjective dawn, and the 1-hour light pulse is given at various times after dawn : at hours 11, 14, 17 and 20.

The group receiving the light pulse during hour 17 after dawn has significantly higher prolactin levels than all the other groups. For example, in rams preconditioned in short photoperiods and after 5 weeks of light pulse treatment, the mean prolactin levels are  $71 \pm 4$  ng (hour 11) ;  $68 \pm 5$  ng (hour 14) ;  $225 \pm 22$  ng (hour 17) and  $110 \pm 16$  ng (hour 20).

The prolactin level is higher during the dark phase than during the light phase ( $272 \pm 15$  ng vs  $126 \pm 8$  ng in the group with light pulse at hour 17). This increase is essentially due to one important release resulting from the change in the light-dark sequence. Another release only occurs after the light pulse of one hour, if given at hour 17. However, these two prolactin releases are not sufficient to entirely explain this increase.

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In domestic mammals, prolactin secretion can be influenced by different factors. In the male or the female, stress (Raud *et al.*, 1971), estrogens (Schams and Reinhardt, 1973) and TRH (Debeljuk *et al.*, 1973) cause an increase in the level of plasma prolactin. Secretion of this hormone also depends on external factors such as ambient temperature and relative humidity (Tucker and Wetteman, 1976). However, one external factor-light-has been shown to be important in the ram (Pelletier, 1973) and in the bull (Bourne and Tucker, 1975), but has not received much attention in other species. This paper describes the effects of light on the secretion of prolactin in the sheep.

## 1. Seasonal variations in the level of plasma prolactin in the sheep. Photoperiodic effect

Seasonal variations in plasma prolactin have been already described in cattle (Tucker *et al.*, 1973 ; Schams and Reinhardt, 1974) and in goats (Buttle, 1974 ; Hart, 1975). One of us (Ravault, 1976) has shown such seasonal variations in the sheep. For that purpose, 9 Ile-de-France lambs, born in autumn, were reared in pens under natural conditions of light and temperature. Blood samples (5 ml) were collected weekly for two years, then every fifteen days during the third year. Blood collection was always carried out prior to feeding between 9 and 9.30 a.m. The results of prolactin determination by radioimmunoassay (Kann, 1971) in the samples are shown in figure 1.

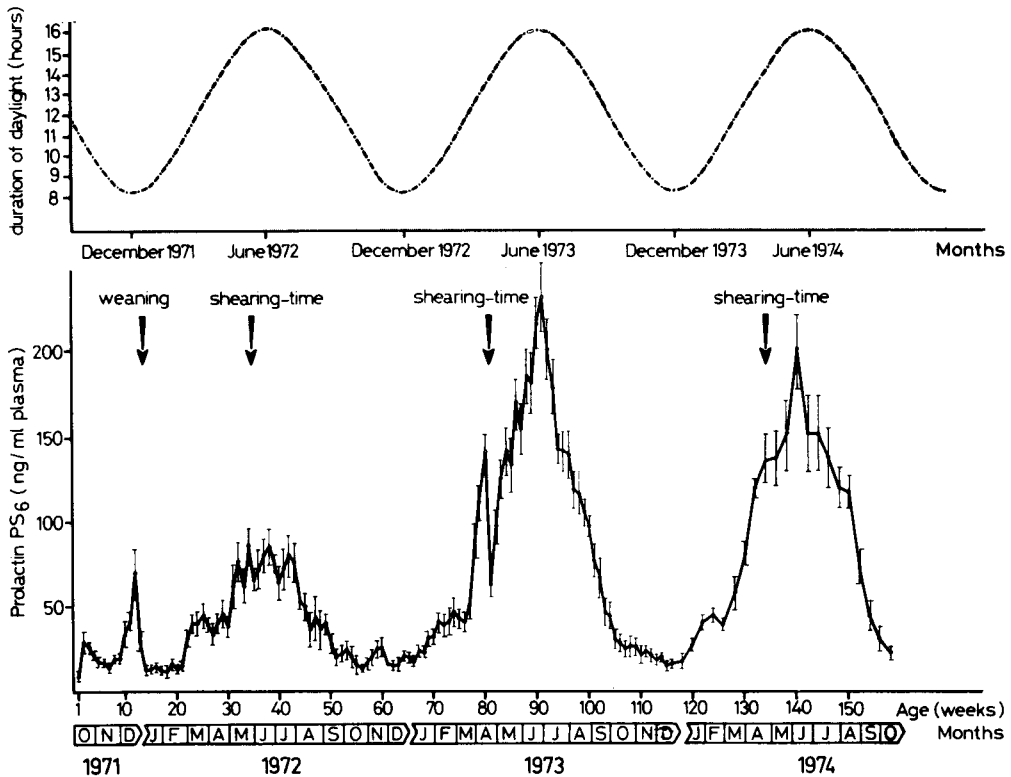


FIG. 1. — Seasonal variations of prolactin in the peripheral blood plasma of Ile-de-France rams ( $N = 9$  ;  $m \pm SE$ ) (from Ravault, 1976).

After a sharp increase about week 12, independent of the season (Ravault and Courot, 1975), the level of plasma prolactin fell rapidly to a basal level. From 15 weeks of age, variations in prolactin parallel variations in the daylength, showing a regular increase up to June, followed by a decline to a minimum in December. Similar variations were observed in the second and third years, but with an increased amplitude.

These results indicate that prolactin secretion is maximum in summer (long days and high temperature) and minimum in winter (short days and low temperature).

However, it seems that the variations of temperature are not responsible for this pattern of prolactin secretion. Alberio and Ravault (unpublished communication) subjected other rams to either constant temperature (between 19° and 21 °C) or to variable temperatures between 1° and 24 °C, according to the season. The results (table 1) show that temperature did not significantly affect the level of prolactin.

TABLE 1

Mean level of plasma prolactin (ng PS6/ml  $\pm$  SE) in rams submitted to natural variations of temperature (minimum in December or 8 hours light, maximum in June or 16 hours light) or to constant temperature (20  $\pm$  1 °C)

Month (duration of light)	Temperature		
	not constant	constant	
<b>1st year :</b>			
— December (8 h light) . . . . .	12 $\pm$ 2	20 $\pm$ 9	NS *
— June (16 h light) . . . . .	90 $\pm$ 8	100 $\pm$ 10	NS *
<b>2nd year :</b>			
— December (8 h light) . . . . .	15 $\pm$ 3	28 $\pm$ 4	NS *
— June (16 h light) . . . . .	220 $\pm$ 20	190 $\pm$ 22	NS *

\* NS : no significant.

On the other hand, daily daylength appears to influence the secretion of prolactin in entire or castrated adult rams (Pelletier, 1973). In this study, animals were subjected to a shortened annual photoperiodic cycle. The maximum and minimum periods of

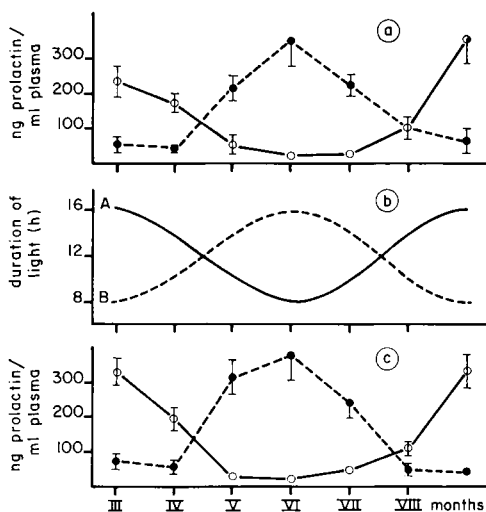


FIG. 2. — Experimental regimens (A and B) of « shortened » annual photoperiodic rhythm (b) and the mean plasma prolactin levels ( $\pm$  SE) of 2 groups of 8 entire (a) and 7 castrated (c) rams submitted to 2 opposite light regimens. Continuous line : animals under light regimen A ; broken line : animals under light regimen B (from Pelletier, 1973).

daily illumination were 16 h and 8 h, respectively, and animals were subjected to two opposite patterns of gradually changing daylight hours between these extremes. A complete cycle took 6 months and temperature remained constant. The results (fig. 2) show the parallelism between variations in the light photoperiod and the plasma prolactin level in intact rams (correlation coefficient  $r = 0.888$  ;  $P < 0.001$ ) and castrated animals ( $r = 0.887$  ;  $P < 0.001$ ). Maximum prolactin values were found when animals were exposed to light for 16 hours. Such results have been confirmed by Forbes *et al.* (1975). Thus, light appears to be a strong modulator of prolactin secretion. An increase in daily daylength stimulates this secretion, while a decrease is inhibitory. How does this light act ?

## 2. Attempt to determine a photoinducible phase for prolactin secretion during the diurnal rhythm

How can the physiological system measure the daily variation of illumination ? Different hypotheses have been given : the most explicit theories are those of Bunning (1960), Pittendrig and Minis (1964), Pittendrig (1966) and Pittendrig and Minis (1971). These authors have suggested that there is an endogenous daily rhythm in

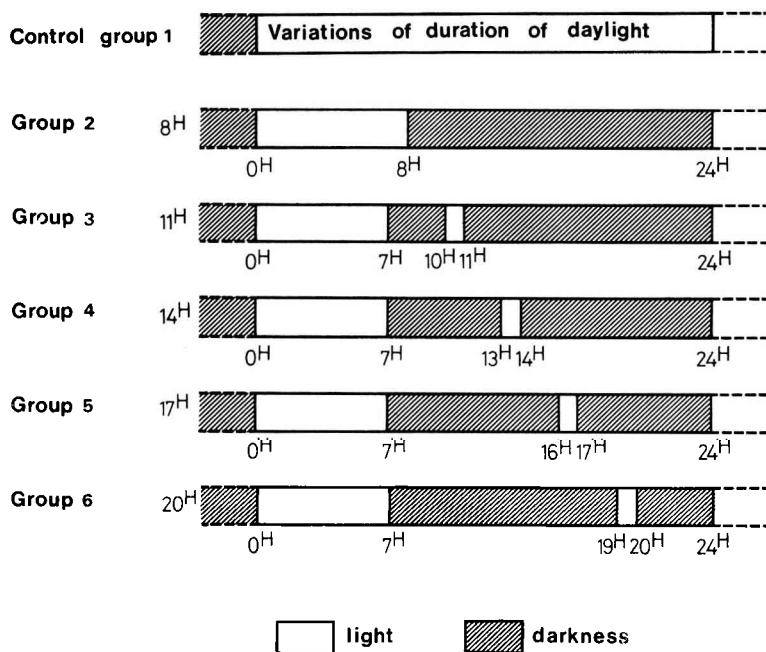


FIG. 3. — Protocol for the research of a photoinducible phase. Position of the light pulses after the dawn.

- Group 1 : control group, normal variations of duration of daylight.  
 Group 2 : 8 continuous hours.  
 Group 3 : 11 h, light pulse hour 11 after the dawn.  
 Group 4 : 14 h, light pulse hour 14 after the dawn.  
 Group 5 : 17 h, light pulse hour 17 after the dawn.  
 Group 6 : 20 h, light pulse hour 20 after the dawn.

photosensitivity ; this rhythm is imagined as having two halves per cycle : during one time the organism is light « insensitive », and during the other it becomes « sensitive » A physiological process is stimulated only if light coincides with the sensitive phase of the daily cycle. In winter, the photoperiods are too short to reach the sensitive phase but, as they lengthen in spring, light begins to progressively reach the phase, and so automatically to stimulate the physiological process. The existence of such a photoinducible phase, determined in relation to dawn, has been shown in birds (Hamner, 1963, 1964 ; Farner, 1965 ; Menaker, 1965 ; Follett and Sharp, 1969 ; Follett, 1973 ; Lofts and Lam, 1973). Could there not be a photoinducible phase for prolactin secretion in the sheep ? To determine this, we interrupted a 24-hour « night » cycle at different times with a short illumination (light pulse), a physiological response to the illumination thus indicating the presence of a sensitive phase.

Six lots of 5 adult Préalpes rams were used. Prior to the experiment they were all subjected to normal variations of daylength with an illumination of 300 lux at eye level. While a control group continued with a regime of normal daylight variation, the other five groups received 8 hours of illumination in two fractions, one of 7 and one of 1 hour duration. The beginning of the 7-hour period was considered to be a subjective dawn ; the 1-hour light pulse was given at various times after dawn (fig. 3).

Prior to light pulse and approximately five weeks and 3 months after the beginning of the light treatment, plasma samples (5 ml) were collected from the rams every hour for 25 hours. Collections in the dark were effected without added illumination. After cold centrifugation, the plasmas were stored at  $-20^{\circ}\text{C}$  until analyzed for prolactin titer by radioimmunoassay (Kann, 1971).

Two experiments were conducted : in the first, rams were preconditioned to short photoperiods (i.e. experiment begun January 1) and in the second, to long photoperiods (i.e. experiment begun June 8).

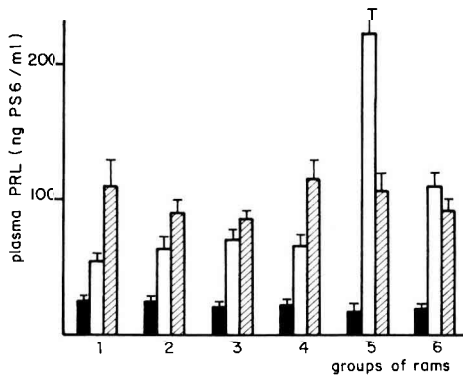


FIG. 4. — Daily mean of prolactin level after 5 weeks and 3 months of various light treatments in rams previously subjected to short photoperiods.

- before treatment (daily daylength : 8 h 15 mn).
- 5 weeks after the beginning of light treatment.
- ▨ 3 months after the beginning of light treatment.

1 : control group (daily daylength after 5 weeks treatment : 9 h 45 mn and after 3 months : 13 h 15 mn).  
 2 : 8 hours continuous light per day.  
 3, 4, 5, 6 : 7 hours of light + 1 hour of light pulse respectively hours 11, 14, 17 and 20 after dawn.  
 T : standard error.

A) Rams preconditioned to short photoperiods (fig. 4).

There were no significant differences in the mean prolactin levels for the 25 consecutive blood samples obtained before the light treatment commenced. The prolactin titer varied around  $20 \pm 5$  ng/ml, a normal value under the light conditions used (8 h 15 mn light).

In contrast, after about 5 weeks of light pulse treatment, the mean prolactin level increased and significant differences were found between the treatment groups. In particular, group 5, receiving the light pulse during hour 17 after dawn, had significantly higher levels of prolactin than all other groups, including the one receiving a pulse hour 20 after dawn (group 6).

After 3 months of treatment, there was no significant difference between the groups. In the control group (1), the prolactin titer continued to augment with increased daylength. However, the prolactin level decreased in group 5 which received strong, high stimulation.

B) Rams preconditioned to long photoperiods (fig. 5).

As in the previous experiment, the mean prolactin levels, before light treatments began, were not significantly different between the groups. The mean value of  $150 \pm 10$  ng/ml is normal for animals in a 15 h 45 mn illumination.

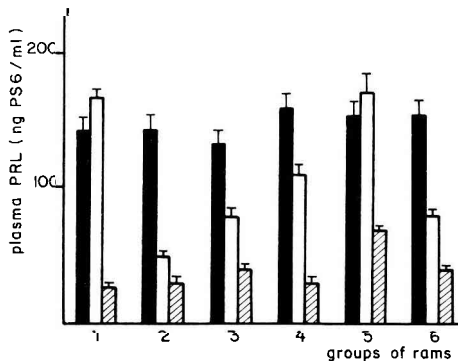


FIG. 5. — Daily mean of prolactin level after 4 weeks and 3 months of various light treatments in rams previously subjected to long photoperiods.

- before treatment (daily daylength : 15 h 45 mn).
- 4 weeks after the beginning of light treatment.
- ▨ 3 months after the beginning of light treatment.

1 : control group (daily daylength after 4 weeks treatment : 16 h and after 3 months : 12 h 15 mn).  
 2 : 8 hours continuous light per day.  
 3, 4, 5, 6 : 7 hours of light + 1 hour of light pulse respectively hours 11, 14, 17 and 20 after dawn.  
 T : standard error.

After 4 weeks of pulse treatments, the prolactin levels varied significantly between groups. During the treatment, the levels of prolactin did not increase significantly in the control group ( $165 \pm 9$  ng/ml) and in group 5 ( $168 \pm 12$  ng/ml). On the other hand, the prolactin levels decreased in all the groups, but most markedly in those which had the light pulse farthest from hour 17 after dawn.

After 3 months of pulse treatments, the prolactin levels fell in all groups and were not significantly different, except for group 5 (higher values). At this time, the control group received 12 h 45 mn illumination per day.

### C) Conclusions.

The results of these two experiments established that light pulses affect the secretion of prolactin in the ram. When the light pulse was given hour 17 after dawn, prolactin levels were maximal and comparable to those observed during long photoperiods (16 h), even though the total daily illumination was only 8 hours. With 8 continuous hours of light or with other pulse treatments, the prolactin levels were lower.

Thus, there seems to be a photoinducible phase for prolactin secretion during the diurnal rhythm. However, sensitivity is not constant, and there is evidence of « fatigue » effect.

The question remaining is : what is the pattern of prolactin secretion during diurnal rhythm in the ram ?

### 3. Comparison between prolactin levels during light and dark phases

Several authors have established variations in prolactin secretion during the diurnal cycle with the maximum secretion of prolactin being observed at night. This effect has been shown in the male rat (Dunn *et al.*, 1972 ; Ronnekleiv, Krulich and

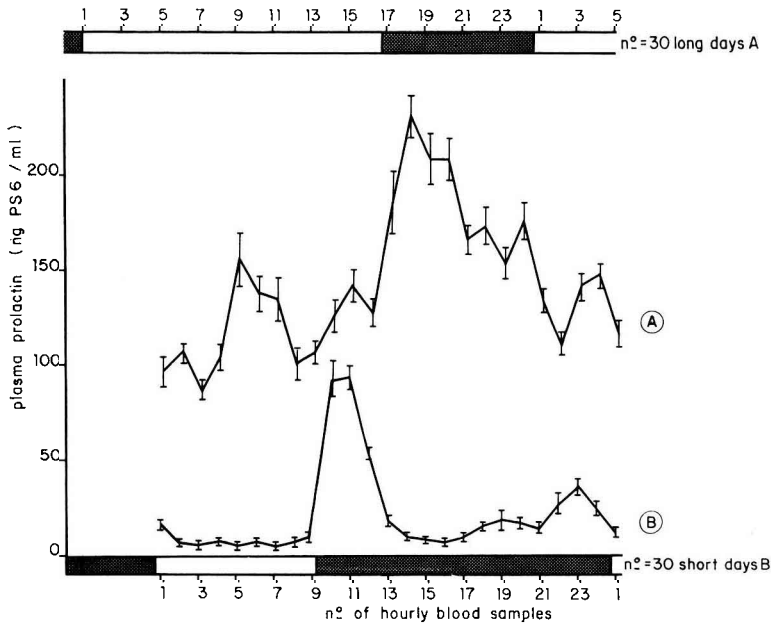


FIG. 6. — Hourly mean prolactin levels in rams preconditioned in short photoperiods (B) or in long photoperiods (A). ( $m \pm SE$ ).

□ light ; ■ darkness.

MacCann, 1973 ; Simionescu, Sahleanu and Oprescu, 1974 ; Kizer *et al.*, 1975), in the pregnant rat (Butcher, Fugo and Collins, 1972) or in the pseudopregnant rat (Freeman and Neill, 1972), in the ewe during the luteal phase (Davis and Borger, 1972), in the lactating cow (Koprowski, Tucker and Convey, 1970, 1972), in the rhesus monkey (Quadri and Spies, 1976) and in man (Sassin *et al.*, 1972 ; Vanhaelst *et al.*, 1973 ; Rubin *et al.*, 1975). But some authors have determined either an inverse pattern in the rat (Hefco and Lackey, 1973), or no circadian rhythm in the ram (Chamley *et al.*, 1974) and in goat (Buttle, 1974).

We have studied the evolution of plasmatic prolactin level during the diurnal cycle in rams before the beginning of light pulse treatment, using either long photoperiods (June 8) or short photoperiods (December 15), and then on the same rams about five weeks after the beginning of the light pulse treatments.

In all groups, the mean prolactin level was higher in the dark than in the light phase. For example, in the pretreatment period (fig. 6), the mean prolactin level in 30 rams was  $190 \pm 9$  ng/ml (long photoperiods) and  $32 \pm 4$  ng/ml (short photoperiods) during the dark, compared with  $112 \pm 5$  ng/ml and  $6 \pm 1$  ng/ml during the light phases. The results after about one month of light treatment are shown in table 2.

TABLE 2

*Mean prolactin level (ng PS6/ml  $\pm$  SE) during the light and dark phase after about 5 weeks light pulse treatments (groups 1, 2, 3, 4, 5, 6 : see protocol fig. 3)*

Group	Rams preconditioned in			
	short photoperiods		long photoperiods	
	light phase	dark phase	light phase	dark phase
1	$36 \pm 3$	$67 \pm 5^*$	$147 \pm 6$	$202 \pm 10^*$
2	$51 \pm 4$	$70 \pm 7$	$45 \pm 5$	$50 \pm 4$
3	$37 \pm 2$	$84 \pm 5^*$	$51 \pm 3$	$96 \pm 6^*$
4	$41 \pm 2$	$79 \pm 4^*$	$90 \pm 6$	$114 \pm 6^*$
5	$126 \pm 8$	$272 \pm 15^*$	$107 \pm 7$	$201 \pm 8^*$
6	$71 \pm 8$	$125 \pm 8^*$	$76 \pm 3$	$79 \pm 2$

\* significantly different from light phase.

This difference is due to the sharp increase in prolactin in the hour after the change from light to dark (fig. 7, 8). A high level is subsequently maintained for 2-3 hours, then falls gradually. The short duration of this peak explains why several authors have not found diurnal variations : blood sampling was not frequent enough.

Other low but significant peaks of prolactin were observed either at the end of the dark phase on rams preconditioned to short photoperiods, or during the light phase in rams preconditioned to long photoperiods (fig. 6). In the latter case, the peaks seem to have a 4-5 hour periodicity, but this may not be related to other phenomena, such as feeding (MacAtee and Trenkle, 1971).



Generally, a one-hour light pulse in the dark phase decreases the prolactin level (table 3). The increase in prolactin during the three hours following the light pulse (table 4) is significant when the pulse occurs at hour 17 after dawn. In other cases, the increase is not significant; there is even a decrease when light pulse is situated at hour 11 or hour 20 after dawn.

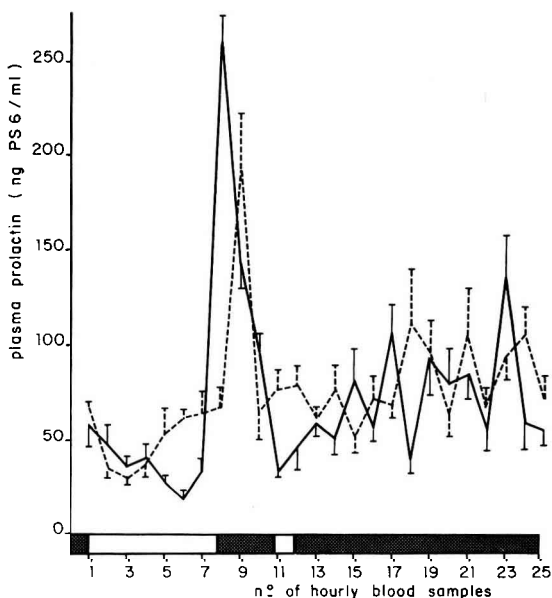


FIG. 7. — Hourly mean prolactin levels in rams preconditioned in short photoperiods (—) or in long photoperiods (---) subjected about 5 weeks, to a light pulse treatment at hour 11 after dawn ( $m \pm SE$ ).

□ light ; ■ darkness.

These phenomena are particularly marked in rams preconditioned to short photoperiods. With rams preconditioned to long photoperiods, the phenomena are less marked, perhaps indicating the beginning of a fatigue effect on the photosensitive system controlling prolactin secretion.

Thus, the high prolactin levels associated with the pulse hour 17 after dawn, are essentially due to two important prolactin releases resulting from two changes in the light-dark sequence. This leads to a good coincidence with the periodic endogenous rhythm previously described, especially in the case of long photoperiods.

What is the mechanism of prolactin rhythm? There are few experiments to provide data. In the rat, pinealectomy abolishes the diurnal rhythm in prolactin secretion (Ronnekleiv *et al.*, 1973) as well as blocking the increase in prolactin observed in rats kept in continuous darkness (Relkin, 1972) and in blind, anosmic rats (Blask and Reiter, 1975). Thus, darkness may modify prolactin secretion by acting on the pineal gland, mediated by dopamine, serotonin or melatonin.

As hypothalamic dopamine inhibits prolactin secretion in the rat (Kamberi, Mical and Porter, 1971) or in the sheep (Davis and Borger, 1973), it seems that the

period of darkness induces inactivation of the dopamine-stimulating system ; an injection of L. Dopa at about 00.30 h suppresses the nocturnal surges of prolactin in the rat (Hsueh and Voogt, 1975). However, there are few studies of diurnal variation in serum dopamine levels. Scheving *et al.* (1968) have suggested a trimodal variation in dopamine secretion in the rat, and if this is also true for sheep, then the light pulse at hour 17 after dawn may modify the dopamine secretion.

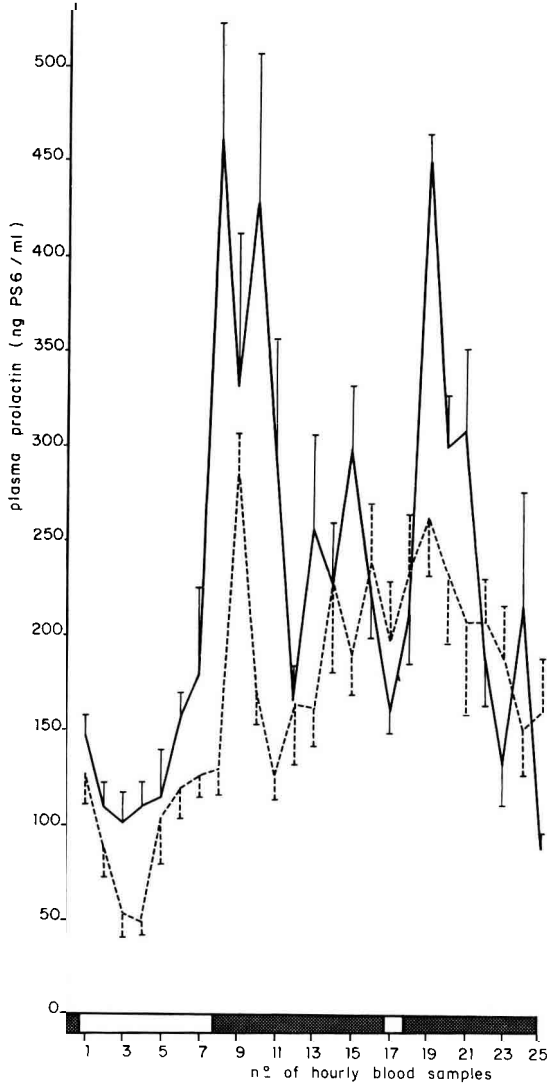


FIG. 8. — Hourly mean prolactin levels in rams preconditioned in short photoperiods (—) or in long photoperiods (- - -) subjected about 5 weeks, to a light pulse treatment at hour 17 after dawn ( $m \pm SE$ ).

□ light ; ■ darkness.

It has also been shown that prolactin secretion is activated by serotonin-stimulating pathways (Lu and Meites, 1973 ; Kordon *et al.*, 1974 ; Lawson and Gala, 1976). In the ferret conditioned to short days, Yates and Herbert (1976) found an increase in serotonin in the pineal gland and hypothalamus at the beginning of the dark phase. The serum serotonin of the rat increases at the beginning of the dark phase, but also at the end of the light phase (Scheving *et al.*, 1972). In man, the highest values of serum serotonin are found during darkness (Sauerbier and Von Mayersbach, 1976), but Halberg *et al.* (1967) observed the zenith of circadian rhythm for serotonin at the beginning of the day. In view of these results, it is difficult to conclude in favor of an hypothalamic effect of serotonin on the secretory rhythm of prolactin.

TABLE 3

Mean prolactin level (ng PS6/ml  $\pm$  SE) in rams preconditioned in short photoperiods during the light pulse treatment and 1 h before and after, 5 weeks after the beginning of light pulse treatment (groups 3, 4, 5, 6 : see protocol fig. 3)

Group	1 h before light pulse	during light pulse	1 h after light pulse
3	95 $\pm$ 10	33 $\pm$ 3	45 $\pm$ 12
4	46 $\pm$ 12	36 $\pm$ 7	68 $\pm$ 10
5	221 $\pm$ 24	160 $\pm$ 8	210 $\pm$ 27
6	185 $\pm$ 19	123 $\pm$ 14	160 $\pm$ 15

The injection of melatonin into the 3rd ventricle increases plasma prolactin levels in the rat (Kamberi, Mical and Porter, 1971) ; serum melatonin in this species is highest during the night (Pang and Ralph, 1975). Melatonin secretion has also been observed to increase at the beginning of darkness in ewes (Rollag and Niswender, 1976) and in man (Pelham *et al.*, 1973). Despite the good correlation between melatonin and prolactin patterns, the relationship between the two needs further research.

TABLE 4

Mean prolactin level (ng PS6/ml  $\pm$  SE) in rams preconditioned in short photoperiods 3 h before beginning and 3 h after the end of darkness, then 3 h before and after light pulse, 5 weeks after the beginning of light pulse treatment (group 3, 4, 5, 6 : see protocol fig. 3).

Group	3 h before beginning of darkness	3 h after the end of darkness	3 h before light pulse	3 h after light pulse
3	27 $\pm$ 3	166 $\pm$ 19 *	166 $\pm$ 19	52 $\pm$ 5 *
4	44 $\pm$ 6	138 $\pm$ 12 *	54 $\pm$ 6	70 $\pm$ 6
5	150 $\pm$ 17	407 $\pm$ 44 *	248 $\pm$ 19	320 $\pm$ 29 *
6	82 $\pm$ 10	158 $\pm$ 9 *	167 $\pm$ 11	132 $\pm$ 10

\* Significantly different from 3 h before the beginning of darkness or light pulse.

Nevertheless, it seems that light treatments affect both excretory and secretory pituitary activity. All the data on our light treatments support these concepts ; for example, the prolactin level observed in animals subjected to a light pulse at hour 17 after dawn is higher than that in all other treatments, even considering the period outside of the peak surges of prolactin due to the passage of darkness.

For this reason, we suggest that the ram has a photosensitive period for the secretion of prolactin.

8<sup>e</sup> Colloque de Neuroendocrinologie. — Table ronde Prolactine.  
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**Résumé.** La sécrétion de prolactine subit d'importantes variations saisonnières chez les ovins. Ainsi, chez le mouton, les taux les plus élevés surviennent en été et les plus bas en hiver. Ces variations de sécrétion sont contrôlées par la lumière car un éclaircissement de durée identique à une longue photopériode entraîne un taux élevé de prolactine ( $220 \pm 20$  ng) et une courte photopériode de faibles taux ( $12 \pm 2$  ng).

En se basant sur l'hypothèse de Bunning, nous avons recherché une phase photosensible au cours du rythme diurne. Pour cela, des béliers préconditionnés en courte ou en longue photopériode recevaient 8 h d'éclaircissement en deux fractions d'une durée de 7 h et de 1 h. Le début de la photopériode de 7 h était considéré comme une aube subjective et l'impulsion lumineuse était donnée à différents moments après l'aube, c'est-à-dire à la 11<sup>e</sup>, 14<sup>e</sup>, 17<sup>e</sup> et 20<sup>e</sup> heure.

Le groupe qui recevait l'impulsion lumineuse à la 17<sup>e</sup> heure après l'aube avait des taux significativement plus élevés que ceux des autres groupes. Chez des béliers préconditionnés en courte photopériode et après 5 semaines de traitements lumineux, les taux moyens de prolactine sont de  $71 \pm 4$  ng (11<sup>e</sup> heure) ;  $68 \pm 5$  ng (14<sup>e</sup> heure) ;  $225 \pm 22$  ng (17<sup>e</sup> heure) et  $110 \pm 16$  ng (20<sup>e</sup> heure).

Le taux de prolactine est plus haut pendant la phase obscure que pendant la phase claire ( $272 \pm 15$  ng contre  $126 \pm 8$  ng dans le groupe avec l'impulsion lumineuse à la 17<sup>e</sup> heure). Cette augmentation est due essentiellement à une importante décharge résultant du changement lumière-obscurité. Une telle décharge se produit après l'impulsion lumineuse de 1 heure si celle-ci est donnée à la 17<sup>e</sup> heure. Cependant, ces deux décharges de prolactine ne sont pas suffisantes pour expliquer cette augmentation. Il semble exister une phase photosensible pour la sécrétion de prolactine.

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