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Influence of Photoperiod and Protein Diet on Growth Hormone Secretion in Rams (42550)

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Abstract. Studies of sheep were undertaken to determine the effects of photoperiod and protein diet on growth hormone (GH) secretion. Rams were subjected to either a control (R1) or an inverted (R2) 6-month (semestral) light regime. In both light regimes day lengths varied gradually between 8 and 16 hr. Within each light regime group of animals, the rams received either a low (L) or a high (H) protein diet containing the same level of energy. Plasma GH profiles consisting of 13 hourly samples were determined at regular intervals corresponding to known day lengths. Analysis of variance indicated that (i) there was a significant effect of day length ($P < 0.01$) and protein diet ($P < 0.05$) on GH secretion, (ii) the two light regimes R1 and R2 were equivalent with respect to GH secretion, and (iii) there were no interactions among the three experimental factors. Although mean GH secretion was consistently higher in L groups than in H groups, there was a similar trend in all the animals of increasing GH secretion as day length was increased. GH secretion was maximum when the day length reached 13 hr 20 min in increasing photoperiods in L groups (15.6 ± 1.6 ng $\times$ h $\times$ ml^{-1}) and 16 hr in H groups (13.0 ± 1.2 ng $\times$ h $\times$ ml^{-1}). From these results we conclude that both an increasing day length and a deficiency in protein diet stimulate GH secretion in rams but the GH response to these two factors may involve different regulatory processes and may have different functions.

Homeostasis and homeorhesis have been defined previously as different biological functions involved in the regulation of growth (1). Homeostasis describes those regulatory mechanisms that compensate for the effects of factors disrupting the steady state, while homeorhesis is the coordination of the metabolism of tissues in support of developmental or physiological processes. Both of these functions require the partitioning of nutrients which is influenced by hormones such as growth hormone (GH) and prolactin (PRL). The objective of this study was to determine the effect on sheep GH secretion of factors (proteins in the diet and photoperiod) which affect homeostatic and homeorhetic functions.

It has been well established that nutritional regimes modify body growth and plasma concentration of GH in sheep (2–4). In numerous species, GH secretion appears to be stimulated when nutrient availability is declining. This has been observed after a fall in free fatty acid (FFA) concentrations in the circulation (5) and after chronic protein deprivation (6, 7).

On the other hand, we have recently demonstrated that photoperiod influences GH secretion in rams (8) and that stimulation of growth during increasing day lengths correlates well with changes in plasma GH levels (9). We now present data to show that GH secretion is still responsive to photoperiod even when it has been increased by a low protein diet and that both factors have independent and additive effects.

Material and Methods. *Experimental design.* The experiment had a factorial design with two light regimes (R1 and R2) and two nutritional planes (H and L). Twenty-four Ile-de-France rams (12 ± 1 months old) were divided at random into four groups of six (R1H, R1L, R2H, R2L). On the 23rd of December, the animals were placed in two light-proof rooms to begin a semestral light regime reproducing in 6 months the annual day length variations. Day length was controlled by automatic switches which turned the light directly on or off. It varied by changing the dusk in quarter-hour steps at appropriate intervals. Light intensity was 350 lx. Day length varied from a minimum of 8 to a maximum of 16 hr. Animals in the first room (groups R1H and R1L) began the experiment at the same day length as that which they were experienc-

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ing outdoors but those in the second room (groups R2H and R2L) passed directly from 8 hr of natural light to 16 hr of artificial light and began a sinusoidal light regime, the inverse of that of the other room. Animals were accustomed to their light regime during a 3-month equilibration time (Fig. 1). During the equilibration time, rams were fed 1200 g of a daily maintenance diet of straw, 42%; soybean meal, 4%; oat grain, 8%; and maize, 46%. At the beginning of the sampling time, groups R1H and R2H received a diet consisting of the same ingredients but in a proportion calculated to give an intake of protein 50% above maintenance. Groups R1L and R2L received a diet calculated to give a level of protein 25% less than maintenance. The diets were calculated to have the same level of energy.

Sequences (S_1–S_7) of 13 blood samples were taken from each ram by venipuncture of a jugular vein in heparinized tubes at hourly intervals. Sampling commenced 2 hr after the artificial dawn. During the dark phase, blood was collected under a weak green light. Sampling sequences were performed when the animals were exposed to 8, 10.40, 13.20, or 16 hr of day length during increasing or decreasing photoperiods (Fig. 1).

GH radioimmunoassay. Plasma was separated by centrifugation and stored at −20°C until assayed. Ovine GH (oGH) was measured by specific radioimmunoassay using a double-antibody separation method. oGH standard and the first antibody were supplied by the National Hormone and Pituitary Program (NIADDK, Bethesda). The second antibody (sheep anti-rabbit γ-globulin serum) was a gift of Dr. M. Blanc (INRA-Nouzilly, France). The sensitivity of the assay was 1 ng/ml and the intraassay variation was 5%.

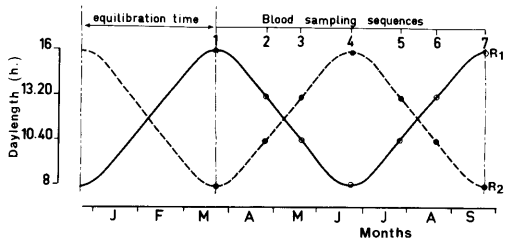

FIG. 1. Experimental design of the experiment. Rams were subjected to either a control (R1) or an inverted (R2) light regime reproducing in 6 months the annual photoperiodic variations. The points show when the blood sampling sequences were performed. They consisted of a series of 13 hourly consecutive samples.

Statistical analysis. The effects of experimental factors (light regime, nutritional regime, day length, and all possible interactions) were subjected to analysis of variance (ANOVA) and tested by the least significant difference (10) using the computer program GENSTAT. Analyses were based on results expressed as ng × h × ml^{-1} (obtained by measuring the area under the line that was plotted for GH concentration against time) in order to take into account both the basal concentration and the episodic discharges which could not be estimated separately using hourly blood samples (8).

Results. Mean plasma GH levels for the four groups during the different sequences of blood sampling are depicted in Fig. 2. During S_1 in the two groups receiving 16 hr of light daily (R1), plasma GH concentrations were markedly above those found in animals receiving only 8 hr (R2). Similar results were observed during S_7 (data not shown) where the day length was identical to S_1 for both R1 and R2.

No difference between the groups in R1 and R2 was observed during S_2 where day lengths were 13 hr 20 min in the decreasing phase in R1 and 10 hr 40 min in the increasing phase in R2. During S_3, plasma GH concentrations in animals of the two groups submitted to R2 (where day length increased up to 13 hr 20 min) were also above those found in animals submitted to R1 (where the day length was decreasing to 10 hr 40 min).

Data obtained during S_4, S_5, and S_6 were very similar to those described during S_1, S_2, and S_3, respectively, except that the results were inverted with respect to R1 and R2. Means of the areas under the curves depicted by GH values of the 13 bleedings were calculated for each group at different sequences. They were then subjected to analysis of variance (Table I). There was no significant interaction among the three experimental factors (light regime, sequence, and protein diet). A significant effect of day length (sequence) ($P < 0.01$) and protein diet ($P < 0.05$) on plasma GH concentrations was seen but there was no effect of the light regime (Table I).

Since there was no effect of the light regime,

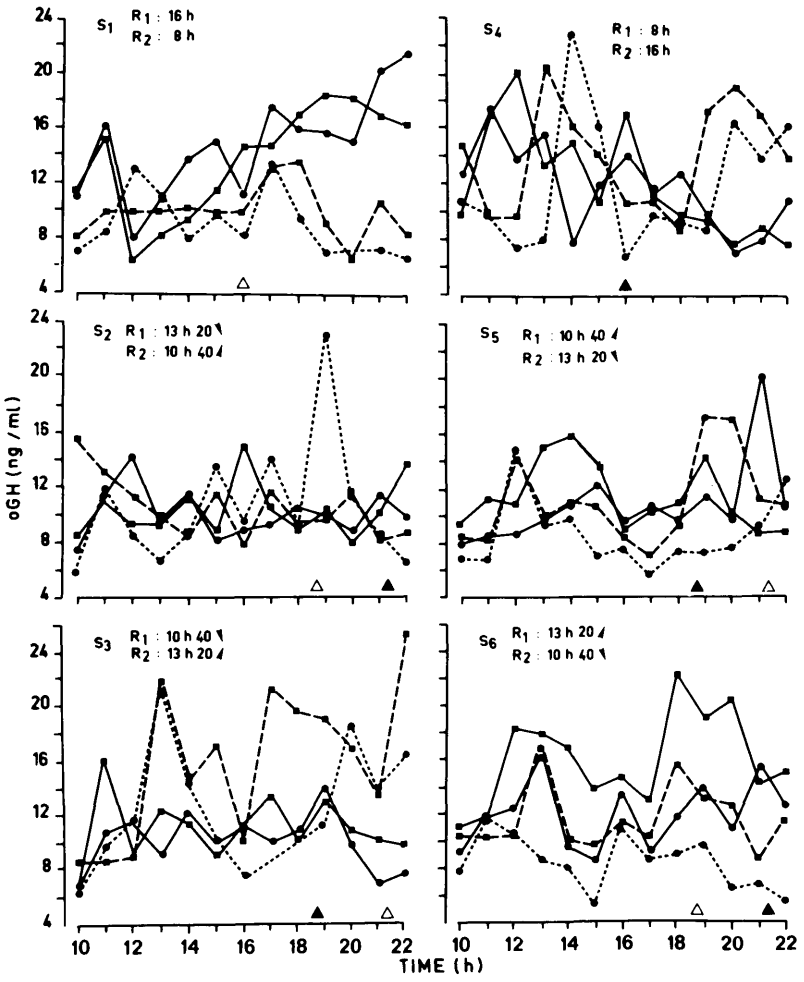

FIG. 2. Profiles of plasma GH concentrations in rams during the different sequences of blood sampling. Day length in each light regime group is given for each sequence (S_1–S_6). Arrows indicate whether the profile is during increasing (↗) or decreasing (↘) day lengths. Extinction of the light in R1 (▲) and R2 (△). Groups: R1H (● — ●), R1L (■ — ■), R2H (● - - - ●), R2L (■ - - - ■) (Mean, $n = 6$)

data in R1 and R2 were pooled as depicted in Fig. 3. In animals receiving the high protein diet, plasma GH concentrations significantly decreased from 13.0 ± 1.2 to 9.1 ± 1.0 ng × h × ml^{-1} ($P < 0.05$) when day lengths were reduced from 16 hr to 13 hr 20 min. GH concentrations then increased when day lengths increased from 8 to 16 hr. In animals fed a low protein diet, plasma GH concentrations were increased significantly ($P < 0.05$) when the day length was augmented from 10 hr 40 min (11.0 ± 1.0 ng × h × ml^{-1}) to 13 hr 20 min (15.6 ± 1.6 ng × h × ml^{-1}). Maximum GH concentrations were observed in H and L groups when the animals were under 16 hr (13.0 ± 1.2 ng × h × ml^{-1}) and 13 hr 20 min (15.6 ± 1.6 ng × h × ml^{-1}) of light daily in increasing photoperiods, respectively (Fig. 3). Minimum GH concentrations were about 9 ng × h × ml^{-1} in H groups and 10.5 ng × h × ml^{-1} in L groups when day lengths were 13 hr 20 min in decreasing photoperiods. The difference between H and L groups was significant ($P < 0.05$) only when the day length was 13 hr 20 min in increasing photoperiods.

Discussion. It is clear that overall plasma GH concentrations in sheep are simultaneously influenced by photoperiod and pro-

TABLE I. MULTIFACTORIAL ANALYSIS OF VARIANCE OF THE INFLUENCE OF LIGHT REGIME, DAY LENGTH, AND PROTEIN DIET ON GH SECRETION

	D.F.[a]	V.R.[b]	S.S.[c]
Light regime (R)	1	1.78	NS[d]
Sequence or day length (D)	5	4.18	$P < 0.01$
Protein diet (P)	1	5.96	$P < 0.05$
R × D	5	0.33	NS
R × P	1	1.32	NS
D × P	5	0.65	NS
R × D × P	5	0.50	NS
Residual	120	—	

[a] Degrees of freedom.
[b] Variance ratio expressed as residual mean square.
[c] Statistical significance.
[d] Not statistically significant.

tein level in the diet. We do not know exactly which of the GH secretory parameters were affected because hourly blood sampling does not allow a precise determination of baseline levels, amplitude, and number of episodic discharges (11). On the other hand, time trends could not demonstrate any effect of repeated venipunctures on plasma GH concentrations in sheep (8, 10). Therefore, the apparent increase in GH release within the sampling period did not reflect a response to consecutive blood sampling.

Reversed photoperiodic regimes produced parallel changes in GH secretory profiles. This indicates that within each group, changes in plasma GH concentrations were caused by day length changes. In both protein diet groups, GH concentrations were maximum at approximately the same day length, 13 hr 20 min. These results agree with our previous findings when we compared plasma GH changes during either long vs short photoperiods (8) or 6-month vs annual light regimes (9). In all the animals, there was a similar trend of increasing GH secretion as day length was increased, whatever the protein diet.

Plasma GH concentrations were consistently higher in rams fed the low protein diet. This agrees with the elevation of GH secretion previously reported in fasted sheep by several investigators (2–4). High circulating GH concentrations have also been found during nutritional deficits in pigs (7), chickens (12), and heifers (13). However, other investigators did not observe any stimulating effect of fasting upon GH secretion in sheep and steers (14, 15). One reason for such a discrepancy may be that the fasting did not last long enough in these last experiments. Indeed, circulating FFA levels increase (15) at the early stage of fasting and this may inhibit GH release (16).

Several parameters such as the inhibition of a neural reflex (17) or rumen distention (18) were reported to influence GH secretion. They may well be involved in the mediation of the effects of fasting. Our data support the concept that chronic protein deprivation acts as a stimulus to GH release in sheep as previously shown in humans, pigs, and chickens (6, 7, 12). This finding seems to be in conflict with the fact that infusion of certain amino acids, such as arginine or leucine, also stimulates GH release (19, 20); however, different regulatory mechanisms may be involved.

Actually, high GH secretion in response to low protein diet may represent an emergency mechanism of regulation to improve nitrogen accretion (21, 22). Thus, it would compensate somewhat for the negative effect of nutritional deficit on growth. This corresponds to the previous definition of homeostasis (1). In contrast, the increase in GH release during infusion of amino acids or during increasing day lengths may serve a physiological role in stimulating the incorporation of amino acids into proteins (20). This kind of phenomenon supports some patterns of growth and corresponds to the definition of homeorhesis (1).

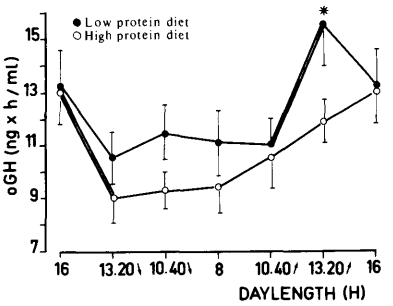

FIG. 3. Plasma concentrations of growth hormone in rams receiving high or low protein diets throughout a semestral light regime. Results show pooled data from R1 and R2 as a function of day length. The arrows indicate whether it is during increasing (↗) or decreasing (↘) day lengths. The double line between two points indicates significant differences between those two points ($P < 0.05$). * Significant difference between the two groups ($P < 0.05$). (Mean ± SEM, $n = 12$)

It has been postulated that a faster growth rate is associated with high concentrations of PRL and low concentrations of GH (3, 13). This was suggested by the fact that animals fed *ad libitum* have higher serum concentrations of PRL and lower GH than animals receiving a restricted diet. In this case, plasma GH concentrations were obviously negatively correlated to weight gain. However, homeostatic factors are not involved in the regulation of growth (1). Thus, the variations in GH secretion in response to these factors cannot be correlated to body growth. This is the case when there is a modification of the nutritional regime (3, 13) or physiological state, as during gestation or lactation (23, 24). Conversely, a positive correlation was found between overall mean GH and weight gain in rams selected for growth rate (25) and according to photoperiodic variations (9). This suggests a link between GH secretion and the pattern of growth in response to homeorhetic factors.

Protein deficiency and increasing day length lead to additive effects on GH concentrations. Indeed, plasma GH concentrations underwent photoperiodic variations in the animals receiving a low protein diet although GH secretion was already stimulated by the restriction of proteins. Interestingly, GH concentrations rose rapidly during increasing day lengths but began to decrease before the end of the increasing day lengths in animals fed the low protein diet. In contrast, it increased slowly in increasing day lengths and then fell rapidly during decreasing day lengths in animals receiving the high protein diet. The reason for such a difference between the two groups of rams is unknown. However, it indicates that homeostatic and homeorhetic factors, which involve different physiological processes (1), have independent effects on GH secretion.

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