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Secretory profiles and production rate of growth hormone in ruminant lambs

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Summary. Secretory profiles and production rates of growth hormone (GH) were determined in 6 ruminant lambs during winter. The mean GH concentrations (3.78 ± 2.17 ng/ml) calculated were based upon blood sampling obtained every 3 min using a withdrawal pump. Body clearance (0.162 ± 0.031 l/h/kg) was calculated from bolus intravenous oGH administration. The data were analysed by non-linear regression analysis ; a bicompartmental model was selected to describe the data. Production rate was 14.6 ± 7.98 µg/kg/24 h. It has been emphasized that the experimental design used gave an accurate estimate of GH production rate.

Introduction.

Production rates of growth hormone (GH) have been evaluated in several species, including ruminants (Yousef *et al.*, 1969 ; Wallace *et al.*, 1972, 1973 ; Trenkle, 1971, 1976, 1977 ; Gopinath and Kitts, 1984). Accurate evaluation of 24-hour GH production requires a valid estimate of two parameters, body clearance (Cl_B) and mean plasma concentration. The experimental design and mathematical treatment of data to estimate clearance have been well established (Tait and Burnstein, 1964 ; Gibaldi and Perrier, 1975). Nevertheless, a review of the literature indicates some inconsistencies in the calculation of GH clearance, especially when single administration and compartmental analysis are used. Some of the most frequent errors are the confusion between distribution and elimination processes, an excessively limited number of data points, and the inappropriate use of linear regression to fit data to a non-linear equation. The correct evaluation of mean GH concentration also merits attention. The episodic nature of GH secretion has been identified in different species including sheep (Davis and Borger, 1974 ; Davis *et al.*, 1977). In addition, relationships between other physiological events and GH secretion have been established. A close association has been demonstrated between the occurrence of the first episode of slow-ware sleep and the highest peak of plasma GH concentration in man (Rubin *et al.*, 1974 ; Weitzman 1976). Consequently, correct overall evaluation of mean plasma GH concentrations for a 24-hour period cannot be carried out on a few samples

obtained just before GH administration ; this evaluation requires a sampling design which will quantify plasma concentrations throughout the period under study.

The aim of the present work was to evaluate the production rate of GH in ruminant lambs.

Material and methods.

Six ruminant lambs (2 males and 4 females) of the Merinos d'Arles breed were used. They were 4 to 5 months old and weighed 30.8 ± 2.99 kg on the day of continuous sampling. One month before bleeding, the lambs were put in individual cages in the same room and allowed to adapt to the experimental conditions. They were fed with hay *ad libitum* and received about 400 g of high-energy pelleted ration in a single meal at 7:30 a.m. Clean tap water was provided *ad libitum*. Natural photoperiod (January) was respected.

Twenty-four hours before the sampling session, indwelling jugular catheters containing heparin were inserted into both jugular veins. The next day blood samples were obtained continuously using a withdrawal pump connected to one of the catheters. Every 3 min a sample of about 1 ml was collected and kept in a fraction collector for a maximal time of 2 h. Thereafter, the blood was centrifuged and the plasma stored at − 20 °C until assay. In order to avoid coagulation, heparin (Heparine Roche, Neuilly, France) was injected regularly *via* the other catheter ; a total dose of about 100 000 IU was used during the 24-hour sampling period. The total amount of blood removed was about 450 ml, *i.e.* approximately 15 % of the calculated blood volume. About one month later, 6 control blood samples were obtained at 10-min intervals. Ovine growth hormone (oGH) (NIH GH5, 1 IU/mg) was then administered at a rate of 18.46 ± 10.17 µg/kg *via* an indwelling catheter in the right jugular vein. Just before administration, oGH was dissolved in a bovine albumin solution. Blood samples (5 ml) were obtained from an indwelling catheter previously inserted in the left jugular vein at 1, 2, 4, 8, 15, 30, 45, 60, 90 min and 2, 3, 4, and 5 h after oGH administration. Blood samples were collected into heparinized tubes and rapidly centrifuged. Plasma was stored at − 20 °C until assay. oGH was measured by specific radioimmunoassay using a double antibody separation method. Reagents for the oGH assay were supplied by the National Hormone and Pituitary Program (NIADDK, Bethesda), except for the second antibody (sheep/anti-rabbit gammaglobulin) which was prepared in our laboratory. Assay sensitivity was 0.5 ng/ml and intraassay variation was 5 %. All the samples from the same experiment were run on the same assay at the appropriate dilution.

Data from the intravenous study were subjected to both compartmental and non-compartmental analysis. Kinetic analysis was done with a desk computer (Apple II plus), using a program for non-linear regression analysis (Koeppe and Hamann, 1980). Plasma concentrations were fitted to the general polyexponential equation 1 :

$$Cp = C_0 + \sum_{i=1}^{n} Yi\, e^{-\lambda it}.$$

In equation 1 Cp represents plasma concentration at time ti ; Co the control GH concentration ; Y_i the coefficient of the i^{th} exponential term and λi the exponent of the i^{th} exponential term. Initial estimates were obtained using the linear regression method. For non-linear regression analysis, the data points were weighted according to equation 2 :

$$W_i = 1/\hat{y}_i^2$$

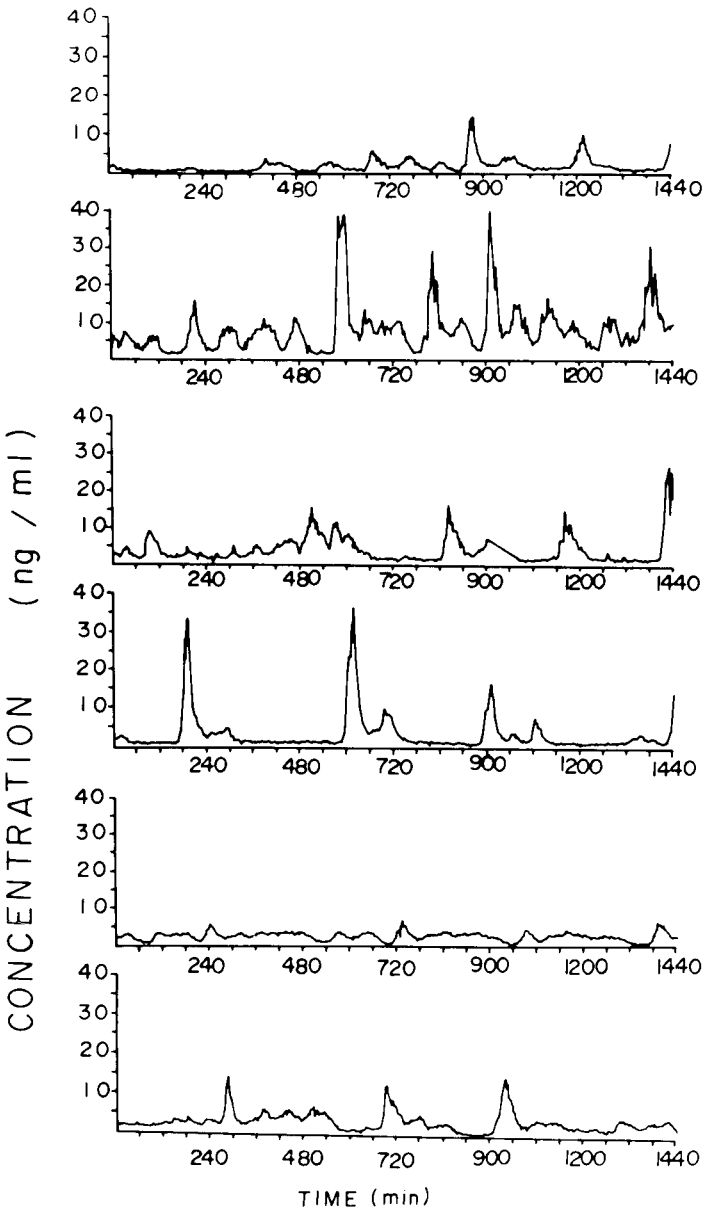

FIG. 1. — *Individual 24-hour secretory profiles of GH in six ruminant lambs.*

where Wi is the weight and Y_i the fitted value of the i^{th} observation. The number of exponents (n) needed for each set of data was determined by the application of Akaike's information criterion (Yamaoka *et al.*, 1978). The area under the plasma concentration-time curve (AUC (IV)) was calculated using the linear trapezoidal rule. Basal GH concentration was taken into account. The area under the plasma curve for 24-hour continuous sampling (AUC (0-24 h)) was calculated by a linear trapezoidal rule. The daily production rate of oGH (PR) was estimated by equation 3 :

$$PR = \frac{AUC\ (0 - 24\ h)}{AUC\ (IV)} \cdot \text{dose IV}\ .$$

Others parameters, such as microconstants of transfer, volume of distribution, volume of central compartment, were calculated according to classic equations (Gibaldi and Perrier, 1975). Mean residence time, *i.e.* the mean time needed for the intact oGH molecule to transit through the body (Riegelman and Collier, 1980), was calculated using a program described by Chan and Wnuck (1983).

Results.

All the sheep supported the experimental conditions and less than 3 % of the samples were lost due to technical difficulties. Figure 1 shows the individual GH secretion profiles of the 6 lambs. Visual inspection clearly shows that GH secretion was not continuous but episodic. Depending on the lamb, plasma concentration fluctuated from a basal level (0.5 to 1 ng/ml) to peak values (8 to 40 ng/ml). The mean area under the plasma GH profile was

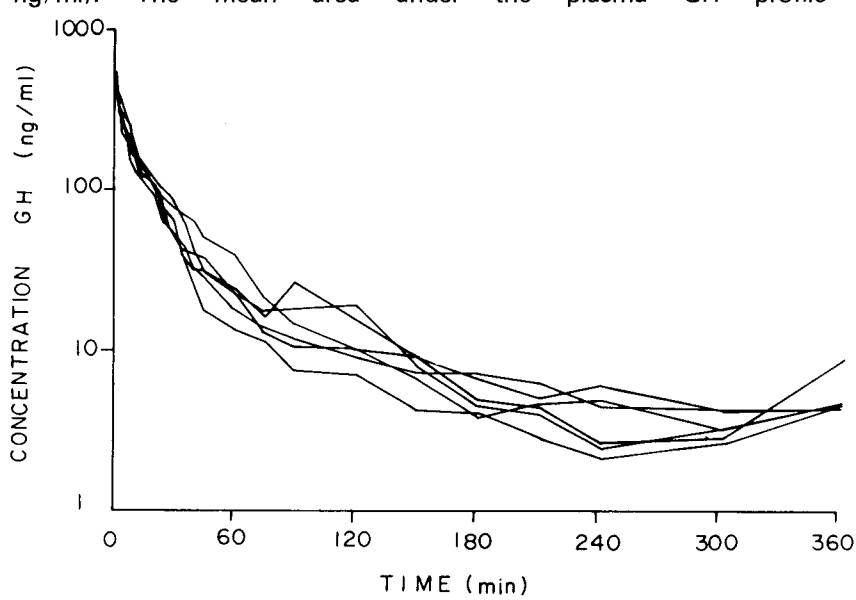

FIG. 2. — *Plasma concentration of GH in six lambs after intravenous administration of oGH at a dose of 18.46 ± 10.17* µg/kg.

TABLE 1

Production rate and mean concentration of GH in six ruminants lambs (AUC : area under the plasma curve).

Parameter, unit	A	B	C	D	E	F	Mean ± SD
AUC (0-24 h) (ng.mn/ml)	2 717.75	11 477.85	5 726.7	4 480.1	3 640.7	4 593.8	5 439.65 ± 3 124.67
Production rate (µg/kg/24 h)	6.73	28.65	18.70	8.93	12.48	12.13	14.60 ± 7.98
Mean daily concentration (ng/ml)	1.89	7.97	3.98	3.11	2.53	3.19	3.78 ± 2.17

5439 ± 3124 ng.min/ml, corresponding to an overall mean concentrations of 3.78 ± 2.17 ng/ml. Individual values are presented in table 1. The plasma concentration (ng/ml) of GH after intravenous administration of oGH is shown for the 6 lambs in figure 2. According to Akaike's information criterion, a biexponential equation (eq. 4) must be selected to fit the data :

$$C_p = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t}.$$

Consequently, a 2-open compartment model with elimination form the central comportment can be used to described the disappearance of GH from the plasma. Individual parameters for the 6 lambs are expressed in table 2 ; the mean elimination half-time (t1/2 λ2, *i.e.* 0.693/λ2) was 43.50 ± 11.15 min (harmonic mean and SD) and body clearance (Cl_B = dose/AUC (IV)) was 0.162 ± 0.03 l/h/kg. According to non-compartmental analysis, mean residence time was 36.46 ± 7.09 min. Daily production was 14.60 ± 7.98 µg/kg (range value : 6.73-28.65 µg/kg). The correlation coefficient between the mean 24-hour concentration and production rate was highly significant ($r = 0.95$; $P < 0.01$). In contrast, the correlation between plasma clearance and mean 24-hour concentration was not significant ($r = 0.12$; $P < 0.05$).

TABLE 2

Kinetic parameters describing the disposition of GH after an intravenous administration of oGH in six ruminant lambs.

Parameter, unit	A	B	C	D	E	F	Mean ± SD
Y_1 (ng/ml)	348.6	346.7	356.5	412.5	321.5	395.3	363.5 ± 33.85
Y_2 (ng/ml)	110.00	54.24	22.17	68.82	18.42	43.83	52.91 ± 33.80
Y_0 (ng/ml)	4.61	1.03	3.64	0.705	2.36	2.21	2.42 ± 1.50
λ_1 (min^{-1})	0.119	0.102	0.0845	0.0974	0.0793	0.0930	0.0959 ± 0.0140
λ_2 (min^{-1})	0.0231	0.0132	0.0124	0.0142	0.0143	0.0166	0.0156 ± 0.0039
t1/2, λ_2 (mn)	30.00	52.35	55.88	48.73	43.41	41.59	43.50 ± 11.15*
MRT (mn)	32.36	45.92	35.41	44.38	29.00	31.36	36.40 ± 7.09
Cl_B (1/h/kg)	0.149	0.150	0.196	0.120	0.205	0.158	0.162 ± 0.0310
$Vd_{(area)}$ 1/kg	0.179	0.180	0.249	0.135	0.227	0.410	0.23 ± 0.097
K_{10} (min^{-1})	0.596	0.0534	0.0631	0.0531	0.0636	0.0638	0.0594 ± 0.0050
V_c (1/kg)	0.0428	0.0439	0.0533	0.0399	0.0533	0.0410	0.0457 ± 0.0060

Y_1 and Y_2 are preexponential constants and λ_1 and λ_2 are the exponential constants for the biexponential equation describing the plasma level curve ; Co is the basal control concentration time ; $t1/2\lambda_2$ is the plasma half-time ; MRT : mean residence time ; Cl_B : body clearance ; Vd (area) : volume of distribution ; K_{10} : first order rate constant of elimination from the central compartment ; V_c : volume of the central compartment ; * : harmonic mean.

Discussion.

The daily production rate of GH (15 µg/kg) calculated for ruminant lambs in the present experiment is difficult to compare with previously published values for ruminants. Indeed, this is the first estimate of GH production taking into account the entire 24-hour GH profile. In newborn lambs, body clearance was evaluated as

0.18 l/kg/h, a value very close to that found in our ruminant lambs, and daily production rate was estimated as about 34 μg/kg (Wallace *et al.*, 1973). A higher clearance has been calculated by Trenkle (1976) in fed adult sheep (0.373 1/kg/h) but the daily production rate of 19 μg/kg, based on the measurement of basal concentration obtained before GH administration, was close to our estimate. In this respect it must be noted that, considering the basal levels of plasma GH evaluated immediately before the clearance study (6 samples at 10-min intervals), our estimate of daily production should be significantly lower than the true value (9.8 ± 6.24 μg/kg *vs* 14.6 ± 7.98 μg/kg). A lower daily production rate (4 μg/kg) has been calculated in adult sheep by Wallace *et al.* (1972). This is due to both a low clearance value (0.08 1/kg/h) and a low basal GH concentration (2 ng/ml).

In our experimental conditions, production rate was highly correlated with mean plasma concentration but not with clearance. This suggests that plasma concentrations are indicative of secretion rate and that plasma concentration differences must be explained in terms of pituitary activity rather than in terms of GH metabolism. Such a conclusion has been drawn by Trenkle (1971) studying cattle. One of the prerequistes for accurate estimation of GH production rate is the similarity between endogenous GH and that administered to evaluate clearance. Different molecular forms of GH have been demonstrated in man, the 22 000-dalton form being the dominant one secreted during a secretion episode, while GH fragments may become the dominant proportion of total immunoreactivity under basal conditions (Baumann *et al.*, 1985a). As body clearance varies greatly among different GH forms (Baumann *et al.*, 1985b), the validity of the current evaluation of the GH production rate in man must be questioned. Similar information is lacking in sheep.

In *conclusion*, if the oGH administered is metabolically equivalent to spontaneously secreted GH, the present estimate of GH production rate for ruminant lambs in winter can be considered as accurate.

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Résumé. *Profil de sécrétion et taux de production de l'hormone de croissance chez l'agneau ruminant.*

Les profils de sécrétion et les taux de production de l'hormone de croissance (GH) ont été établis chez 6 agneaux ruminants pendant la période d'hiver. Les concentrations moyennes de GH(3,78 ± 2,17 ng/ml) ont été calculées à partir de prélèvements sanguins effectués automatiquement toutes les 3 minutes. La clairance corporelle de la GH(0,162 ± 0,03 l/h/kg) a été évaluée par une administration intraveineuse d'oGH. L'analyse des données a été réalisée par régression non linéaire, un modèle bicompartimental ayant été sélectionné pour décrire les données. Le taux de production calculé est de 14,6 ± 7,98 μg/kg/24 h. L'importance du protocole expérimental retenu dans l'estimation correcte du taux de production de la GH est discutée.

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