Aust. J. Biol. Sci., 1988, **41**, 177–87

Effects of Growth Hormone Administration on Wool Growth in Merino Sheep

P. C. Wynn,[AB] *A. L. C. Wallace,*[B] *A. C. Kirby*[C] *and E. F. Annison*[A]

[A]Department of Animal Husbandry, Sydney University, Camden, N.S.W. 2570.
[B]Division of Animal Production, CSIRO, P.O. Box 239, Blacktown, N.S.W. 2148.
[C]Department of Biometry, Sydney University, N.S.W. 2006.

Abstract

The effects of daily administration of 10 mg of highly purified ovine growth hormone (GH) for a period of 4 weeks on wool growth have been measured in 12 Merino ewes fed either a calculated maintenance energy intake or 1·6 times this amount (six on each ration). Concentrations of hormones, glucose, urea, α-amino N and amino acids in the blood were monitored and faeces and urine collected for measurement of nitrogen balance.

Wool growth rate decreased by 20% during the 4 weeks of GH treatment in sheep fed the high energy diet, largely because of reduced wool fibre diameter. This was followed by restoration of normal growth and then an increase of up to 20% above control levels, a response which persisted for 12 weeks following cessation of GH administration, and which was due to increases in both fibre length and diameter. GH administration caused marked increases in plasma concentrations of GH, insulin and somatomedin C, glucose and free fatty acids, all of which returned to basal levels following cessation of GH administration. No consistent changes in plasma concentration of T_3, T_4, cortisol, prolactin or α amino N were detected. Plasma urea and methionine levels decreased during GH treatment and returned to, or were raised above, basal levels after the GH treatment period. GH injection also resulted in a net retention of N during treatment, followed by a transient period of net N loss.

The GH-induced changes in wool growth may be caused by a change in the partitioning of amino acids between the muscle mass and the skin. No other contributing factor(s) were identified.

Introduction

In view of its known anabolic functions, considerable interest has been generated in the use of GH for improving efficiency of animal production by the increased availability of relatively inexpensive synthetic hormone. The present study further investigates its use as a possible stimulant to wool growth. Initial observations using daily injections of crude pituitary extracts showed that wool growth decreased by 17% during the second fortnightly treatment period, but that, following cessation of injections, there was a protracted period of stimulation of wool growth of up to 30% above basal levels for the subsequent 24 weeks (Ferguson 1954). Similar results were obtained by Ferguson *et al.* (1965) using crude extracts of sheep and ox pituitaries. Wheatley *et al.* (1966) confirmed that the causative agent was GH by using a highly purified ovine GH preparation to obtain the same response. The cause of this response in wool growth is not yet known.

Since these early reports, radioimmunoassays have been developed for measuring circulating levels of ovine hormones. Moreover, it is now recognized that many of the physiological actions of GH are mediated by insulin-like anabolic peptides, the somatomedins (Van Wyk *et al.* 1974; Phillips and Vassilopoulou-Sellin 1980).

The present study used radioimmunoassays for GH, insulin, thyroxine, prolactin, and cortisol and a bioassay for somatomedin C to investigate changes in endocrine status which may explain the wool growth responses to long-term ovine GH administration. Changes in metabolic status have also been monitored.

0004-9417/88/020177$03.00

Materials and Methods

Twelve 3-year-old Merino ewes of similar body weight (34·3 ± 2·8 kg) were housed in metabolism crates at 23 ± 1°C under constant lighting and fed once daily a maintenance level (low plane, LP) or 1·6 times this amount (high plane, HP) of a pelleted concentrate ration [Feedlot No. 5, Allied Feeds Ltd, Australia; 90% wheat, sorghum, oats and barley; metabolizable energy, ME, 10·5 MJ kg^{-1}; crude protein (N × 6·25), 17·4%]. Animals were fed for a period of 12 weeks before commencement of the experiment to ensure stability of wool growth rate. The amount fed was calculated according to Agricultural Research Council standards (Anon. 1975). Indwelling catheters were inserted into both jugular veins 48 h before blood sampling. Blood samples (8 ml), taken daily before feeding, were collected in heparinized tubes (10 U ml^{-1} of blood), cooled in ice and centrifuged at 2°C and the plasma separated.

Plasma samples (10 ml) were collected weekly for amino-acid analysis. Protein was removed with 500 mg sulfosalicylic acid following addition of a standard (25 μl ml^{-1} of 2·5 mM DL-norleucine); the supernatant was stored at −20°C. After 4 days of blood sampling, three sheep from each of the high- and low-plane nutritional groups received a daily subcutaneous injection of GH in saline (10 mg in 2 ml) for 28 days whereas the remaining three sheep from each group received saline alone. Daily blood sampling continued for 14 days after cessation of GH treatment and thereafter twice weekly for a further 28 days.

Nitrogen Balance

Urine and faeces were collected each 48 h for 14 days prior to the GH treatment period and for 28 days post-treatment. Urine was collected in 10 ml 5N HCl and faeces dried at 60°C, and both were subsampled and stored at −20°C prior to analysis. N content was determined by a micro Kjeldahl procedure or by the use of an automatic N analyser (A-S.N. Foss, Denmark). N balance was calculated by subtracting total urinary and faecal N from N consumed, with an appropriate adjustment for N collected during washing of cages (1·47 ± 0·18%) and is expressed as the change relative to the pretreatment period.

Wool Growth

Wool growth was measured at 14-day intervals by clipping tattooed areas of *c.* 100 cm^2 on the midside and shoulder region of the right side of each animal.

Wool samples were conditioned at 20°C and 65% humidity for 72 h, placed in a 20-μm nylon-mesh filter and washed successively with a hot solution (60°C) of 0·3% (v/v) non-ionic detergent (Nonidet P40; Shell Chemicals, Australia), 0·2% Na_2SO_4 (w/v) six times, warm water six times and absolute alcohol twice. Samples were again conditioned for 72 h and weighed, and wool weight expressed as clean dry wool.

In order to determine changes in fibre dimensions by autoradiography, L-^{35}S-cystine (Amersham) was dissolved in 1 M HCl (1·8 MBq in 0·4 ml per injection) and administered intravenously at 7-day intervals for 14 days before, and for 42 days after, the cessation of GH treatment. At the completion of experimentation, a total of 40 wool fibres was plucked from five representative sites on the left side of each animal.

The distance along wool fibres between individual bands of [^{35}S]cystine incorporation was measured using an image analyser (Ernst Leitz, Gmbh, Wexlar, West Germany); the diameter at each point was calculated from the fibre length and diameter measurements, assuming the fibres were cylindrical (Reis *et al.* 1983).

GH

The method of preparation has been previously described (Wallace 1961; Wallace and Ferguson 1963). Biological potency was measured in a 4-point hypophysectomized rat tibial cartilage assay (n = 4 for each point) by the method of Evans *et al.* (1943) and found to be 1·4 i.u. mg^{-1} when compared with the standard NIH-GH-S7.

Radioimmunoassay of Plasma Hormones

GH was measured by the method of Wallace and Bassett (1970) using powdered talc to separate bound and free hormone (Rosselin *et al.* 1966). The sensitivity determined by the method of Burger *et al.* (1972) was 1 μg l^{-1}. The intra-assay coefficient of variation (CV) varied from 1·8 to 3·8% (n = 10) for the range of concentrations measured in the assay, and the inter-assay CV was 14·8% (n = 17).

Insulin was determined as described by Bassett and Thorburn (1971). Sensitivity was 2 m U l^{-1}, intra-assay CV was 2·5–4·0% (n = 10) and inter-assay CV was 13·9% (n = 15).

Somatomedin C was measured using the rat weanling cartilage bioassay of Yde (1968) as modified by Stuart *et al.* (1976). A pool of normal Merino wether plasma was used as a reference standard with a designated concentration of 1·0 U ml^{-1}. Plasma somatomedin concentrations were corrected for differences in plasma inorganic sulfate levels which were determined by the method of Henry *et al.* (1974).

T_3 was determined by the method of Eastman *et al.* (1975). The sensitivity of the assay was 0·02 μg l^{-1}, intra-assay CV was 1·9–4·5% (n = 10) and inter-assay CV was 14·2% (n = 11).

T_4 was measured by the method of Wallace *et al.* (1978). The sensitivity was 0·13 μg l^{-1}, intra-assay CV was 1·1–3·7% (n = 10) and inter-assay CV was 14·2% (n = 11).

Cortisol was determined by the method of Farmer and Pierce (1974). The sensitivity of the assay was 0·32 μg l^{-1}, intra-assay CV was 3·3–8·1% (n = 6) and inter-assay CV was 14·5% (n = 8).

Prolactin was measured as previously described (Gow *et al.* 1983) with a sensitivity of 1 μg l^{-1}, intra-assay CV of 1·6–4·3% (n = 3) and inter-assay CV of 7% (n = 12).

Metabolites in Plasma

Glucose was measured by the glucose oxidase method of Huggett and Nixon (1957), free fatty acids by Annison's (1960) modification of the method of Dole (1956), urea by the technique of Chaney and Marbach (1962), and α-amino nitrogen using the method of Oddy (1974). Amino acids in plasma were analysed by ion-exchange chromatography using a Dionex D-300 analyser (Dionex, U.S.A.).

Statistical Analyses

Data from this experiment were non-orthogonal, as analyses were not performed on every sample and two sheep in the HP group were withdrawn midway through the GH treatment period as they consumed less than 90% of their ration. Therefore, means are given with standard deviations calculated from the original data as the number of observations differs between variables. An analysis of variance was used on a randomized complete-block split-plot-in-time design (Searle 1971); results were divided into three treatment periods, namely pre-treatment, during GH administration, and post-treatment. 'Conservative' tabular values of the f-ratio (Gill and Hafs 1971) were used for all time-by-treatment effects, in view of the correlation between errors induced by repeated measurements from the same animals.

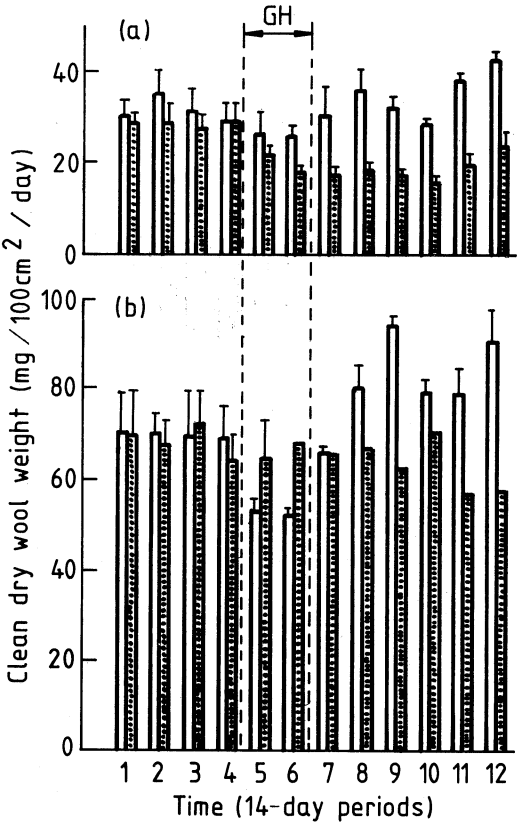

Fig. 1. Mid-side and shoulder patch wool growth (mean ± s.e.) for sheep fed (*a*) a calculated maintenance energy requirement or (*b*) 1·6 × maintenance, and receiving daily intramuscular injections of GH (open bars) or saline (stippled bars).

Results

Wool Growth

Wool growth rate, as measured by the patch technique, changed significantly ($P < 0.01$) with time in response to GH treatment (Fig. 1). Although wool growth did not change markedly in the LP group during the period of GH administration (Fig. 1*a*), it was depressed by 20% in the HP group (Fig. 1*b*). In both treated groups, wool growth returned to pre-treatment rates within 14 days of cessation of GH administration, and rose to 20% above this level for the subsequent 10 weeks (Fig. 1). Sheep in the LP group received 13% more GH per unit bodyweight than the HP group due to differences in liveweight at the commencement of the GH treatment (Table 3). Wool yield, calculated from the ratio of clean to greasy wool weight, remained constant in all groups (70–80%) throughout the studies.

Analyses of fibre length and diameter determined by autoradiography demonstrated that the ratio of fibre length : fibre diameter varied significantly with time ($P < 0.05$; Table 1). Although there was a marked interaction between GH treatment and energy intake over time,

Table 1. Changes in wool fibre length, diameter, length : diameter ratio and fibre volume (mean ± s.d.) as determined by autoradiography for a 7-day period during: (1) the pre-treatment period, (2) the first 14-day period of growth hormone (GH) injections, (3) the second 14-day period of GH injections and (4) the post-treatment period

	Period	High energy intake		Low energy intake	
		10 mg GH day^{-1}	Control	10 mg GH day^{-1}	Control
Fibre length (μm)	1	362 ± 47	322 ± 43	301 ± 43	305 ± 58
	2	364 ± 50	339	307 ± 47	302 ± 61
	3	370 ± 51	335	298 ± 50	299 ± 60
	4	396 ± 45	337	321 ± 48	303 ± 60
Fibre diameter (μm)	1	20·9 ± 3·4	19·8 ± 3·8	19·2 ± 4·9	18·9 ± 5·4
	2	19·5 ± 3·2	19·6	17·8 ± 4·3	18·2 ± 5·5
	3	20·5 ± 3·2	18·7	18·0 ± 4·4	17·9 ± 5·2
	4	21·7 ± 3·4	19·6	19·0 ± 5·4	17·3 ± 5·0
Fibre length: diameter ratio	1	17·7 ± 2·6	16·7 ± 3·3	16·6 ± 3·9	16·6 ± 2·7
	2	19·1 ± 2·9	17·9	18·0 ± 1·6	17·2 ± 2·9
	3	18·9 ± 2·6	17·8	17·4 ± 4·0	17·2 ± 3·0
	4	18·5 ± 2·8	17·6	17·6 ± 4·1	19·4 ± 3·6
Fibre volume growth ($\mu m^3 \times 10^{-3}$ day^{-1})	1	132 ± 56	104·6 ± 48·0	97·0 ± 63·6	101·5 ± 88·3
	2	116 ± 50	108	85 ± 52	94 ± 86
	3	123 ± 49	95	85 ± 56	89 ± 76
	4	158 ± 61	107	106 ± 82	88 ± 71

this was not significant using conservative f values. However, it was highly significant ($P < 0.01$) using normal f values and therefore it was probable that this was a true interaction over time between GH and energy intake. The increase in the ratio during GH treatment was due largely to a decrease in fibre diameter, whereas the post-treatment decrease in this ratio to control values was caused by the proportional increases in both fibre diameter and length resulting in the protracted stimulation of wool growth during this period. The changes in fibre length and diameter (Table 1) during and after GH administration were consistent with the changes in wool growth monitored by the patch technique, in both LP and HP groups (Fig. 1) and by estimation of fibre volumes (Table 1).

Endocrine Status

Circulating hormone levels observed during the different treatment periods of the experiment are given in Table 2. GH injection resulted in a significant treatment-by-time interaction ($P < 0.05$) in plasma GH levels measured 24 h after injection, although the increase was more

marked in the LP group. Levels of insulin and somatomedin C showed significant variation with time ($P < 0.01$) in response to both the level of energy intake and GH treatment (insulin, $P < 0.01$; somatomedin, $P < 0.05$). The magnitude of the response was greater in HP sheep. The elevated levels of GH, insulin and somatomedin C observed during the period of GH injection were not sustained after cessation of treatment.

Table 2. Changes in plasma hormone levels (mean ± s.d.) during: (1) the pre-treatment period, (2) the 28-day period of growth hormone (GH) injection and (3) the post-treatment period

	Period	High energy intake		Low energy intake	
		10 mg GH day^{-1}	Control	10 mg GH day^{-1}	Control
GH (μg l^{-1})	1	4·1 ± 2·2	4·0 ± 2·5	2·5 ± 2·5	4·9 ± 1·8
	2	5·1 ± 3·2	3·2	8·7 ± 4·6	4·2 ± 1·5
	3	3·2 ± 1·6	2·8	2·8 ± 2·8	3·7 ± 2·1
Insulin (mU l^{-1})	1	32 ± 19	20 ± 9	30 ± 18	25 ± 3
	2	132 ± 95	21	46 ± 42	32 ± 25
	3	40 ± 21	17	17 ± 9	20 ± 12
Somatomedin (U ml^{-1})	1	0·65 ± 0·11	0·53 ± 0·18	0·59 ± 0·08	0·51 ± 0·20
	2	1·46 ± 0·45	0·58	0·93 ± 0·32	0·61 ± 0·15
	3	0·86 ± 0·27	0·46	0·45 ± 0·15	0·53 ± 0·18
T_3 (μg l^{-1})	1	0·58 ± 0·14	0·64 ± 0·13	0·48 ± 0·15	0·37 ± 0·16
	2	0·59 ± 0·11	0·52	0·39 ± 0·11	0·35 ± 0·18
	3	0·56 ± 0·11	0·51	0·46 ± 0·14	0·34 ± 0·13
T_4 (μ l^{-1})	1	74 ± 20	97 ± 32	83 ± 18	56 ± 7
	2	54 ± 20	67	76 ± 13	50 ± 11
	3	52· ± 12	58	83 ± 16	47 ± 16
Molar T_4/T_3 ratio	1	116 ± 49	134 ± 49	164 ± 80	153 ± 78
	2	80 ± 30	117	186 ± 96	164 ± 118
	3	81 ± 18	101	164 ± 59	144 ± 80
Prolactin (μg l^{-1})	1	107 ± 30	97 ± 50	63 ± 31	43 ± 16
	2	127 ± 58	79	64 ± 38	55 ± 23
	3	126 ± 47	62	63 ± 47	47 ± 16
Cortisol (μ l^{-1})	1	5·6 ± 3·9	12·5 ± 12·2	4·0 ± 3·4	14·0 ± 6·1
	2	3·7 ± 2·3	13·4 ±	3·6 ± 2·3	13·0 ± 7·8
	3	1·6 ± 0·9	17·0 ±	2·5 ± 2·5	11·8 ± 9·4

Plasma prolactin levels were significantly greater ($P < 0.05$) in the HP group, and GH administration also resulted in a significant elevation ($P < 0.05$) during the treatment period. GH treatment *per se* did not influence circulating T_3 and T_4 levels nor the ratio of these hormones, but plasma T_3 levels were elevated in the high energy-intake groups ($P < 0.05$).

Circulating concentrations of cortisol were significantly lower ($P < 0.01$) in the GH-treated groups over all periods, although this difference was not attributable to GH administration as no interaction with treatment period was detected.

Metabolic Status

Observed changes in metabolites in the plasma are shown in Table 3. Both plasma glucose and free fatty acid levels varied significantly ($P < 0.01$) between treatment periods; GH injection tended to increase concentration, whereas the level of energy intake was without effect.

By contrast, plasma urea levels tended to decrease during GH administration and either increased above (HP group) or returned to basal levels (LP group) following cessation of GH administration.

Plasma amino-acid analyses were conducted on samples collected from three GH-treated animals, 2 from the HP group and one from the LP group at each of the treatment periods with the exception of the post-treatment period when only one sample was analysed (Table 4). Methionine concentrations decreased by 20% during GH administration in both energy

Table 3. Changes in plasma metabolite levels (mean ± s.d.) and liveweight (mean ± s.d.) during: (1) the pre-treatment period, (2) the 28-day period of growth hormone (GH) injection and (3) the post-treatment period

	Period	High energy intake		Low energy intake	
		10 mg GH day^{-1}	Control	10 mg GH day^{-1}	Control
Glucose (mg %)	1	61 ± 6·0	61 ± 9	65 ± 17	65 ± 20
	2	74 ± 8·0	65	74 ± 12	71 ± 11
	3	64 ± 5·9	63	62 ± 8	66 ± 11
Urea (mg %)	1	7·2 ± 2·4	6·1 ± 0·9	7·3 ± 1·9	7·3 ± 2·1
	2	6·2 ± 1·1	5·2	6·1 ± 1·6	7·3 ± 2·3
	3	8·6 ± 1·2	5·5	6·9 ± 2·1	7·4 ± 2·1
α-amino N (mg %)	1	4·8 ± 1·1	4·0 ± 1·3	4·2 ± 2·1	4·2 ± 1·2
	2	4·4 ± 1·2	5·7	3·8 ± 1·2	3·7 ± 0·8
	3	3·6 ± 1·2	5·5	3·6 ± 1·0	3·1 ± 0·4
Free fatty acids (μ Eq l^{-1})	1	798 ± 342	907 ± 436	1045 ± 254	812 ± 362
	2	1129 ± 505	998	1178 ± 421	652 ± 208
	3	663 ± 300	921	918 ± 224	445 ± 148
Liveweight (kg)	1	36·7 ± 7·2	36·8 ± 4·0	31·9 ± 2·4	31·7 ± 6·0
	2	40·0 ± 7·2	38·2	32·9 ± 3·5	32·5 ± 5·3
	3	40·3 ± 7·9	37·4	30·6 ± 1·2	30·7 ± 4·1

intake groups and rose to pre-treatment concentrations following cessation of GH injections. No consistent variation was observed in the plasma concentrations of other amino acids, although data were not obtained on lysine, histidine, aspartic acid, glutamic acid, proline, glycine and alanine.

Table 4. Plasma amino-acid concentrations (mol l^{-1}; mean ± s.e.m.) in sheep fed high and low energy intakes: (1) during the pre-treatment period, (2) after 14 days of growth hormone (GH) treatment, (3) after 28 days of GH treatment and (4) 14 days after cessation of GH administration

	Period			
	1	2	3	4
High plane				
Threonine	141 ± 4	146 ± 10	144 ± 7	143 ± 8
Serine	211 ± 33	319 ± 70	195 ± 33	175 ± 33
Cystine	77 ± 5	86 ± 6	72 ± 4	67 ± 7
Valine	145 ± 11	145 ± 11	142 ± 7	161 ± 10
Methionine	18 ± 2	15 ± 1	15 ± 1	19 ± 2
Isoleucine	59 ± 5	67 ± 6	67 ± 3	63 ± 1
Leucine	72 ± 5	73 ± 5	71 ± 4	74 ± 1
Tyrosine	66 ± 2	62 ± 4	58 ± 5	60 ± 4
Phenylalanine	53 ± 1	52 ± 3	51 ± 2	52 ± 4
Arginine	117 ± 12	146 ± 19	125 ± 10	201 ± 35
Low plane				
Threonine	88 ± 18	98 ± 6	107 ± 13	106
Serine	179 ± 6	207 ± -	261 ± 27	166
Cystine	71 ± 4	69 ± 8	64 ± 19	65
Valine	149 ± 4	125 ± 21	128 ± 18	104
Methionine	18 ± 1	14 ± 2	12 ± 1	15
Isoleucine	63 ± 2	77 ± 14	65 ± 4	73
Leucine	95 ± 1	67 ± 12	70 ± 11	63
Tyrosine	54 ± 3	54 ± 6	41 ± 1	51
Phenylalanine	46 ± 2	50 ± 8	47 ± 3	43
Arginine	135 ± 11	150 ± 30	122 ± 3	119

The mean 48-h N balances for fortnightly periods (Fig. 2) demonstrated a net retention of N during GH administration in both energy-intake groups relative to the pre-treatment period; the magnitude of this was greater in the HP group. This was followed by a loss of N in the subsequent 14-day period, which persisted for a further 14 days in LP sheep.

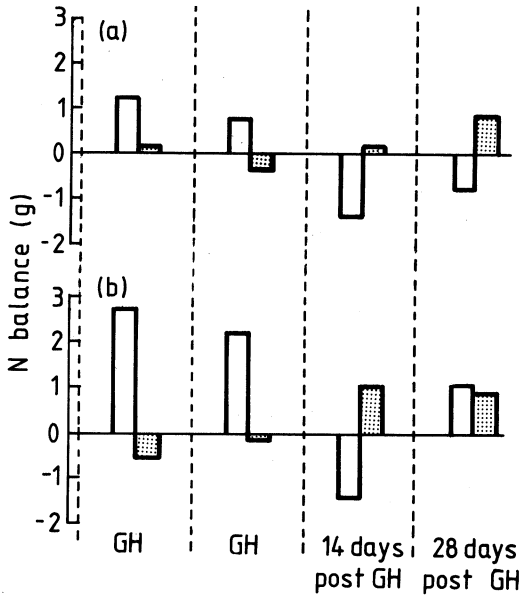

Fig. 2. The mean 48-hourly change in N balance relative to the pre-treatment period in GH-treated (open bars) and control (stippled bars) groups of animals fed (*a*) a calculated maintenance energy requirement or (*b*) 1·6 × maintenance. N balance figures are calculated by subtracting the figure for the pre-treatment period from the value for each treatment and post-treatment period.

The positive N balance during GH administration is reflected by a small increase in liveweight relative to control groups, which was greater in the HP group (Table 3).

Discussion

The depression in wool growth during treatment with GH and the subsequent protracted increase following the cessation of GH treatment is consistent with the findings of Ferguson *et al.* (1965), Wheatley *et al.* (1966) and Wallace (1979). However, while the observation that the decrease in wool growth resulted mainly from a decrease in fibre diameter agrees with the finding of Wallace (1979), the observation that the post-treatment surge in wool growth resulted from a proportional increase in both fibre length and diameter does not. Wallace (1979) found that only fibre diameter increased during the post-treatment period. The mean variation in the fibre length : diameter ratio during this period did not exceed 9% although individual fibres displayed considerably greater variability. This variation is comparable to the 15% reported by Downes and Sharry (1971) in response to a change in nutritional status, and suggests that this wool growth response may be due to increased nutrient availability.

Significant increases in clean wool weight in response to bovine GH injections (1 mg kg^{-1} every 10 or 15 days until weaning at 100 days) have been measured in growing lambs by Reklewska (1974), although this response may be explained by an increase in feed intake in the GH-treated animals. Nevertheless, the observation that feed intake remained stimulated for 15 weeks post-treatment suggested that GH had a persistent metabolic effect long after circulating GH concentrations had returned to basal levels, an effect which is consistent with the wool growth responses in the present study.

A similar study has been conducted with lambs by Johnsson *et al.* (1985). Wool growth was significantly stimulated by daily GH injections (0·1 mg kg^{-1} liveweight) for a period of 12 weeks; this response was decreased by the simultaneous administration of bromocryptine. In contrast to the report of Reklewska (1974), these results could not be explained by an increase in feed intake in the GH-treated animals, although greater efficiency of feed conversion in

GH-treated animals may in part account for the improved wool growth. The present results were derived from mature animals provided with a defined energy intake for an extended period to ensure stability of wool growth. Therefore, wool growth rates from the two studies are not comparable. However, the reported inhibitory effect of bromocryptine on GH-stimulated wool growth provides circumstantial evidence that the GH response may be mediated by somatomedin production which, in addition to GH, is modulated by circulating prolactin (Schalch *et al.* 1979) and insulin (Daughaday *et al.* 1976). In the study by Johnsson *et al.* (1985), plasma levels of both of these hormones were reduced by bromocryptine administration.

In searching for an explanation for the wool growth response to GH administration in the present study, we have measured the circulating concentrations of several hormones. Blood samples were collected 23·5 h after each daily GH injection. Changes in the pattern of hormone release in response to GH may have diminished by the time of blood sampling, as blood levels of GH returned to basal levels 12 h after a subcutaneous injection (unpublished data).

Comparison of plasma GH concentrations in the group fed the lower energy level with those in the sheep fed the higher level suggests that an inverse relationship may exist between nutritional status and clearance rate of GH from the circulation. It has been shown that GH is cleared more slowly from fasted animals compared with fed controls (Trenkle 1976). In addition, fasting is associated with an increase in both the mean concentration and instability of GH levels (Bassett 1974; Driver and Forbes 1978).

A number of studies have shown that measurement of plasma immunoreactivity may not accurately reflect circulating GH biological activity (Ellis and Grindeland 1974; Vodian and Nicoll 1977). This finding may result from aggregation of the exogenous GH in the circulation (Beitins *et al.* 1977) into the various oligomeric forms (Sinha 1980; Stolar *et al.* 1984). These various forms of GH may be regulated by the nutritional status of the animal, the polymeric forms acting as a source of monomeric GH, the form with the greatest biological activity (Gordon *et al.* 1976).

The marked increases in plasma insulin levels after GH administration (Table 2) were consistent with the reports of Wallace and Bassett (1966) and Davis *et al.* (1970*b*). GH administration must have had a sustained influence on the β cells of the pancreatic islets, in view of the time between each injection and blood sampling and the relatively short half life (12–13 min) of circulating insulin in sheep (Trenkle 1971).

In view of the dependence of somatomedin C activity on plasma GH (Salmon and Daughaday 1957) and insulin (Daughaday *et al.* 1976) concentration, it is not surprising that activity of this growth factor was increased in plasma during GH administration. The greater responsiveness in animals fed the higher energy level is consistent with the nutritional dependence of plasma somatomedin activity (Takano *et al.* 1978; Phillips and Vassilopoulou-Sellin 1979; Maes *et al.* 1984).

Although GH administration resulted in increases in plasma concentrations of GH, insulin and somatomedin during treatment, the return to basal plasma levels of these hormones soon after cessation of GH injections appears to eliminate their involvement in the post-treatment increases in wool growth.

Despite the established role of the thyroid hormones and glucocorticoids in the control of wool growth (Ferguson *et al.* 1965; Wallace 1979), no change in circulating levels of these hormones resulting from GH administration was detected. It is unlikely that the fluctuations in prolactin levels caused changes in wool growth, as previous reports have been unable to establish a role for this hormone in wool growth (Ferguson *et al.* 1965; Wallace 1979).

The previously established diabetogenic and lipolytic effects of GH administration in sheep (Bassett and Wallace 1966; Davis *et al.* 1970*a*) have been corroborated by the present study.

The net retention of N during the period of GH administration is in accord with previous studies in the sheep (Manns and Boda 1965; Wheatley *et al.* 1966; Davis *et al.* 1970*b*). The mean 48-hourly retention of 2·5 and 1·1 g N for sheep fed the high and low energy intakes respectively was reflected by increased body weight in the treated groups of animals. The transient loss of N during the first 14-day period following cessation of GH injections probably resulted from homeostatic mechanisms causing mobilization of extra muscle protein synthesized as

a result of prolonged GH administration.

That GH administration stimulated the deposition of body protein was suggested by decreased plasma urea concentrations; these rose above pre-treatment levels in the post-treatment period. Similar results were obtained by Wheatley *et al.* (1966) and Davis *et al.* (1970*b*). Yet no such trend was observed in plasma α-amino N levels, although amino-acid analysis showed a change in methionine level.

S-amino acids are the first rate-limiting nutrients for wool growth (Reis and Schinckel 1963, 1964). Hence, the 20% depression in plasma methionine concentration observed during GH administration, which results from preferential uptake of amino acids for protein synthesis in muscle tissue, may account for the GH-induced depression in wool growth. The decrease in wool growth resulted largely from a depression in fibre diameter, however, and therefore is not in accord with previously reported responses to reduced nutrient intakes (Downes and Sharry 1971).

We have been unable to detect any endocrine factor likely to explain the wool growth responses. It is likely that the protracted post-treatment stimulation of wool growth resulted from homeostatic mechanisms causing mobilization of tissue protein and increased amino-acid availability to the wool follicle.

Acknowledgments

We wish to acknowledge: the skilled technical assistance of Mrs Jenny Ryan; the support of, and many helpful discussions with, Associate Professor G. H. McDowell; and the use of facilities and technical advice of Dr N. Jackson and Mrs Helen Engel for analysis of wool samples and Dr Y. Mollah for amino-acid analyses. Financial support was provided by the Australian Wool Corporation.

References

Annison, E. F. (1960). Plasma non-esterified fatty acids in sheep. *Aust. J. Agric. Res.* **11**, 58–64.

Anon. (1975). Energy allowances and feeding systems for ruminants. Min. Agric. Fish. Food Tech. Bull. No. 33. (Her Majesty's Stationery Office: London.)

Bassett, J. M. (1974). Diurnal patterns of plasma insulin, growth hormone, corticosteroid and metabolite concentrations in fed and fasted sheep. *Aust. J. Biol. Sci.* **27**, 167–81.

Bassett, J. M., and Thorburn, G. D. (1971). The regulation of insulin secretion by the ovine foetus *in utero*. *J. Endocrinol.* **50**, 59–74.

Bassett, J. M., and Wallace, A. L. C. (1966). Short-term effects of ovine growth hormone on plasma glucose, free fatty acids and ketones in sheep. *Metab. Clin. Exp.* **15**, 933–44.

Beitins, I. Z., Rattazzi, M. C., and MacGillivray, M. H. (1977). Conversion of radiolabeled human growth hormone into higher molecular weight moieties in human plasma *in vivo* and *in vitro*. *Endocrinology* **101**, 350–9.

Burger, H. G., Lee, V. W. K., and Rennie, G. C. (1972). A generalized computer program for the treatment of data from competitive protein-binding assays including radioimmunoassays. *J. Lab. Clin. Med.* **80**, 302–12.

Chaney, A. L., and Marbach, E. P. (1962). Modified reagents for determination of urea and ammonia. *Clin. Chem.* **8**, 130–2.

Daughaday, W. H., Phillips, L. S., and Mueller, M. C. (1976). The effects of insulin and growth hormone on the release of somatomedin by the isolated rat liver. *Endocrinology* **98**, 1214–19.

Davis, S. L., Garrigus, V. S., and Hinds, F. C. (1970*a*). Metabolic effects of growth hormone and diethylstilbestrol in lambs. I. Acute effects of ovine growth hormone on plasma metabolite levels in fasted lambs. *J. Anim. Sci.* **30**, 229–35.

Davis, S. L., Garrigus, V. S., and Hinds, F. C. (1970*b*). Metabolic effects of growth hormone and diethylstilbestrol in lambs. II. Effects of daily ovine growth hormone injections on plasma metabolites and nitrogen retention in fed lambs. *J. Anim. Sci.* **30**, 236–40.

Dole, V. P. (1956). A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *J. Clin. Invest.* **35**, 150–4.

Downes, A. M., and Sharry, L. F. (1971). Measurement of wool growth and its response to nutritional changes. *Aust. J. Biol. Sci.* **24**, 117–30.

Driver, P. M., and Forbes, J. M. (1978). Plasma growth hormone and spontaneous meals in sheep. *Proc. Nutr. Soc.* **37**, 100A.

Eastman, C. J., Corcoran, J. M., Ekins, R. P., Williams, E. S., and Nabarro, J. D. N. (1975). The radioimmunoassay of triiodothyronine and its clinical application. *J. Clin. Pathol. (Lond.)* **28**, 225–30.

Ellis, S., and Grindeland, R. E. (1974). Dichotomy between bio and immunoassayable growth hormone. In 'Advances in Human Growth Hormone Research'. (Ed. S. Raiti.) pp. 409–22. (U.S. Government Printing Office: Washington, D.C.).

Evans, H. M., Simpson, M. E., Marx, W., and Kibrick, E. A. (1943). Bioassay of the pituitary growth hormone. Width of the proximal epiphyseal cartilage of the tibia in hypophysectomized rats. *Endocrinology* **32**, 13–16.

Farmer, R. W., and Pierce, C. E. (1974). Plasma cortisol determination: radioimmunoassay and competitive protein binding compound. *Clin. Chem.* **20**, 411–14.

Ferguson, K. A. (1954). Prolonged stimulation of wool growth following injections of ox growth hormone. *Nature (Lond.)* **174**, 411.

Ferguson, K. A., Wallace, A. L. C., and Lindner, H. R. (1965). Hormonal regulation of wool growth. In 'Biology of the Skin and Hair Growth'. (Eds A. G. Lyne and B. F. Short.) pp. 655–77 (Angus and Robertson: Sydney.)

Gill, J. L., and Hafs, H. D. (1971). Analysis of repeated measurements of animals. *J. Anim. Sci.* **33**, 331–6.

Gordon, P., Lesniak, M. A., Eastman, R., Hendricks, S., and Roth, J. (1976). Evidence for higher proportion of 'little' growth hormone with increased radioreceptor activity in acromegalic plasma. *J. Clin. Endocrinol. Metab.* **43**, 364–73.

Gow, C. B., McDowell, G. H., and Jenkin, G. (1983). The importance of prolactin for initiation of lactation in the pregnant ewe. *Aust. J. Biol. Sci.* **36**, 357–67.

Henry, R. J., Cannon, D. C., and Winkelman, J. W. (1974). 'Clinical Chemistry: Principles and Technics.' 2nd Edn. (Harper and Rowe: New York.)

Huggett, A. St. G., and Nixon, D. A. (1957). Use of glucose oxidase, peroxidase and *o*-dianisidine in determination of blood and urinary glucose. *Lancet* **ii**, 368–70.

Johnsson, I. D., Hart, I. C. and Butler-Hogg, B. W. (1985). The effects of exogenous bovine growth hormone and bromocryptine on growth, body development, fleece weight and plasma concentrations of growth hormone, insulin and prolactin in female lambs. *Anim. Prod.* **41**, 207–17.

Maes, M., Underwood, L. E., and Ketelslegers, J. -M. (1984). Low serum somatomedin C in protein deficiency: relationship with changes in liver somatogenic and lactogenic binding sites. *Mol. Cell. Endocr.* **37**, 301–9.

Manns, J. G., and Boda, J. M. (1965). Effects of ovine growth hormone and prolactin on blood glucose, serum insulin, plasma non-esterified fatty acids and amino nitrogen in sheep. *Endocrinology* **76**, 1109–14.

Oddy, V. H. (1974). A semi-automated method for the determination of plasma alpha amino nitrogen. *Clin. Chim. Acta* **51**, 151–6.

Phillips, L. S., and Vassilopoulou-Sellin, R. (1979). Nutiritional regulation of somatomedin. *Am. J. Clin. Nutr.* **32**, 1082–96.

Phillips, L. S., and Vassilopoulou-Sellin, R. (1980). Somatomedins (Part 1). *New Engl. J. Med.* **302**, 371–80.

Reis, P. J., and Schinckel, P. G. (1963). Some effects of sulphur-containing amino acids on the growth and composition of wool. *Aust. J. Biol. Sci.* **16**, 218–30.

Reis, P. J., and Schinckel, P. G. (1964). The growth and composition of wool. II. The effect of casein, gelatin and sulphur-containing amino acids given per abomasum. *Aust. J. Biol. Sci.* **17**, 532–47.

Reis, P. J., Tunks, D. A., Rigby, R. D. G., Morton, T. C., and Munro, S. G. (1983). Investigation of some amino acid analogues and metabolites as inhibitors of wool and hair growth. *Aust. J. Biol. Sci.* **36**, 157–70.

Reklewska, B. (1974). A note on the effect of bovine somatotrophic hormone on wool production in growing lambs. *Anim. Prod.* **19**, 253–5.

Rosselin, G., Assan, R., Yalow, R. S., and Berson, S. A. (1966). Separation of antibody-bound and unbound peptide hormones labelled with iodine-131 by talcum powder and precipitated silica. *Nature (Lond.)* **212**, 355–8.

Salmon, W. D., and Daughaday, W. H. (1957). A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage *in vitro*. *J. Lab. Clin. Med.* **49**, 825–36.

Schalch, D. S., Heinrich, V. E., Draznin, B., and Johnson, C. J. (1979). Role of the liver in regulating somatomedin activity: hormonal effects on the synthesis and release of insulin-like growth factor and its carrier protein by the isolated perfused rat liver. *Endocrinology* **104**, 1143–51.

Searle, S. (1971). 'Linear Models.' (John Wiley: New York.)
Sinha, Y. N. (1980). Molecular size variants of prolactin and growth hormone in mouse serum: strain differences and alterations of concentrations by physiological and pharmacological stimuli. *Endocrinology* **107**, 1959–69.
Stolar, M. W., Amburn, K., and Bauman, G. (1984). Plasma 'big' and 'big-big' growth hormone (GH) in man: an oligomeric series composed of structurally diverse GH monomers. *J. Clin. Endocr. Metab.* **59**, 212–18.
Stuart, M. C., Lazarus, L., Moore, S. S., and Smythe, G. A. (1976). Somatomedin production in the neonatal rat. *Horm. Metab. Res.* **8**, 442–5.
Takano, K., Hizuka, N., Kawai, K., and Shizume, K. (1978). Effect of growth hormone and nutrition on the level of somatomedin A in the rat. *Acta Endocrinol.* **87**, 485–94.
Trenkle, A. (1971). Postprandial changes in insulin secretion rates in sheep. *J. Nutr.* **101**, 1099–104.
Trenkle, A. (1976). Estimates of the kinetic parameters of growth hormone metabolism in fed and fasted calves and sheep. *J. Anim. Sci.* **43**, 1035–43.
Van Wyk, J. J., Underwood, L. E., Hintz, R. L., Clemmons, D. R., Voina, S. J., and Weaver, R. P. (1974). The somatomedins: a family of insulinlike hormones under growth hormone control. *Recent Prog. Horm. Res.* **30**, 259–318.
Vodian, M. A., and Nicoll, C. S. (1977). Growth hormone releasing factor and the bioassay–radioimmunoassay paradox revisited. *Acta Endocrinol.* **86**, 71–80.
Wallace, A. L. C. (1961). Preparation of sheep growth hormone. *Nature (Lond.)* **190**, 535–6.
Wallace, A. L. C. (1979). The effect of hormones on wool growth. In 'Physiological and Environmental Limitations to Wool Growth'. (Eds. J. L. Black and P. J. Reis.) pp. 257–268. (Univ. New England: Armidale.)
Wallace, A. L. C., and Bassett, J. M. (1966). Effect of sheep growth hormone on plasma insulin concentration in sheep. *Metab. Clin. Exp.* **15**, 95–7.
Wallace, A. L. C., and Bassett, J. M. (1970). Plasma growth hormone concentrations on sheep measured by radioimmunoassay. *J. Endocrinol.* **47**, 21–36.
Wallace, A. L. C., and Ferguson, K. A. (1963). The preparation of sheep growth hormone. *J. Endocrinol.* **26**, 259–63.
Wallace, A. L. C., Gleeson, A. R., Hopkins, P. S., Mason, R. W., and White, R. R. (1978). Plasma thyroxine concentrations in grazing sheep in several areas in Australia. *Aust. J. Biol. Sci.* **31**, 39–47.
Wheatley, I. S., Wallace, A. L. C., and Bassett, J. M. (1966). Metabolic effects of ovine growth hormone in sheep. *J. Endocrinol.* **35**, 341–53.
Yde, H. (1968). A simplified technique for the determination of growth hormone dependent sulphation factor, using intact animals. *Acta Endocrinol.* **57**, 557–64.

Manuscript received 23 June 1986, revised 27 January 1987, accepted 11 November 1987