### THE GROWTH AND COMPOSITION OF WOOL

## II. THE EFFECT OF CASEIN, GELATIN, AND SULPHUR-CONTAINING AMINO ACIDS GIVEN PER ABOMASUM\*

## By P. J. REIS<sup>†</sup> and THE LATE P. G. SCHINCKEL<sup>†</sup>

#### [Manuscript received December 11, 1963]

#### Summary

The effect on wool growth and the sulphur content of wool of abomasal supplements of casein, gelatin, and of L-cysteine and DL-methionine added to the proteins has been examined. The supplements were administered as a continuous infusion over a period of 10-12 hr each day.

Supplements of 60 g casein per day, given to three sheep for 9 weeks, resulted in substantial increases in wool growth (123–181%). In contrast 60 g gelatin per day increased wool growth by only 17–24%. When sulphur-containing amino acids (Samino acids) were added to the proteins wool growth was further increased (16–37%). The percentage of the supplementary S-amino acids which could be accounted for in the wool grown during supplementation was high, viz. 48–51% of the S-amino acids in casein, 29–35% of the S-amino acids in gelatin, and 13–35% of the supplements of cysteine or methionine. An increase in fibre cross-sectional area accounted for c. 30-40% of the increase in wool growth during supplementation with protein or protein plus S-amino acid. In some sheep a much higher proportion of the increase in wool growth, due to S-amino acids alone, could be accounted for by an increase in cross-sectional area.

The sulphur content of the wool was increased 9–19% during case in supplementation, but was not increased during gelatin supplementation. During protein plus S-amino acid supplementation the sulphur content of the wool was increased 17–30%.

There was a consistent increase in the dry matter digestibility of the basal diet during the infusion of gelatin or casein into the abomasum. The mean dry matter digestibility was 55.0% on the basal diet alone and 57.8% during protein supplementation.

It is concluded that the substantial increases in wool growth rate obtained during casein supplementation per abosmaum are due specifically to essential amino acids. Previously observed effects of S-amino acids on wool growth and sulphur content of wool have been confirmed.

# I. INTRODUCTION

Earlier work showed that casein and sulphur-containing amino acids (S-amino acids), administered directly into the abomasum, produced considerable increases in wool growth and in the sulphur content of the wool (Reis and Schinckel 1961, 1963). Casein produced a much greater increase in the amount of wool grown than a supplement of S-amino acid given alone, similar in amount to the S-amino acid supplied

\* Part I of this series was published in Aust. J. Biol. Sci., 1963, 16, 218.

<sup>†</sup> Division of Animal Physiology, CSIRO, Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W.

Aust. J. Biol. Sci., 1964, 17, 532-47

by the casein. It was suggested that the greater wool growth response with casein was due to the presence of other essential amino acids.

The present work was carried out to test the hypothesis that the wool-growth response to abomasal protein supplements is dependent on the content of essential amino acids, especially the S-amino acids. The effect on the growth and sulphur content of wool of equal amounts of casein and gelatin was compared. Gelatin was selected as it has a low content of S-amino acids and is also deficient in several other essential amino acids. Further, the effect of supplementing both proteins with two levels of S-amino acids was examined. The previously observed effects of casein and S-amino acids on the growth and sulphur content of wool were confirmed. In contrast, gelatin supplements produced only a slight increase in wool growth and the sulphur content of the wool was not significantly changed.

## II. MATERIALS AND METHODS

## (a) General

The experimental animals were mature Merinos (SC8 and SC29, wethers; E107, ewe) and mature English Leicester-Merino crossbred wethers (1390, 1391, 1392, 1393, and SD67). Each sheep had an abomasal fistula fitted with a catheter. The sheep were kept in metabolism cages in a room maintained at a temperature of  $23\pm1^{\circ}$ C. A supplement of 1,000,000 i.u. of vitamin D was administered to each sheep once every 3 months. Facces were collected over 10-day periods as described by Reis and Schinckel (1961). The sheep were fed once daily, between 9 and 10 a.m.; the diet consisted of equal parts lucerne chaff and wheaten chaff. Each sheep received a constant amount of the basal diet; E107, 610 g/day; all other sheep, 800 g/day. A continuous check was made on the nitrogen content of the diet. The mean nitrogen content was 1.85 g/100 g dry matter (range 1.77-1.94 g/100 g dry matter), which corresponded to 10.6% crude protein on an air-dry basis.

## (b) Abomasal Infusions

Abomasal infusions were administered as gravity drips over a period of 10–12 hr each day, commencing when the sheep were fed. The casein supplement was Casinal (Glaxo Laboratories); 1 litre of a 6.6% w/v solution in water supplied 60 g protein, calculated as nitrogen  $\times$  6.37. Gelatin used was Davis Edible Gelatine, grade B, which contained 84.5% protein (nitrogen  $\times$  5.6) and 14.2% moisture. Thus, 60 g protein was supplied by 2 litres of a 3.5% w/v solution of gelatin in 0.02M HCl. The use of dilute and acidified solutions of gelatin was necessary to overcome the tendency of gelatin solutions to form a gel. S-amino acids were prepared as aqueous solutions and were added to the protein solutions each day. The daily supplements were  $3 \cdot 7$  g DL-methionine and 1.5 or 3.0 g L-cysteine (supplied as L-cysteine hydrochloride, 1.95 g supplies 1.5 g L-cysteine). When the amino acids were added to the protein, the amount of protein given was reduced by 1.5 or 3.0 g so that the total amount of supplement remained at 60 g/day. When casein was supplemented with cysteine it was necessary to infuse the cysteine from a separate bottle and through a separate tube into the abomasum, as the hydrochloric acid present would precipitate the casein and block the tube. The volume of all infusions of protein plus amino acid was 2 litres.

The amount of hydrochloric acid given in the infusions (with gelatin or as cysteine hydrochloride) did not exceed 1.46 g/day. This is less than the amount of acid normally secreted by the abomasum and the probable effect of the infusion would be a reduction in the amount of acid secreted (Ash 1961).

Abomasal supplements of protein were administered to six sheep for a period of 18 weeks. During the first 9 weeks, three sheep received 60 g casein per day and three sheep received 60 g gelatin per day. During the last 9 weeks of supplementation *S*-amino acids were added to the protein so that two sheep received 1.5 g L-cysteine per day, two sheep received 3.0 g L-cysteine per day, and two sheep received 3.7 g DL-methionine per day (equivalent to 3.0 g cysteine in terms of sulphur). The three levels of *S*-amino acid were given to one sheep on each type of protein. Sheep SC29 received 60 g casein per day for a further 9 weeks after supplementation with casein plus DL-methionine had ceased. During the experiment sheep SD67 received an abomasal infusion of 2 litres/day of 0.02M HCl for a period of 6 weeks, as a check on any effect of the acid used to administer gelatin. The wool growth of an untreated control sheep (E107) was measured throughout the experiment.

## (c) Nitrogen Estimation

Nitrogen in casein, gelatin, feed, faeces, and wool was estimated by the Kjeldahl method. The feed was prepared and sampled as described by Reis and Schinckel (1961). Nitrogen in faeces was estimated on bulked 10-day samples, prepared as described by Reis and Schinckel (1961). Dry matter content of feed and faeces was determined by drying in a hot air oven at 95–100°C.

# (d) Wool Growth

Wool growth was measured on each sheep from areas defined by tattooing; the wool was removed at intervals of 3 weeks with small animal clippers (Oster, size 40). Each sheep had two tattoo patches approximately  $100 \text{ cm}^2$  placed on either the shoulder, mid-side, or thigh. The patch areas were measured on two occasions during the experiment. The mean of the two estimates, which did not differ significantly, was used to express wool growth as milligrams clean dry wool/cm<sup>2</sup>/day. Total wool growth per sheep (expressed as grams/day) was estimated by multiplying the tattoo patch production by a factor relating patch production to whole fleece production. The relationship between total wool growth and patch wool growth was established by closely shearing the sheep on two occasions. The wool was cleaned as described by Reis and Schinckel (1961).

Fibre diameter was measured on clean wool samples, removed from the tattoo patches, by a modification of the air-flow technique of Anderson (1954); version B was modified by Chapman (unpublished data) to measure diameter in samples of 0.5, 1.0, or 2.5 g wool.

## (e) Sulphur in Wool

Sulphur was estimated as described by Reis and Schinckel (1963). The results are expressed as percentage sulphur in the clean dry wool. It was found unnecessary to clean the wool further with ether, as had been done previously. The mean difference

between duplicate estimates of sulphur content was 0.04 (S.D.  $\pm 0.029$ )% sulphur, in wool containing 3-4% sulphur.

# III. RESULTS

# (a) Effect of Abomasal Supplements on Wool Growth

The effects of the protein and S-amino acid supplements on wool growth are shown in Figure I and the results are summarized in Table 1. The administration of 60 g gelatin per day resulted in only a slight increase in wool growth, whereas administration of 60 g casein per day more than doubled the rate of wool growth. The actual amount of extra wool grown by the three sheep receiving each type of protein supplement was similar  $(0 \cdot 9 - 1 \cdot 3 \text{ g/day}$  on gelatin and  $6 \cdot 5 - 6 \cdot 7 \text{ g/day}$  on casein). The response to casein was immediate, but with gelatin no increase in wool growth was observed during the first 3 weeks. The increases were calculated from the mean value during the 9 weeks prior to treatment and the wool growth during the last 3 weeks of protein supplementation.

The addition of S-amino acids to the protein supplements increased wool growth in all sheep and to the same extent on both casein and gelatin. The increases given in Table 1 were calculated by comparing the wool growth during the last 3 weeks of protein supplementation with that during the last 3 weeks of S-amino acid supplementation. It is difficult to make an accurate estimate of the increases obtained with S-amino acids as it is not certain that the wool growth rate had stabilized by the end of protein supplementation. One sheep (SC29) was maintained on 60 g casein per day for 9 weeks after S-amino acid supplementation had ceased (Fig. 1). The wool growth rate fell after S-amino acids were removed from the supplement, but not to the level previously obtained on casein alone. After supplementation stopped, the wool growth rate of all sheep fell, and was similar to the pretreatment wool growth rate after 12 weeks.

The wool growth rate of the control sheep (E107) showed sufficient variation to suggest that the order of increase in wool growth obtained with gelatin alone would be barely significant. The administration of acid per abomasum (sheep SD67, Fig. 1) had no effect on wool growth.

Throughout the experiment the sheep were weighed once a week, before feeding. The mean increases in body weight during the periods of supplementation were: 4.5 kg (casein), 3.0 kg (gelatin), and 2.9 kg (S-amino acids). The type or amount of S-amino acid supplement did not affect the result, the range of increases observed being 2.6-3.3 kg. The increases were calculated from the body weights during the 3 weeks prior to supplementation and during the last 3 weeks of each supplementation period. The body weight of the control sheep (E107) did not change during the experiment.

A high percentage of the S-amino acid in the supplement, whether supplied as protein or protein plus S-amino acid, can be accounted for in the wool grown during supplementation (Table 2). To make these estimates the cystine content of the wool was calculated from the sulphur content by assuming that all the sulphur was present as cystine, and the amount of cystine supplied by the protein supplements was calculated from data given by Block and Bolling (1951). Accordingly, gelatin contains

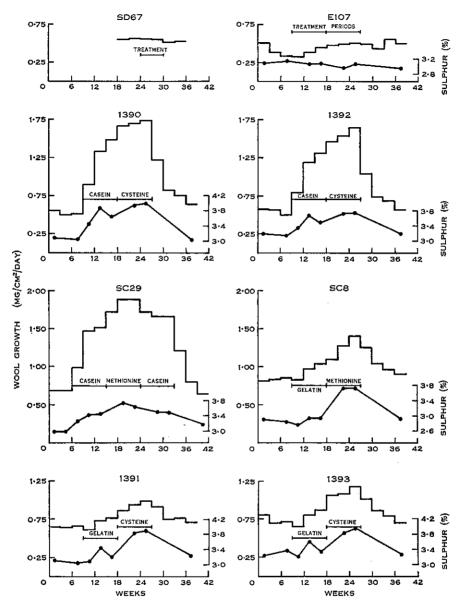


Fig. 1.—Effect of proteins and S-amino acids per abomasum on wool growth and sulphur content of wool. All sheep received a diet consisting of lucerne chaff and wheaten chaff (50:50); E107, 610 g/day; all other sheep, 800 g/day. Abomasal supplements were 60 g/day casein or gelatin during the first treatment period; during the second treatment period the protein was continued, supplemented as follows: 1390 and 1391, 3.0 g L-cysteine per day; 1392 and 1393, 1.5 g L-cysteine per day; SC8 and SC29, 3.7 g pL-methionine per day. SC29 received 60 g casein per day during a third treatment period. SD67 received 2 litres/day 0.02m HCl, per abomasum, during the treatment period. E107 was untreated. Wool growth is expressed as milligrams clean dry wool; sulphur is expressed as the percentage in clean dry wool.

the equivalent of 0.74% cystine (0.1% cystine and 0.8% methionine), and casein contains the equivalent of 2.8% cystine (0.4% cystine and 3.5% methionine); methionine is converted to cystine on the basis of its sulphur content. Thus 60 g

### TABLE 1

INCREASE IN WOOL GROWTH DUE TO NITROGENOUS SUPPLEMENTS GIVEN PER ABOMASUM Data are derived from the experiment presented in Figure 1; experimental details are given in the legend to the figure. The method of calculating the data is described in the text

			Increase in			Wool Growth		
Sheep No.	Treatment	Growth of Clean Dry Wool (g/day)	Due to Supple		Due to S-Amino Acid Only			
			(g/day)	(%)	(g/day)	(%)		
1392	None Casein Casein + 1 · 5 g	4·8 11·5	6.7	140		_		
	cysteine	14.4	9∙6	200	2.9	25		
1390	None Casein Casein + 3 · 0 g	3·7 10·4	6.7	181	_			
	cysteine	12.1	8∙4	227	1.7	16		
SC29	None Casein Casein + 3 · 7 g	5·3 11·8	6.5	123	_			
	methionine	14.6	9.3	176	$2 \cdot 8$	24		
1393	None Gelatin Gelatin + 1 · 5 g	5·5 6·5	1.0	18	_	_		
	cysteine	8.8	3 · 3	60	2.3	35		
1391	None Gelatin Gelatin +3.0 g	$5 \cdot 2 \\ 6 \cdot 1$	0.9	17	_			
	cysteine	7 • 9	2.7	52	1.8	30		
SC8	None Gelatin	5·5 6·8	1.3	24		_		
	Gelatin + 3 · 7 g methionine	9.3	3.8	69	2.5	37		

gelatin supplied 0.44 g cystine and 60 g casein supplied 1.92 g cystine. Also, a high percentage of the casein nitrogen and a very low percentage of the gelatin nitrogen can be accounted for in the wool grown during supplementation (Table 2). The addition of S-amino acids to both proteins increased the extent of conversion of protein nitrogen into wool. These calculations were based on a nitrogen content in the clean dry wool of 16.2%. This value was obtained by determining the nitrogen content of six samples of wool, from each of two sheep (1390 and 1391), grown on two

Sheep	Treatment	Cystine in Clean Dry	Recovery Acid in V	Recovery of	
No.	Treatment	Wool (%)	From Total Supplement	From S-Amino Acid Only	Nitrogen in Wool (%)*
1392	None	11.9			<u> </u>
	Casein	13.0	48		11.5
	Case in $+1.5$ g				
	cysteine	$14 \cdot 0$	42	35	$16 \cdot 5$
1390	None	· 11·5			
	Casein	13.6	51	_	11.5
	$Casein + 3 \cdot 0 g$				
	cysteine	14.9	28	13	14.4
SC29	None	11.2			
	Casein	13.0	49		$11 \cdot 2$
	Casein+3·7 g methionine	14.1	30	18	16.0
1393	None	12.4			
1000	Gelatin	12.6	31		1.5
	Gelatin + 1.5 g		01		10
	cysteine	14.8	32	32	$5 \cdot 0$
1391	None	11.6			
	Gelatin	12.0	29	_	1-4
	Gelatin $+3.0$ g				
	cysteine	14.6	16	14	4 · 1
SC8	None	10.8			
	Gelatin	11.0	35	<u> </u>	$2 \cdot 0$
	Gelatin + 3 · 7 g methionine	14.0	21	18	5.7

\* i.e. percentage of the S-amino acid or the percentage of nitrogen in the supplement accounted for in the wool grown during supplementation.

sites on the basal diet alone, during protein supplementation and during protein plus S-amino acid supplementation. The mean nitrogen content of the clean dry wool from the two sheep was 16.22% (range 16.02-16.44%) and 16.21% (range 16.13-16.14%)

TABLE 2

16.37%). This procedure was adopted as published values for the nitrogen content of wool vary widely (Simmonds 1954; Block and Weiss 1956; Spector 1956).

#### TABLE 3

CONTRIBUTION OF FIBRE CROSS-SECTIONAL AREA TO INCREASED WOOL GROWTH The data refer to the experiment presented in Figure 1; experimental details are given in the legend to the figure. Fibre diameter is the mean of two sites, determined on 3 weeks growth of wool collected 3 weeks before treatment commenced and at the end of each treatment period. The method of calculating the remainder of the data is described in the text

Sheep	Treatment .	Fibre Diameter (µ)	Increase in C Area	ross-sectional (%)	Cross-sectional Area (%)*		
No.			Due to Total Supplement	Due to S-Amino Acid Only	Due to Total Supplement	Due to S·Amino Acid Only	
1392	None	20.9	<u>_</u>				
	Casein Casein $+1.5$ g	26.4	60		42		
	cysteine	$27 \cdot 8$	77	11	38	<b>42</b>	
1390	None	$20 \cdot 2$					
	Casein	25 • 7	61	—	33	-	
	Casein + 3 0 g			10		100	
	cysteine	28.0	91	19	39	100	
SC29	None	$22 \cdot 0$					
	Casein	$25 \cdot 8$	37		30		
	Casein + 3 · 7 g methionine	$28 \cdot 0$	62	19	35	76	
1393	None	19.8					
	Gelatin	20.6	7	_	38		
	Gelatin $+1.5$ g		2.9			10	
	cysteine	$22 \cdot 0$	23	14	40	<b>40</b>	
1391	None	$23 \cdot 6$					
	Gelatin	$23 \cdot 0$	-5		0	—	
	$\operatorname{Gelatin} + 3 \cdot 0 \text{ g}$	<u> </u>	1-				
	cysteine	$25 \cdot 5$	17	23	33	77	
SC8	None	20.6					
	Gelatin	$20 \cdot 9$	3	_	10	_	
	Gelatin $+3.7$ g methionine	23.3	28	24	40	68	
	<u></u>	200		41	<u> </u>	00	

\* i.e. percentage of increase in wool growth due to increase in fibre cross-sectional area.

## (b) Contribution of Fibre Cross-sectional Area to Increased Wool Growth

Fibre diameter was measured on wool grown prior to protein supplementation and on wool grown during the last 3 weeks of protein and protein plus S-amino acid

539

supplementation. The cross-sectional areas of the fibres were calculated assuming that the fibres were circular in cross section. The air-flow technique used gives the best estimate of the true mean diameter of a group of fibres. The cross-sectional area was substantially increased during case in supplementation but was not significantly altered during gelatin supplementation (Table 3). The addition of S-amino acids to both casein and gelatin increased the cross-sectional area. Columns 6 and 7 of Table 3 show the percentage of the wool-growth increase obtained during abomasal supplementation which can be accounted for by the increase in fibre cross-sectional area. The remainder of the increase in wool growth can be assumed to be due to an increased length growth rate. These values were computed from the tattoo patch wool growth, assuming no change in the specific gravity of the wool fibres during the experiment. An increase in cross-sectional area accounts for c. 30-40% of the wool growth increase obtained during supplementation with protein or protein plus S-amino acid. Due to the small wool-growth increases, the values calculated for gelatin alone cannot be regarded as reliable. Hence, no significance can be attached to the values of 0 and 10%for 1391 and SC8 (column 6). In some sheep a much higher proportion of the increase in wool growth, due to S-amino acids alone, could be accounted for by an increase in cross-sectional area. Higher values were consistently found when  $3 \cdot 0$  g cysteine or 3.7 g methionine were given.

## (c) Effect of Abomasal Supplements on the Sulphur Content of Wool

The sulphur content of the wool clipped from the two tattoo patches was measured at intervals throughout the experiment. Sulphur analyses were also carried out on wool collected from two further sites (right and left thigh) on sheep 1390, 1391, 1392, and 1393. The sulphur values plotted on Figure 1 are the means for each group of sites. The sulphur content of the wool was increased 9–19% during casein supplementation and 17–30% during protein plus S-amino acid supplementation, but was not increased during gelatin supplementation (Table 4). The small increase in the sulphur content of the wool of 1391 and 1393 during the second 3 weeks of gelatin supplementation has been disregarded. This increase coincides with a high sulphur value obtained for 1390 and 1392; however, there was no change in the sulphur content of the wool of sheep SC8, SC29, and E107 (control) at this time. The reason for this variation is not known. The sulphur content had returned to about the pretreatment level 12 weeks after treatment ceased.

The sulphur content of wool from all sites prior to treatment and at the end of each treatment period is given in Table 4. The mean difference between sample sites (i.e. the greatest difference when four sites were involved), calculated from all analyses carried out during the experiment, was 0.10 (S.D. $\pm 0.072$ )% sulphur. These differences appear to be random as the mean difference between all thigh samples and all shoulder samples, irrespective of sheep and treatment, was +0.025% sulphur. The mean difference between casein supplemented and control periods was +0.44 (S.E. $\pm 0.08$ )% sulphur, calculated within sheep, while the mean difference between S-amino acid supplemented and control periods was +0.75 (S.E. $\pm 0.05$ )% sulphur, calculated within sheep. These values were calculated from the data presented in Table 4.

## GROWTH AND COMPOSITION OF WOOL. II

## TABLE 4

## SULPHUR CONTENT OF WOOL

Sulphur content of wool grown during the experiment presented in Figure 1; experimental details are given in the legend to the figure. Samples 1 and 2 were collected prior to treatment, while samples 3 and 4 were grown during the last 3 weeks on treatment

		le Treatment	- - -	Increase				
Sheep No.	Sample		Sampling Position					in Sulphur Content due to
			Right Shoulder	Left Shoulder	Right Thigh	Left Thigh	Mean	Treatment (%)
1392	1	None	3.12	3.16	3.30	3.20	3.20	
	2	None	3.17	3.17	3.10	$3 \cdot 15$	3.15	1
	3	Casein	3.44	$3 \cdot 46$	3.50	$3 \cdot 50$	3.48	9.4
	4	Casein + 1.5 g						1
		cysteine	3.66	3.67	3.77	3.81	3 73	17.3
1390	1	None	3.02	<b>3</b> ⋅00	3.22	$3 \cdot 14$	3.10	
	2	None	3.05	$3 \cdot 04$	3.09	$2 \cdot 97$	$3 \cdot 04$	
	3	Casein	3.57	3.58	3.73	3 · 66	$3 \cdot 64$	18.6
	4	Casein + 3.0 g					ł.	
		cysteine	3.94	3.96	4.00	4.00	3.98	29-6
SC29	1	None	3.01		2.98		3.00	
	2	None	3.00		2.99		3.00	
	3	Casein	3.46		3.47		3.46	$15 \cdot 3$
	4*	$Casein + 3 \cdot 7 g$					Į	
		methionine	3.78		3.72		3.75	$25 \cdot 0$
1393	1	None	3.14	3.12	3.37	3.34	3.24	
	2	None	3 · 42	$3 \cdot 42$	$3 \cdot 34$	$3 \cdot 34$	3.38	
	3	Gelatin	3.28	$3 \cdot 23$	$3 \cdot 52$	3 • 43	3 · 36	1.5
	4	Gelatin + 1.5 g						
		cysteine	3.98	$3 \cdot 94$	3.92	3.98	3.95	19.3
1391	1	None	3.06	3.10	3.16	3.17	3.12	
	2	None	<b>3</b> ⋅09	3.09	3.01	3.07	3.06	
	3	Gelatin	3.11	$3 \cdot 11$	3.31	3.30	$3 \cdot 21$	3.9
	4	$Gelatin + 3 \cdot 0 g$						
		cysteine	3.92	3.88	3.84	3.86	3.88	$25 \cdot 6$
SC8	1	None	2.92		2.88†		2.90	
	2	None	2.81		$2.87^{+}$		$2 \cdot 84$	
	3	Gelatin	$2 \cdot 95$		2.94†		2.94	2.4
	4	Gelatin+3·7 g				{		
		methionine	3.72		3.71†		3.72	29.6
E107	1	None	3.13		3.02†		3.08	
	2	None	$3 \cdot 20$		3.06		3.13	
	3	None	3.14		3.03†		3.08	
	4	None	3.07		3.04†	l	3.06	

\* Sample 4 was collected after 6 weeks on treatment.

† Wool samples collected from right mid-side.

## (d) Effect of Abomasal Protein Supplements on Dry Matter Digestibility

Previous work showed that casein given per abomasum was completely digested and absorbed (Reis and Schinckel 1961). Accordingly, faecal nitrogen excretion was measured during gelatin supplementation (Table 5). There was only a slight increase in faecal nitrogen output during gelatin supplementation and it can be concluded that at least 95% of the gelatin was digested and absorbed. Some increase in faecal nitrogen output during gelatin supplementation would be expected due to the small increase in nitrogen intake from the basal diet (nitrogen digestibility of the basal diet was 65-68% and was unaffected by protein supplementation).

TABLE 5					
EFFECT OF ABOMASAL GELATIN SUPPLEMENTS ON FAECAL NITROGEN					
EXCRETION					

Faecal nitrogen was estimated during 10-day collection periods on the basal diet and on the basal diet plus gelatin (during the last 2 weeks of supplementation). Experimental details are given in the legend to Figure 1

<u>61</u>	Treatment	Nitrogen	Faecal		
Sheep		Basal Diet	Gelatin	Total	Nitrogen (g/day)
1391	None Gelatin	13·44 13·91	 10·74	$13 \cdot 44 \\ 24 \cdot 65$	$4 \cdot 44 \\ 4 \cdot 51$
1393	None Gelatin	$13 \cdot 44 \\ 13 \cdot 91$	 10•74	$13 \cdot 44 \\ 24 \cdot 65$	$4 \cdot 32 \\ 4 \cdot 75$
SC8	None Gelatin	$12 \cdot 92 \\ 13 \cdot 63$	 10·74	$12 \cdot 92 \\ 24 \cdot 37$	$4 \cdot 20 \\ 4 \cdot 82$

There was an increase in the dry matter digestibility of the basal diet in all sheep, during the infusion of gelatin or casein into the abomasum (Table 6). The effect was the same with both types of protein. The mean dry matter digestibility was  $55 \cdot 0\%$  on the basal diet alone and  $57 \cdot 8\%$  during protein supplementation.

# IV. DISCUSSION

The substantial increases in the rate of wool growth during the administration of casein per abomasum confirm previous results (Reis and Schinckel 1961, 1963). Indirect evidence was given (Reis and Schinckel 1963) that the extra energy available from the casein was far too small to account for the increases in wool growth obtained. The big difference in the response of wool growth to casein and gelatin supplementation provides direct evidence that the increase in wool growth during casein supplementation is due specifically to the essential amino acids supplied by the protein. The gelatin can be regarded as an isocaloric control for the casein infusions. Downes (1961) showed that the tissues of the sheep very probably require the same amino acids as those regarded as essential for the growing rat, with the exception that the sheep was able to synthesize arginine. Gelatin has a very low content of all these essential amino acids, except lysine and histidine. The wool growth rate during both casein and gelatin supplementation was increased to a similar extent by the addition of S-amino acids. This confirms previously observed effects of abomasal supplements of S-amino acids on wool growth (Reis and Schinckel 1963). Moreover, the increases in wool growth rate during S-amino acid supplementation were quantitatively similar to those previously obtained. While it is obvious that supplements of both cysteine and methionine have increased the wool growth rate, there are insufficient data to compare the relative responses to the

#### TABLE 6

### EFFECT OF ABOMASAL PROTEIN SUPPLEMENTS ON DRY MATTER DIGESTIBILITY

Dry matter digestibility was measured during 10day collection periods on the basal diet and on the basal diet plus protein (during the last 2 weeks of supplementation). Experimental details are given in the legend to Figure 1

		the second s	
Sheep No.	Treatment .	Dry Matter Digestibility (%)	Increase in Percentage Dry Matter Digestibility
1392	None Casein	54 · 5 57 · 6	3 · 1
1390	None Casein	$55 \cdot 8$ 57 \cdot 8	2.0
SC29	None Casein	56 · 8 59 · 8	3.0
1393	None Gelatin	$55 \cdot 4$ $58 \cdot 6$	$3 \cdot 2$
1391	None Gelatin	$53 \cdot 9$ $57 \cdot 0$	3.1
SC8	None Gelatin	53 · 8 55 · 9	2 · 1

two amino acids. Although the number of sheep involved is small it appears that a supplement of 1.5 g cysteine stimulated wool growth to a greater extent than a supplement of 3.0 g cysteine. The failure of the higher level of cysteine to stimulate wool growth further, and in fact the probable smaller effect of 3.0 g cysteine, may be associated with an amino acid imbalance. Such effects are well known in growth studies with rats (Harper 1958).

It is possible that as the level of S-amino acid in the supplement is increased other amino acids become limiting for wool growth. Next to methionine, threenine is the most limiting amino acid in casein for growth in rats (Harper 1959). A comparison of the amino acid composition of casein and of wool (Block and Bolling 1951) suggests that this may also be the case for wool growth. With gelatin, the very low content of most of the essential amino acids would presumably limit the wool growth response substantially. However, the high ratio of non-essential to essential amino acids in gelatin, and particularly the high glycine content, may also be limiting the wool growth response to gelatin. Thus, a high proportion of non-essential amino acids and a supplement of glycine can depress the nutritive value of proteins for growth in rats (Hier, Graham, and Klein 1944; Swendseid, Hickson, and Friedrich 1962).

The importance of amino acid composition is demonstrated by the fact that the wool growth rate was much greater during casein supplementation than it was during supplementation with gelatin, to which S-amino acids had been added to give the same content of S-amino acids as in casein. Thus, casein alone (which contained  $3\cdot 2\%$  cystine, i.e. total S-amino acids expressed as cystine) was superior to gelatin plus  $1\cdot 5$  g cysteine ( $3\cdot 2\%$  cystine), and casein plus  $1\cdot 5$  g cysteine ( $5\cdot 6\%$  cystine) was superior to gelatin plus  $3\cdot 0$  g cysteine or  $3\cdot 7$  g methionine ( $5\cdot 7\%$  cystine).

The rates of wool growth obtained with supplements of casein plus S-amino acids are high (1.64-1.88 mg clean dry wool/cm<sup>2</sup>/day), and are probably approaching the limit of which these sheep are capable. Thus, Schinckel (1963) quotes a figure of  $1.8 \text{ mg/cm}^2$ /day as the maximum wool growth rate of which sheep are capable, whereas Williams (personal communication) observed that the wool growth rate of some sheep at pasture exceeded  $2.0 \text{ mg/cm}^2$ /day for short periods. These values suggest that sheep can grow wool at their maximum rate, even on moderate intakes of feed, provided a satisfactory supply of good quality protein is available for digestion and absorption at the intestinal level.

The wool growth responses to case and S-amino acid supplements were obtained with sheep fed only one type of diet, at a moderate level of intake. The diet supplied c. 85 g crude protein per day but no estimate can be given of the amount of amino nitrogen (free and protein-bound) reaching the abomasum. This will depend on the extent of breakdown and resynthesis of protein, associated with microbial growth, in the rumen. The magnitude of the response to case and S-amino acid supplements will obviously depend on how nearly the wool growth rate on the basal diet approaches the genetic potential for wool growth. In these experiments the intake of the basal diet was kept at a moderate level so that wool growth would be well below the maximum. Substantial increases in wool growth rate during case supplementation per abomasum had also been obtained on a different diet, containing 40% cracked maize and 60% chaff (Reis and Schinckel 1961) and it is probable that a response would be obtained in any situation where wool growth rate is below the genetic potential.

In agreement with previous findings (Reis and Schinckel 1963) the S-amino acids in the supplements were very efficiently incorporated into wool. Thus, 48-51%of the S-amino acids in casein and 29-35% of the S-amino acids in gelatin could be accounted for in the extra wool grown during supplementation. These values were calculated on the assumption that all the extra sulphur incorporated into wool during

544

supplementation was derived from the supplement. The big difference in the efficiency of conversion of the two protein supplements into wool is shown by the fact that up to 16.5% of the nitrogen from a supplement of casein plus S-amino acid was incorporated into wool, while as low as 1.4% of the nitrogen from a gelatin supplement was incorporated into wool. However, the efficiency of conversion of the S-amino acid supplements into wool was the same when they were added to either protein supplement.

The values given in Table 2 for the efficiency of conversion of S-amino acids into wool were calculated on the basis that all the sulphur in wool is present as cyst(e)ine. The authors have previously used a value of 90% as a conservative estimate of the proportion of the total sulphur in wool present as cystine. As methionine accounts for c. 3% of the sulphur in wool the maximum value for cystine in wool would be 97% of the sulphur content. It would now appear that there is sufficient evidence to state that all the sulphur in wool, apart from methionine, is present as cyst(e)ine; the small amounts of other sulphur-containing compounds found in wool are derived from cystine (Leach 1959; Wibaux, Mazingue, and Van Overbèke 1960; Fletcher, Robson, and Todd 1963). It was therefore considered that the error involved in assuming that all of the sulphur in wool was present as cystine would be negligible.

The small increase in dry matter digestibility of the basal diet observed during casein and gelatin infusion may have been due to nitrogen from the protein supplements recycled to the rumen (Somers 1961). However, the nitrogen content of the basal diet was slightly higher during protein supplementation and this may also have influenced digestibility. Moir and Harris (1962) observed that duodenal supplements of casein, given to maintain nitrogen intake at about 12 g/day, prevented the substantial fall in the digestibility of dry matter and of crude fibre which occurred when the nitrogen content of a semi-synthetic diet was lowered. Up to 8 g casein nitrogen per day were infused and it was estimated that at least  $3 \cdot 5$  g nitrogen per day were recycled to the rumen. It is evident that any effect of recycled nitrogen on wool growth must be small as both gelatin nitrogen and casein nitrogen should be recycled to a similar extent.

The observation that 30-40% of the increase in wool growth during protein supplementation can be accounted for by an increase in fibre cross-sectional area is in general agreement with the report of Schinckel (1962). He observed an increase of 71% in fibre cross-sectional area during casein administration per abomasum and this increase accounted for 46% of the increased wool growth. The indication from the data, that a higher proportion of the increase in wool growth due to S-amino acids alone can be accounted for by an increase in fibre cross-sectional area, needs further examination. As pointed out by Schinckel (1962), there is at present no way of determining the relative contributions of rate of cell production and of cell volume to changes in cross-sectional area and length of fibres. Measurements of mitotic rate or of final cell volume in fibres grown during the period of administration of S-amino acids, in those sheep in which the wool growth increase was mainly one of crosssectional area, should provide information on whether rate of proliferation or changes in cell volume or both factors were limiting wool growth.

### P. J. REIS AND THE LATE P. G. SCHINCKEL

As was previously observed (Reis and Schinckel 1963), the sulphur content of wool was considerably increased during administration of casein and S-amino acids. The fact that there were no significant differences in sulphur content between the four sites examined is evidence that an accurate estimate was made of the sulphur content of the whole fleece. An earlier suggestion by the authors that "the altered sulphur content of the whole fibres observed here was probably associated with a relatively greater synthesis of a high-sulphur component of wool protein" has been confirmed (Gillespie, Reis, and Schinckel 1964). It was found that abomasal supplements of S-amino acids or casein greatly altered the composition of the wool proteins. The proportion of the high-sulphur proteins in wool was increased, and within the group of high-sulphur proteins there was increased formation of the components richer in sulphur. Moreover, the increase in sulphur content of the wool could be completely accounted for by this increased synthesis of sulphur-rich components. Thus, while supplements of case or S-amino acids greatly increase the rate of synthesis of all protein components of keratin, the synthesis of the sulphur-rich proteins is increased to the greatest extent. An increase in the rate of synthesis of the high-sulphur proteins due to S-amino acids could be explained on the basis of an increase in the supply of limiting substrate, but this does not seem likely in the case of the low-sulphur proteins. However, another possible effect of S-amino acids may be a stimulation of mitotic rate in the follicle bulb, with a resultant increase in the rate of synthesis of all protein fractions. Alternatively, the synthesis of the high-sulphur proteins may be the rate limiting step in keratin synthesis. If this was so, increased synthesis of the highsulphur proteins would automatically result in an increase in the rate of wool growth.

### V. ACKNOWLEDGMENT

The authors are indebted to Mr. D. A. Tunks for technical assistance throughout the course of this work.

#### VI. References

BLOCK, R. J., and WEISS, K. W. (1956).—"Amino Acid Handbook. Methods and Results of Protein Analysis." (C. C. Thomas: Springfield, Illinois.)

DOWNES, A. M. (1961).-Aust. J. Biol. Sci. 14: 254.

FLETCHER, J. C., ROBSON, A., and TODD, J. (1963).-Biochem. J. 87: 560.

GILLESPIE, J. M., REIS, P. J., and SCHINCKEL, the late P. G. (1964).-Aust. J. Biol. Sci. 17: 548.

HARPER, A. E. (1958).-Ann. N.Y. Acad. Sci. 69: 1025.

HARPER, A. E. (1959).-J. Nutr. 67: 109.

HIER, S. W., GRAHAM, C. E., and KLEIN, D. (1944).-Proc. Soc. Exp. Biol., N.Y. 56: 187.

LEACH, S. J. (1959).-Biochim. Biophys. Acta 33: 264.

MOIR, R. J., and HARRIS, L. E. (1962).-J. Nutr. 77: 285.

REIS, P. J., and SCHINCKEL, P. G. (1961).-Aust. J. Agric. Res. 12: 335.

REIS, P. J., and SCHINCKEL, P. G. (1963).-Aust. J. Biol. Sci. 16: 218.

546

ANDERSON, S. L. (1954).-J. Text. Inst. 45: P312.

ASH, R. W. (1961).-J. Physiol. 156: 93.

BLOCK, R. J., and BOLLING, D. (1951).—"The Amino Acid Composition of Proteins and Foods." 2nd Ed. (C. C. Thomas: Springfield, Illinois.)

SCHINCKEL, P. G. (1962).-Anim. Prod. 4 (1): 122.

SCHINCKEL, P. G. (1963).—Proc. World Conference on Animal Production. Rome, September 1963. Vol. 1. pp. 199-239.

SIMMONDS, D. H. (1954).-Aust. J. Biol. Sci. 7: 98.

SOMERS, M. (1961).-Aust. J. Exp. Biol. Med. Sci. 39: 145.

SPECTOR, W. S. (1956).—"Handbook of Biological Data." (Ed. W. S. Spector.) pp. 90-1. (W. B. Saunders Co.: Philadelphia and London.)

SWENDSEID, M. E., HICKSON, J. B., and FRIEDRICH, B. W. (1962).-J. Nutr. 78: 115.

WIBAUX, G., MAZINGUE, G., and VAN OVERBÈKE, M. (1960).-Bull. Inst. Text. Fr. 86: 41.