# SOME EFFECTS OF SULPHUR-CONTAINING AMINO ACIDS ON THE GROWTH AND COMPOSITION OF WOOL

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### Summary

The effect on wool growth and the sulphur content of wool of supplements of L-cysteine, DL-methionine, and casein, given per abomasum as a continuous infusion, has been examined.

Daily supplements of 2 g L-cysteine or an equivalent amount of DL-methionine (2.46 g), given for 6 weeks, resulted in increases in the rate of wool growth from tattoo patches of 35-130% in four sheep, during the last 3 weeks of treatment. The estimated increases in the weight of wool grown by the sheep during this period ranged from 1.7 to 3.9 g per day (basal levels 3.0-8.3 g per day). The efficiency of recovery of the supplementary sulphur in the wool grown during the period of supplementation was high, 15-29%. The treatments also resulted in increases in the wool of 24-35%.

Supplements of 60 g casein per day per abomasum for 6 weeks resulted in very substantial increases in the amount of wool grown by two sheep. This amount of casein supplied about 1.75 g per day of sulphur-containing amino acid (S-amino acid), expressed as cystine. The increases in the rate of wool growth from tattoo patches were 84 and 102%, during the last 3 weeks of treatment, and the estimated increases in the weight of wool grown by the sheep during this period were 6.6 and 4.6 g per day respectively (basal levels 7.9 and 4.5 g per day). The efficiency of recovery in the wool, of the supplementary sulphur from the casein, was also exceptionally high, viz. 50 and 40%. The casein also produced an increase in the sulphur content of the wool of 15-20%.

Possible mechanisms by which cysteine, methionine, and casein, administered per abomasum, act to stimulate wool growth and to increase the sulphur content of wool are discussed. It is concluded that protein, made available for absorption by the sheep, does specifically stimulate wool growth and that the S-amino acids may be especially important in this regard.

### I. INTRODUCTION

Wool growth is considerably influenced by nutrition, but there is still much disagreement regarding the important nutritional factors limiting wool growth. Marston (1948) demonstrated the effect of food intake on the rate of wool growth, but concluded that this effect was due to the protein, and more especially the essential amino acids, in the diet. The theory that the rate of wool growth was dependent primarily on the dietary supply of essential amino acids, particularly cystine, was further elaborated by Marston (1955). In contrast, other reports (Fraser and Roberts 1933; Slen and Whiting 1952; Ferguson 1959) have provided some evidence that energy is the main nutritional factor limiting wool growth. In previous work the present authors showed that casein administered per abomasum produced a

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considerable increase in wool growth (Reis and Schinckel 1961). However, it was pointed out that no conclusions could be drawn regarding the metabolic reasons for the response to casein, although the increased wool growth obtained with casein appeared to be considerably greater than would be expected if the casein was acting merely as a supply of extra energy.

In the belief that cystine was an essential amino acid and in view of the relatively low level of cystine in dietary proteins, Brailsford Robertson (1928) and Marston (1928b) suggested that cystine was likely to be the primary nutritional factor limiting wool growth under pastoral conditions. Marston (1932a) observed an increase in the level of wool growth by grazing sheep fed a supplement of blood meal and attributed the response to the cystine in the meal. He also believed that the superiority (in terms of increased wool growth) of yeast over casein as a supplement for straw was due to its higher cystine content (Marston 1932b). Dietary supplements of cystine have usually failed to stimulate wool growth (Marston 1932b; Du Toit *et al.* 1935), and Marston believed that "free cystine is not utilized by the animal to anything like the same extent as cystine bound in protein linkage". Moir (personal communication) also failed to obtain a wool growth response from a dietary cystine supplement. In contrast, Marston (1935) later reported increased wool growth (34%), associated with a subcutaneous administration (as opposed to dietary supplementation) of  $1 \cdot 3$  g L-cysteine hydrochloride daily for 10 days to one sheep receiving a chaff diet.

Marston (1928a) claimed that wool contained a constant amount of sulphur (about 3.6% of the dry matter), which was present almost exclusively as cystine. Although there has been considerable uncertainty regarding the accuracy of techniques for measuring sulphur and cystine in wool, the results of other published work suggest that the sulphur content of wool can vary widely. Values ranging from  $2\cdot9$  to  $4\cdot5\%$ sulphur in the dry matter have been reported (Barritt and King 1926, 1929; Simmonds 1954, 1955, 1956; Earland 1961). However, there is little evidence concerning the effect of nutrition, or of other factors, on the sulphur content of keratins. Barritt, King, and Pickard (1930) and Barritt and Rimington (1931) found that daily supplements of 0.05, 0.1, and 0.2 g cystine, given to rabbits, had no significant effect on the sulphur or cystine content of the fur. Koyanagi and Takanohashi (1961) showed that the cystine in hair from children on a low protein diet was much lower than from children on a "well-balanced diet", and that supplements of milk and of vitamin A both increased the cystine content of the hair. Ross (1961) examined the sulphur content of Romney wool, collected from grazing sheep in New Zealand during four successive years. There was some seasonal variation in sulphur content, which may have been due to environment or nutrition. The author believed that the sulphur content was lowest when wool production was highest and vice versa, although this relationship was not invariable.

In view of earlier results (Reis and Schinckel 1961) and the observation by Marston (1935) of a response to subcutaneous cysteine, the effects on wool growth of casein and of sulphur-containing amino acids (*S*-amino acids) administered per abomasum were investigated. The effect of these supplements on the sulphur content of wool was also investigated. Relatively large effects on growth and sulphur content were observed.

### II. MATERIALS AND METHODS

# (a) General

The experimental animals were mature Merinos (SC8, wether; E122 and E107, ewes) and mature English Leicester-Merino crossbred wethers (1390, 1391, 1392, and 1393). Each sheep had an abomasal fistula fitted with a catheter. The sheep were kept in metabolism cages which were housed in a room maintained at a temperature of  $22\pm1^{\circ}$ C. A supplement of 1,000,000 i.u. of vitamin D was administered to each sheep once every 3 months. The sheep were fed once daily, between 9 and 10 a.m.

Protein (casein) and amino acid supplements were administered directly into the abomasum as a gravity drip. The protein supplement was a 6% w/v solution of "Casinal" (Glaxo Laboratories) administered as previously described over a period of approximately 8 hr each day (Reis and Schinckel 1961). The amino acid supplements were administered as aqueous solutions in a volume of 1 litre, over a period of 10–12 hr each day.

### (b) Nitrogen Estimation

Nitrogen in the casein and in the feed was estimated by the Kjeldahl method. The feed was prepared and sampled as described by Reis and Schinckel (1961).

# (c) Wool Growth

Wool growth was measured on each sheep from areas defined by tattooing; the wool was removed at intervals, usually 3 weeks, with small animal clippers (Oster, size 40). Each sheep had two tattoo patches approximately 100 cm<sup>2</sup> placed on either the shoulder or the mid-side. Some patches were subdivided by a dorsoventral line into two approximately equal areas and the wool from these areas was collected and cleaned separately. Some wool samples were lost by misadventure during cleaning and, in consequence, wool growth from the total patch areas was not available in all cases. The area from which wool growth was actually measured is given in the legends to the figures. The wool was cleaned as described by Reis and Schinckel (1961).

# (d) Sulphur in Wool

Duplicate estimates of sulphur content were made on some of the wool samples used for the measurement of wool growth rate. This wool was further cleaned by extracting with ether in a Soxhlet apparatus for 8 hr; after drying in air any trace of foreign matter remaining was removed by hand before taking a sample for analysis. Samples of c. 100 mg were dried at  $105^{\circ}$ C for 16-20 hr and the dry wool was weighed accurately prior to analysis. Sulphur was estimated by the oxygen flask method essentially as described by Macdonald (1959); the sulphuric acid produced was titrated with barium perchlorate using "Thorin" and methylene blue indicator. The concentration of hydrogen peroxide used in the oxidation was 6% w/v as suggested by Lysyj and Zarembo (1958). This technique for estimating sulphur was applied to wool by Earland (1961). The results are expressed as percentage sulphur in the dry wool. The difference between duplicate estimates of sulphur content varied from 0 to 0.08%, mean 0.04%.

### III. RESULTS

### (a) Effect of S-Amino Acid Supplements on Wool Growth

Two experiments were carried out in which supplements of S-amino acids were administered daily per abomasum, for a period of 6 weeks. In the first experiment (Fig. 1) one sheep received  $2 \cdot 0$  g L-cysteine per day. In the second experiment (Fig. 2) two sheep each received  $2 \cdot 0$  g L-cysteine per day, one sheep received  $2 \cdot 46$  g pL-methionine per day (equivalent to 2 g cysteine in terms of sulphur), and one sheep received  $0 \cdot 62$  g glycine plus  $1 \cdot 22$  g L-glutamic acid per day (each is equivalent to 1 g cysteine in terms of amino nitrogen). In both experiments the wool growth from an untreated control sheep was measured at the same time. Each sheep (except



Fig. 1.—Effect of L-cysteine per abomasum on wool growth and body weight. E107 received 610 g per day of a diet consisting of 7 parts lucerne chaff and 3 parts cracked corn; SC8 received 800 g per day of a diet consisting of equal parts lucerne chaff and wheaten chaff. During the treatment period, SC8 also received 2.0 g L-cysteine per day per abomasum; E107 was untreated. Wool growth is expressed as clean dry wool collected from the tattoo patches; the patch areas were 170 cm<sup>2</sup> (SC8) and 210 cm<sup>2</sup> (E107).

E107, experiment 1) received a constant daily diet consisting of equal parts lucerne chaff and wheaten chaff. During experiment 1, E107 was also acting as a control to other experiments run concurrently and its diet consisted of 610 g per day of a mixture of 7 parts lucerne chaff and 3 parts cracked corn. The amount of the chaff diet varied from 600 to 800 g per day for different sheep; details are given in Figures 1 and 2. The nitrogen content of the chaff diet varied from  $2 \cdot 0$  to  $2 \cdot 3$  g/100 g dry matter, which corresponded to  $11 \cdot 3$  and  $12 \cdot 9\%$  crude protein respectively on an air-dry basis.

There was a marked increase in wool growth in response to cysteine and methionine supplements and this increase was substantially greater during the second 3-weekly period of supplementation (Figs. 1 and 2). The rate of decline in wool growth, after the cessation of treatment, showed considerable individual variation. Wool growth returned to the pretreatment level with SC8 and 1390, but the wool growth of 1391 and 1392 stabilized at a higher level than the pretreatment one. The reason for this variability is not known. The control sheep showed no corres-



Fig. 2.—Effect of amino acids per abomasum on wool growth and body weight. All sheep received the chaff diet: E107, 610 g per day; 1390 and 1393, 600 g per day; 1391 and 1392, 700 g per day. Abomasal supplements during the treatment period were as follows: I390 and I392, 2.0 g L-cysteine per day; 1391, 2.46 g DL-methionine per day; 1393, 0.62 g glycine plus 1.22 g L-glutamic acid per day; E107 was untreated. Wool growth is expressed as clean dry wool collected from the tattoo patches; the patch areas were 210 cm<sup>2</sup> (E107), 140 cm<sup>2</sup> (1390), 130 cm<sup>2</sup> (1391), 140 cm<sup>2</sup> (1392), and 180 cm<sup>2</sup> (1393).

ponding increase in wool growth during the periods of cysteine and methionine supplementation. Thus, E107 (untreated control) showed no change in the rate

of wool growth in the first experiment (Fig. 1); in the second experiment (Fig. 2), while there was some variation in the rate of wool growth, the rate actually fell during the period when the other sheep received their supplements. The sheep receiving glycine and glutamic acid (1393, Fig. 2) showed no biologically significant increase in wool growth during the period of supplementation, but there was a

Expt. No.	Sheep No.	Treatment	Growth of Clean	Increase in Wool Growth		S-Amino Acid
			(g/day)	(g/day)	(%)	(%)*
1 (Fig. 1)	SC8	None Cysteine	$\begin{array}{c c} 8 \cdot 3 \\ 11 \cdot 2 \\ 2 \cdot 9 \end{array}$		35	29
2 (Fig. 2)	1390	None Cysteine	3 · 6 6 · 3	2.7 75		25
	1391	None Methionine	3 · 0 6 · 9	3-9	130	29
	1392	None Cysteine	$3 \cdot 2$ $4 \cdot 9$	1.7	53	15
	1393	None Glycine and glutamic acid	$2 \cdot 5$ $2 \cdot 9$	0.4	16	
3 (Fig. 3)	E122	None Casein	4.5 9.1	4.6	102	40
	SC8	None Casein	$7 \cdot 9 \\ 14 \cdot 5$	6.6	84	50

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EFFECT OF NITROGENOUS SUPPLEMENTS GIVEN PER ABOMASUM ON WOOL GROWTH Data are derived from the experiments presented in Figures 1, 2, and 3; experimental details are given with these figures. The method of calculating the data is described in the text

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

marked rise in the level of wool growth after the cessation of treatment. No explanation can be offered for this increased level of wool growth, which was maintained for the 12 weeks observed. The fact that there were no significant increases in the rate of wool growth of any of the control sheep during the treatment period supports the conclusion that the increases in wool growth obtained with the treated sheep were due to cysteine and methionine.

There was a small increase in the body weight during the periods of cysteine and methionine supplementation, but the body weights of the control sheep (E107, 1393) did not change during this period (Figs. 1 and 2).

The responses to the cysteine and methionine supplements are summarized in Table 1, experiments 1 and 2. The percentage increases in wool growth which can be ascribed to the supplements (35-130%) were calculated by using a mean value for wool growth during the periods prior to treatment (6-12 weeks) and using the wool growth during the second 3-weekly period of treatment. This value may not be maximal as the amino acid supplements were not continued until a new stable level of wool growth was reached. The total wool growth per sheep was estimated by multiplying the tattoo patch production for relevant periods by a factor relating patch weight to whole fleece weight. These factors were not experimentally determined in these observations but were the same as those determined previously in another group of sheep of similar size (Reis and Schinckel 1961). The estimates of total wool growth are unlikely to be in error by more than 10%. Any such error will not affect the estimates of the percentage increase in wool growth. The last column of Table 1 shows the percentage of the S-amino acid supplement which can be accounted for in the wool grown during the last 3 weeks of supplementation, assuming that cystine accounts for 90% of the sulphur content. The basis for this assumption is considered later. The estimated cystine contents of the wool grown during supplementation (as a percentage of clean dry wool weight) were: 1390, 13.5%; 1391,  $12 \cdot 8\%$ ; 1392,  $13 \cdot 0\%$ ; SC8,  $12 \cdot 4\%$ .

# (b) Effect of S-Amino Acid Supplements on the Sulphur Content of Wool

The sulphur content of the wool was determined in samples taken prior to treatment (wool clipped 3 weeks before commencement of treatment) and in samples taken during the last 3 weeks of amino acid supplementation (Table 2). There was an increase (24-35%) in the sulphur content during the periods of cysteine and methionine supplementation, in all cases. Moreover, there was no increase in the sulphur content of the two control sheep (E107, untreated; 1393, receiving glycine and glutamic acid) clipped at corresponding intervals. Also, there was no increase in the sulphur content of the wool from 1393 (sample 3, Table 2) clipped 9 weeks after the amino acid supplementation period ended, when wool growth had increased for an unexplained reason. The mean difference between duplicate estimates of sulphur content was 0.04 (S.D.  $\pm 0.022$ )% sulphur, while the mean difference between the two sample sites was 0.05 (S.D.  $\pm 0.030$ )% sulphur. The mean difference between supplemented (cysteine, methionine, or casein) and control periods was  $\pm 0.73$  (S.E.  $\pm 0.08$ )% sulphur (calculated within sheep) and  $\pm 0.73$  (S.E.  $\pm 0.05$ )% sulphur (calculated within sites).

# (c) Effect of a Casein Supplement on Wool Growth and Sulphur Content of Wool

The administration of an abomasal supplement of 60 g "Casinal" per day for 6 weeks resulted in substantial increases in wool growth (84 and 102%) and in body weight in the two sheep studied (Fig. 3 and Table 1). The increased weights of wool grown during case in supplementation shown in Table 1 were calculated in the same manner as the values presented in experiments 1 and 2. The percentage of the S-amino acids, supplied in the case in, which can be accounted for in the wool

grown during supplementation, was very high (40 and 50%). The amount of S-amino acids supplied by the case in supplement was calculated by using the values of Block

#### TABLE 2

# SULPHUR CONTENT OF WOOL

Sulphur content of wool grown during three experiments (see Figs. 1, 2, and 3 for experimental details); the wool samples were collected 3 weeks before the commencement of treatment (sample 1) and at the end of the treatment period (sample 2). Sample 3 (sheep No. 1393) was collected 9 weeks after treatment had ceased

Experiment No.	Sheep No.	Sample	Treatment	Sulphur Content (%)			Increase
				Sampling Position			in Sulphur Content due to
				(a) Right Shoulder	(b) Left Shoulder	Mean	Treatment (%)
1	SC8	I	None	2.92	2.89*	2.90	
(Fig. 1)		2	Cysteine	3.68	3.68*	3.68	26-9
	E107	1	None	3.09	3.04*	3.06	
		2	None	$2 \cdot 98$	3 · 06*	$3 \cdot 02$	
2	1390	1	None	2.99	2.92	2.96	
(Fig. 2)		2	Cysteine	$3 \cdot 97$	<b>4</b> · 01	$3 \cdot 99$	34.8
	1391	1	None	$3 \cdot 05$	3.09	3.07	_
		2	Methionine	$3 \cdot 82$	$3 \cdot 79$	3 · 80	23.8
	1392	1	None	$3 \cdot 07$	3.13	$3 \cdot 10$	
		2	Cysteine	$3 \cdot 83$	3 · 84	3 · 84	$23 \cdot 9$
	1393	1	None	$3 \cdot 41$	$3 \cdot 35$	3.38	_
		2	Glycine and glutamic acid	3.18	3.17	3.18	
		3	None	$3 \cdot 03$	$2 \cdot 99$	3.01	—
	E107	1	None	3.33	3 · 22*	3.28	-
		2	None	$3 \cdot 14$	3.08*	$3 \cdot 11$	—
3	E122	1	None	3.23	3.34	3.28	
(Fig. 3)		2	Casein	$3 \cdot 89$	3.94	$3 \cdot 92$	19.5
	SC8	1	None	$3 \cdot 01$	2.97*	$2 \cdot 99$	_
		2	Casein	$3 \cdot 43$	3 · 46*	$3 \cdot 44$	15 · 1
	1	1					

\* Sampling position (b) was the right mid-side.

and Bolling (1951). Thus, 60 g "Casinal" (equivalent to 54 g protein) contains  $3 \cdot 5$  g methionine and  $0 \cdot 4$  g cystine per 16 g nitrogen. Expressed as cystine equivalents, the "Casinal" supplies c.  $1 \cdot 75$  g cystine per day.

Table 2 (experiment 3) gives the sulphur content of wool prior to treatment (samples clipped 6 weeks before the commencement of casein administration) and of wool grown during the last 3 weeks of casein supplementation. There was an increase (15-20%) in the sulphur content of the wool during the period of casein administration.



Fig. 3.—Effect of casein per abomasum on wool growth and body weight. Both sheep received the chaff diet: E122, 600 g per day; SC8, 800 g per day. Casein, 60 g per day per abomasum, was administered during the treatment period. Wool growth is expressed as clean dry wool collected from the tattoo patches; the patch areas were 190 cm<sup>2</sup> (E122) and 230 cm<sup>2</sup> (SC8). Note that different scales are used for the wool growth ordinates.

### IV. DISCUSSION

### (a) Effects on Wool Growth

The diet of 600-800 g chaff supplied the equivalent of 2-3 g cystine per day (cystine plus methionine expressed as cystine, values from Marston (1948) and Block and Bolling (1951) being used). Thus, the infusion of 2 g cysteine per day is unlikely to be an unphysiological amount although it may represent a substantial increase in the amount of S-amino acid reaching the abomasum.

The supplements of cysteine and methionine resulted in increases of 35-130%in the rate of wool growth. While only a small number of sheep was used in these studies the differences obtained were so large that there is no doubt regarding their biological significance. These results are in agreement with those of Marston (1935), who found that L-cysteine administered subcutaneously stimulated wool growth, and support the concept that the amount of S-amino acids absorbed by the body may be an important factor limiting the rate of wool growth, at least on moderate intakes of feed. The failure of several workers to obtain significant increases in wool growth by adding cystine to the diet was possibly due to microbial breakdown of cystine in the rumen, similar to that reported by el-Shazly (1952), Lewis (1955), and Lewis and Elsden (1955) for several amino acids and casein hydrolysate.

The efficiency of recovery of the cysteine and methionine supplements in the wool ranged from 15 to 29% for individual sheep. This is remarkably high for any biological process. This observation is in agreement with that of Downes (1961b) who was able to recover 30% of an intravenous dose of L-[<sup>35</sup>S]cystine in the wool. The values in Table 1 have been calculated on the assumption that 90% of the sulphur in wool is present as cystine. Since estimates of the amount of sulphur in wool which can be accounted for as cystine are variable (Marston 1928a; Simmonds 1954, 1955, 1956; Earland 1961), the value of 90% was used as a reasonable approximation. Most analyses indicate that methionine accounts for c. 3% of the sulphur in wool, so that the maximum value for cystine would be 97% of the sulphur content. It is probable that sulphur compounds, other than cystine and methionine, isolated from wool hydrolysates are derived from cystine (Earland 1961; Fletcher and Robson 1961), but the position is still uncertain.

Although only one sheep received methionine, it seems clear that DL-methionine can completely replace an equivalent amount of L-cysteine. This was not unexpected as it is known that both D- and L-methionine can be converted to cysteine, via homocysteine and cystathionine (Berg 1953).

The mechanism of action of the cysteine and methionine supplements in stimulating wool growth is obscure, but several possibilities exist:

(1) There may be some general anabolic effect which indirectly affects keratin synthesis in the follicles. There is an indication of such a general anabolic effect from the fact that the body weight rose by  $1 \cdot 5-2$  kg in all sheep receiving cysteine and methionine, during the 6 weeks of supplementation.

(2) There may also be direct effects on keratin synthesis in the follicles. Cyst(e)ine may be the limiting amino acid for keratin synthesis as suggested by Marston (1935, 1948, 1955), and the treatment may simply increase the supply of substrate available for keratin synthesis. The increase in the sulphur content (and hence presumably the cystine) of the fibres, as distinct from the increased rate of fibre growth, is evidence that at least part of the wool growth response obtained is due to augmentation of substrate supply, although this is not necessarily the primary mechanism of action. There may also be some other specific effect of cyst(e)ine, or of the sulphur or sulphydryl component of the molecule, in the follicle. This effect may be a stimulation of mitotic activity in the follicle bulb, as there is much evidence that sulphydryl groups play an important role in mitosis (Stern 1959; Mazia 1959, 1961). Also, there may be increased production of co-factors important in protein or energy metabolism. Thus, cysteine is involved in the synthesis of glutathione and coenzyme A.

(3) Another possible function of cyst(e)ine may be the stimulation of keratinization by the provision of sulphydryl groups. For this mechanism to operate one would need to assume that the S-amino acids are incorporated into preformed fibrous proteins during the keratinization phase and also that this step is rate-limiting. However, DeBersaques and Rothman (1962) have cast doubt on the theory that cyst(e)ine is incorporated into preformed protein.

The observation that the rate of wool growth was greater during the second three weeks of supplementation (Figs. 1, 2, and 3) could be associated with the time lag necessary for the wool to emerge from the skin and appear in the clippings, following stimulation of the growth rate. However, Downes (1961*a*) has suggested that there is a metabolic "pool" of cystine associated with the wool follicles. Thus, wool growth may be buffered to some extent against fluctuations in the supply of cystine and the observed lag in the response to S-amino acids may also be associated with this pool.

The considerable increase in the rate of wool growth obtained when casein was fed per abomasum confirms our previous results (Reis and Schinckel 1961). The extra energy available from the casein was far too small to account for the increases in wool growth which were obtained. Thus, Graham (personal communication), using the same diet, showed that increasing the daily intake from 600 g to 800 g supplied an extra 450 kcal available energy per day (allowing for indigestibility and losses as methane) and resulted in only a 17% increase in the rate of wool growth. In contrast, the casein supplement, which supplied only 310 kcal gross energy, almost doubled the rate of wool growth in the experiments reported here.

The casein supplement supplied approximately 1.75 g cystine daily (cystine plus methionine expressed as cystine). Thus, the casein supplement is approximately equivalent to the S-amino acid supplements given in these experiments in terms of S-amino acids. However, casein supplementation resulted in a greater increase in the rate of wool growth on an absolute weight basis (Table 1). Also, a remarkably high proportion of the S-amino acids in the casein (40-50%) was recovered in the wool grown during supplementation. This recovery was greater than that obtained with the S-amino acid supplements. The stimulation of wool growth obtained when casein is administered per abomasum may be primarily a response to S-amino acids. The greater response obtained with casein, compared with an equivalent amount of cysteine, could be due to the simultaneous presence of other essential amino acids which allowed more efficient utilization of the S-amino acids. The proportion of the casein nitrogen that can be accounted for in the wool grown during supplementation is also high (8.5-12.2%).

The results obtained with supplements of casein and S-amino acids indicate that these sources of nitrogen do result in a substantial increase in wool growth when administered in such a way as to be available to the animal, i.e. by abomasal, intestinal, or parenteral routes. Such supplements may not result in increased wool growth when added to the diet and exposed to runnial microbial activity. This may account for the lack of wool growth response to levels of dietary protein above 8% in the experiments reported by Ferguson (1959). It thus appears that protein, apart from its energy value, can specifically stimulate wool growth and that the S-amino acids may be especially important. However, the specific function of cyst(e)ine in stimulating wool growth is not known at present.

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# (b) Effects on Sulphur Content of Wool

The results in Table 2 show that the sulphur content of wool can vary over a wide range, and that the administration of cysteine, methionine, and casein per abomasum can substantially increase the sulphur content. So far as we are aware, this is the first report that the sulphur content of wool has been varied by controlled experimental means. While there is still some doubt regarding the partition of sulphur in wool it can be assumed that the variations in sulphur content observed in these experiments are largely due to variations in the cystine content.

The increase in sulphur content of the wool during casein supplementation was slightly smaller than that obtained with the cysteine and methionine supplements. This smaller increase in sulphur might be associated with a "dilution" of the sulphur in the wool due to the greater increase in wool growth obtained with casein, possibly as a consequence of the supply of an additional factor limiting wool growth. Thus, there was an increase in the sulphur content of the wool in every case during abomasal administration of casein and S-amino acids and this increase was associated with an increased level of wool growth. These results do not agree with those of Ross (1961), who claimed that the sulphur content of wool was highest when wool production was lowest and vice versa. However, his results do not wholly support this claim. Further, there seems to be no justification for the author's suggestion that "within wide limits it is probable that the availability of sulphur is not a limiting factor in wool production". Thus, an increase in feed intake will increase wool growth and, if sulphur (i.e. cystine) is not adequately available from the extra feed, this increased wool growth might be accompanied by a fall in the sulphur content of the wool. This does not mean that sulphur is not a factor limiting wool growth, and under these conditions a supplement of cystine, available for absorption by the animal, may further increase wool growth and the sulphur content of the wool.

There is no evidence at present whether the variations in the sulphur content of wool are associated with any change in the internal structure of the fibres. Wool fibres can be degraded into several components of which the largest two are a-keratose and  $\gamma$ -keratose (Alexander and Hudson 1954). The a-keratose contains c.  $2\cdot5\%$ sulphur, while the  $\gamma$ -keratose contains c.  $6\cdot0\%$  sulphur. 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, presumably  $\gamma$ -keratose. The alternative would be a uniform change in the sulphur content of these protein fractions.

# V. ACKNOWLEDGMENTS

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### VI. References

- ALEXANDER, P., and HUDSON, R. F. (1954).—"Wool: Its Chemistry and Physics." (Chapman and Hall: London.)
- BARRITT, J., and KING, A. T. (1926) .-- J. Text. Inst. 17: T394.

BARRITT, J., and KING, A. T. (1929).-J. Text. Inst. 20: T151.

- BARRITT, J., KING, A. T., and PICKARD, J. N. (1930).-Biochem. J. 24: 1061.
- BARRITT, J., and RIMINGTON, C. (1931).-Biochem. J. 25: 1072.
- BERG, C. P. (1953).-Physiol. Rev. 33: 145.
- BLOCK, R. J., and BOLLING, D. (1951).—"The Amino Acid Composition of Proteins and Foods." 2nd Ed. (C.C. Thomas: Springfield, Illinois.)
- BRAILSFORD ROBERTSON, T. (1928) .--- Coun. Sci. Industr. Res. Aust. Bull. No. 39. pp. 5-13.
- DEBERSAQUES, J., and ROTHMAN, S. (1962).-Nature 193: 147.
- Downes, A. M. (1961a).-Aust. J. Biol. Sci. 14: 109.
- Downes, A. M. (1961b) .- Aust. J. Biol. Sci. 14: 427.
- DU TOIT, P. J., MALAN, A. I., GROENEWALD, J. W., and BOTHA, M. L. (1935).—Onderstepoort J. Vet. Sci. 4: 229.
- EARLAND, C. (1961).-Text. Res. J. 31: 492.
- FERGUSON, K. A. (1959).-Nature 184: 907.
- FLETCHER, J. C., and ROBSON, A. (1961).-Biochem. J. 80: 37P.
- FRASER, A. H. H., and ROBERTS, J. A. F. (1933).-J. Agric. Sci. 23: 97.
- KOYANAGI, T., and TAKANOHASHI, T. (1961) .--- Nature 192: 457.
- LEWIS, D. (1955).-Brit. J. Nutr. 9: 215.
- LEWIS, D., and ELSDEN, S. R. (1955).-Biochem. J. 60: 683.
- LYSYJ, I., and ZAREMBO, J. E. (1958).—Analyt. Chem. 30: 428.
- MACDONALD, A. M. G. (1959).-Industr. Chem. 35: 33.
- MARSTON, H. R. (1928a) .- Coun. Sci. Industr. Res. Aust. Bull. No. 38.
- MARSTON, H. R. (1928b).-Coun. Sci. Industr. Res. Aust. Bull. No. 39. pp. 13-51.
- MARSTON, H. R. (1932a) .- Coun. Sci. Industr. Res. Aust. Bull. No. 61.
- MARSTON, H. R. (1932b).-Aust. J. Exp. Biol. Med. Sci. 9: 235.
- MARSTON, H. R. (1935) .--- J. Agric. Sci. 25: 113.
- MARSTON, H. R. (1948).-Aust. J. Sci. Res. B1: 362.
- MARSTON, H. R. (1955).—"Progress in the Physiology of Farm Animals." (Ed. J. Hammond.) Ch. 11. (Butterworths Scientific Publications: London.)
- MAZIA, D. (1959).—"Sulfur in Proteins." (Eds. R. Benesch et al.) Ch. VII. I. (Academic Press Inc.: New York.)
- MAZIA, D. (1961).—"The Cell." (Eds. J. Brachet and A. E. Mirsky.) Vol. 3. pp. 77–412. (Academic Press Inc.: New York.)
- REIS, P. J., and SCHINCKEL, P. G. (1961).-Aust. J. Agric. Res. 12: 335.
- Ross, D. A. (1961).-Proc. N.Z. Soc. Anim. Prod. 21: 153.
- EL-SHAZLY, K. (1952).—Biochem. J. 51: 647.
- SIMMONDS, D. H. (1954).-Aust. J. Biol. Sci. 7: 98.
- SIMMONDS, D. H. (1955).-Aust. J. Biol. Sci. 8: 537.
- SIMMONDS, D. H. (1956).—Proc. Int. Wool Text. Res. Conf. Aust. 1955. Vol. C. p. C-65.
- SLEN, S. B., and WHITING, F. (1952) .- J. Anim. Sci. 11: 156.
- STERN, H. (1959).—"Sulfur in Proteins." (Eds. R. Benesch et al.) Ch. VII. 2. (Academic Press Inc.: New York and London.)