THE GROWTH AND COMPOSITION OF WOOL

IV.* THE DIFFERENTIAL RESPONSE OF GROWTH AND OF SULPHUR CONTENT OF WOOL TO THE LEVEL OF SULPHUR-CONTAINING AMINO ACIDS GIVEN PER ABOMASUM

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Summary

Various amounts of DL-methionine or L-cysteine were infused directly into the abomasum of sheep as supplements to a diet of chopped wheaten and lucerne hay, and effects on wool growth and sulphur content of wool were measured.

Small amounts (0·5–2·0 g/day of L-cysteine or equimolar amounts of DL-methionine) increased wool growth by as much as 100%. Similar maximum levels of wool growth were obtained with cysteine or methionine. When larger amounts of L-cysteine (6·0–8·0 g/day) were given, the rate of wool growth was reduced to slightly below the maximum response. Administration of equimolar amounts of DL-methionine caused a substantial reduction in wool growth; in one experiment the rate of wool growth was depressed to slightly below the pretreatment values.

The proportion of the supplementary sulphur accounted for in the extra wool grown was inversely related to the amount of amino acid given. With 2·0 g/day of L-cysteine (or equivalent DL-methionine) 23–49% of the supplementary sulphur was recovered in wool.

D-Methionine and methionine hydroxy analogue (MHA) were also effective in stimulating wool growth; D-methionine appeared to be less effective than DL-methionine whereas MHA was equivalent to DL-methionine.

All sulphur supplements were effective in increasing the sulphur content of wool and similar maximum values were obtained with each supplement. In contrast to effects on wool growth, high levels of L-cysteine or DL-methionine did not depress the sulphur content of wool.

I. Introduction

Previous work has shown that small amounts of sulphur-containing amino acids (S-amino acids), administered directly into the abomasum, substantially increase wool growth and the sulphur content of wool (Reis and Schinckel 1963, 1964). Both L-cysteine and DL-methionine were effective when given as a supplement to a basal diet of chopped hay either alone or added to protein given per abomasum. The importance of the amount of amino acid given was suggested by the observation that 1·5 g/day L-cysteine stimulated wool growth slightly more than did 3·0 g/day L-cysteine (Reis and Schinckel 1964).

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The present study was undertaken to compare the relative effectiveness of cysteine and methionine and to determine the importance of level of S-amino acid supplementation. Various levels of L-cysteine and DL-methionine were given as abomasal supplements to a basal diet of chopped wheaten and lucerne hay, and effects on wool growth and the sulphur content of wool were measured. The effectiveness of D-methionine and of a methionine analogue was also tested. There was an optimal amount of S-amino acid for wool growth, whereas the sulphur content of wool was not reduced at high levels of S-amino acid supplementation.

II. Experimental

(a) *Sheep and Diet*

The experimental animals were mature English Leicester × Merino wethers; each sheep had an abomasal fistula fitted with a cannula. The sheep were kept in metabolism cages in a room maintained at a temperature of 23±1°C. A supplement of 1 million i.u. of vitamin D was administered to each sheep once every 3 months. The diet consisted of equal parts chopped lucerne hay and chopped wheaten hay, and a daily ration of 800 g was offered between 9 and 10 a.m. The nitrogen content of the diet was 1·90 (S.D. ±0·10) g/100 g dry matter (during a 50-week control period), 2·21 (S.D. ±0·12) g/100 g dry matter (experiments 1, 2, and 5), and 1·86 (S.D. ±0·04) g/100 g dry matter (experiments 3, 4, 6, and 7); these values corresponded to 10·8, 12·5, and 10·6% crude protein respectively on an air-dry basis. The sheep were weighed once weekly prior to feeding. The body weights during each experiment were corrected for cumulative wool growth using the data for clean wool growth recalculated to greasy wool.

(b) *Abomasal Supplements*

Aqueous solutions of amino acids (DL-methionine, D-methionine, or L-cysteine) were administered as gravity drips, volume 1–2 litres, over a period of 10–12 hr each day, commencing when the sheep were fed. Amounts of L-cysteine up to 2 g/day were supplied as L-cysteine hydrochloride (1·3 g supplies 1·0 g L-cysteine); amounts above 2 g/day were given as L-cysteine to restrict the amount of acid infused. Thus, 8·0 g/day L-cysteine was given as 2·6 g L-cysteine hydrochloride plus 6·0 g L-cysteine. Methionine hydroxy analogue (MHA) was in the form of a calcium salt (calcium DL-2-hydroxy-4-methylthiobutyrate; Monsanto Chemical Co.). As MHA could not be dissolved completely in aqueous, acidic, or alkaline solutions, it was administered in doses of 0·5 g in a gelatin capsule inserted through the abomasal cannula at intervals of 2 hr commencing at about 8 a.m. each day. Six capsules per day supplied *c*. 0·8 g gelatin, which would have no effect on wool growth or sulphur content of the wool (Reis and Schinckel 1964).

The experimental treatments are described in Table 1. The amounts of methionine infused in experiments 1, 2, and 3 were calculated as molar equivalents of amounts of cysteine ranging from 0·5–8·0 g. Thus, 1·23 g methionine is equivalent to 1·0 g cysteine. The amount of MHA given in experiment 7 (3·0 g) is equivalent to 2·52 g methionine or 2·05 g cysteine, when allowance is made for the calcium content and assuming 95% purity.

Table 1

ABOMASAL SUPPLEMENTS

Expt. No.	Sheep No.	Abomasal Supplement (g/day)	Period of Supplementation	
			No. of Weeks	Time of Year
1	SD67	DL-Methionine		
		0	14	Oct. 4–Jan. 10
		2·46	8	Jan. 10–Mar. 6
		4·92	8	Mar. 6–May 1
		9·84	6	May 1–June 12
		2·46	8	June 12–Aug. 7
		1·23	8	Aug. 7–Oct. 2
2	1024	DL-Methionine		
		0	10	June 12–Aug. 21
		2·46	9	Aug. 21–Oct. 23
		7·38	7	Oct. 23–Dec. 11
		0·615	7	Dec. 11–Jan. 29
	1038	DL-Methionine		
		0	10	June 12–Aug. 21
		7·38	9	Aug. 21–Oct. 23
		2·46	7	Oct. 23–Dec. 11
		0·615	7	Dec. 11–Jan. 29
3	SD67	DL-Methionine		
		0	6	June 24–Aug. 5
		0·615	7	Aug. 5–Sept. 23
		9·84	7	Sept. 23–Nov. 11
	1038	DL-Methionine		
		0	6	Aug. 12–Sept. 23
		7·38	7	Sept. 23–Nov. 11
4	SD67	L-Cysteine		
		0	8	Aug. 20–Oct. 15
		0·5	8	Oct. 15–Dec. 10
		2·0	8	Dec. 10–Feb. 4
		8·0	8	Feb. 4–Apr. 1
5	1392	L-Cysteine		
		0	8	June 24–Aug. 21
		2·0	9	Aug. 21–Oct. 23
		6·0	7	Oct. 23–Dec. 11
6	1038	D-Methionine		
		0	10	Dec. 3–Feb. 11
		1·0	6	Feb. 11–Mar. 25
7	1024	Methionine hydroxy analogue		
		0	8	Dec. 17–Feb. 11
		3·0	8	Feb. 11–Apr. 8

(c) *Wool Growth*

Wool growth was measured on each sheep from areas defined by tattooed lines; the wool was removed at intervals with small animal clippers (Oster, size 40). The interval between clippings was 2 weeks, with the exception of pretreatment samples in experiment 1 (3 and 4 weeks), and the first clipping after a treatment change in experiments 2, 3, and 5 (1 week). The wool from these weekly clippings was discarded as it was considered to be wool grown on the previous treatment. The sheep had a tattooed patch approximately 100 cm^2 (10 by 10 cm) on each shoulder. During the experiments, the patch areas were measured on three occasions (experiments 3 and 5), four occasions (experiments 2, 4, 6, and 7), or seven occasions (experiment 1). The mean of the various estimates, which did not differ significantly, was used to express wool growth as milligrams clean dry wool/cm^2/day.

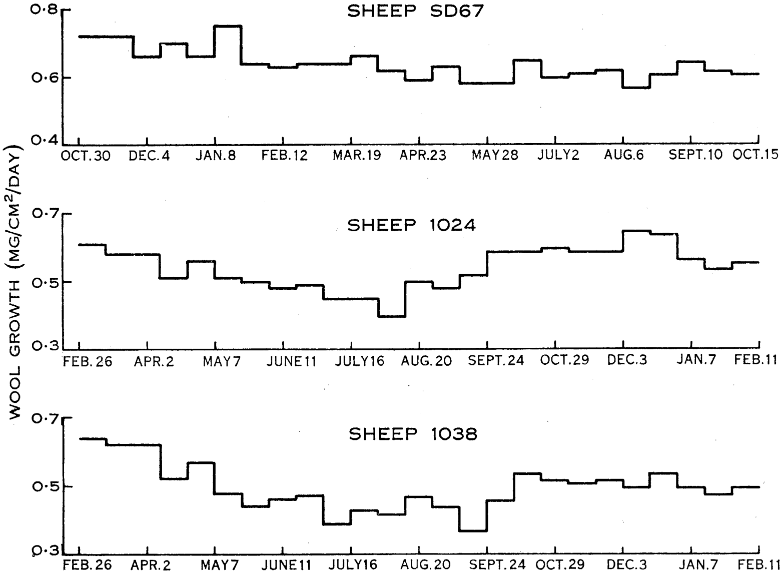

Fig. 1.—Seasonal pattern of wool growth for three sheep receiving 800 g/day chopped hay for 50 weeks. Wool growth is expressed on the basis of clean dry wool.

Total growth of clean dry wool per sheep (g/day) was estimated as described by Reis and Schinckel (1964). Pretreatment wool growth was the mean for 6 weeks prior to treatment (8 weeks, experiment 1); treatment values were the mean wool growth rate for the last 4 weeks of each treatment period. These values for wool growth were used to calculate the proportion of the supplementary sulphur recovered in wool, on the assumption that all the extra sulphur incorporated into wool during supplementation was derived from the supplement. It is recognized that this calculation is an oversimplification and ignores metabolic pools and utilization of endogenous sulphur. In calculating the increased output of sulphur in wool during treatment account was taken of the increased sulphur content of the wool. The sulphur value used was the mean of the two values for each treatment.

Wool samples were cleaned by washing twice with light petroleum (Shell X4, b.p. 56–73°C), once with a wetting agent (*c.* 2% Shell Teepol), followed by thorough rinsing in distilled water and finally ethanol. The clean wool was dried to constant weight at *c.* 80°C.

(*d*) *Analytical*

Sulphur content of wool (expressed as percentage in clean dry wool) was determined in duplicate samples by the procedure of Reis and Schinckel (1963). All values plotted are the mean of values for the two shoulder sites. There were no significant differences between values from the two sites.

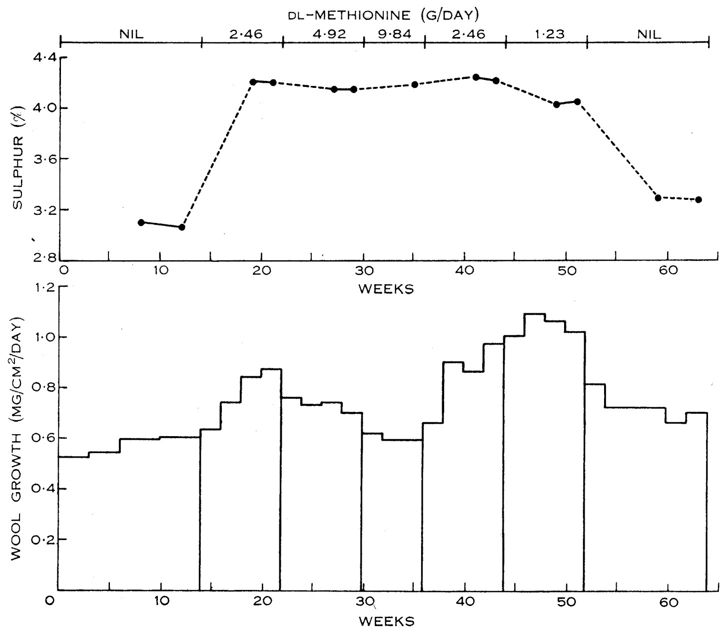

Fig. 2.—Effect of DL-methionine per abomasum on growth and sulphur content of wool. Sheep SD67, experiment 1, diet 800 g/day chopped hay. Broken lines joining sulphur values indicate that some wool samples, collected between these points, were not analysed. Wool growth and sulphur are expressed on the basis of clean dry wool.

Nitrogen content of the diet was determined by a Kjeldahl method as described by Reis and Schinckel (1961).

III. Results

(*a*) *Pattern of Wool Growth during Control Period*

The seasonal patterns of wool growth for three sheep, given the chopped hay diet alone for 50 weeks, are shown in Figure 1. For each sheep the observations commenced 4 weeks after a methionine infusion had stopped and wool growth rate may still have been decreasing following this treatment. After this initial drop, sheep

SD67 showed no seasonal trend in wool growth. Sheep 1024 and 1038 did show some seasonal rhythm of wool growth, with a depressed rate of growth during winter (June–August).

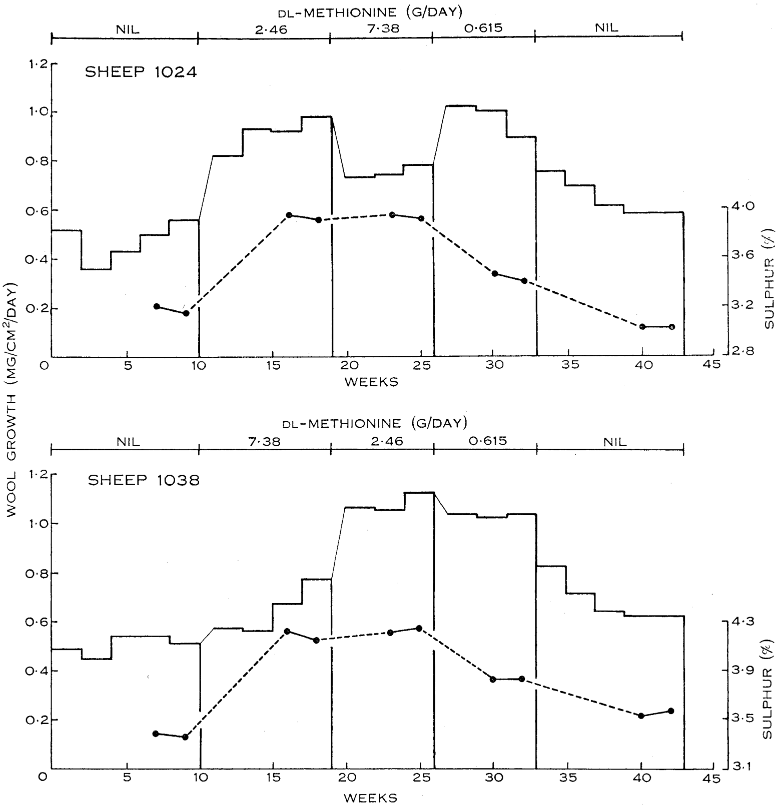

Fig. 3.—Effect of DL-methionine per abomasum on growth and sulphur content of wool. Sheep 1024 and 1038, experiment 2, diet 800 g/day chopped hay. Broken lines joining sulphur values indicate that some wool samples, collected between these points, were not analysed. Wool growth and sulphur are expressed on the basis of clean dry wool.

(b) *Effect of Varying Amounts of Sulphur-containing Amino Acids on Growth and Sulphur Content of Wool*

(i) DL-*Methionine*.—Experiments 1, 2, and 3 (Figs. 2, 3, and 4) show the effects of abomasal administration of amounts of DL-methionine ranging from 0·615–9·84 g/day (equivalent to 0·5–8·0 g L-cysteine). In these experiments there were substantial effects on growth and sulphur content of wool. Wool growth was approximately doubled with the most effective level of methionine supplementation (0·615–2·46 g/day) for each sheep (Table 2). In experiments 1 and 2 (Figs. 2 and 3) levels of DL-methionine above 2·46 g/day produced a smaller increase in wool growth, and with 9·84 g/day (Fig. 2) wool growth was the same as the pretreatment value.

In experiment 3 (Fig. 4) high levels of DL-methionine were repeated and with both sheep wool growth was depressed slightly below pretreatment values. This experiment also showed that 0·615 g DL-methionine, given as the first level of supplementation, produced a response similar to that obtained with 2·46 g

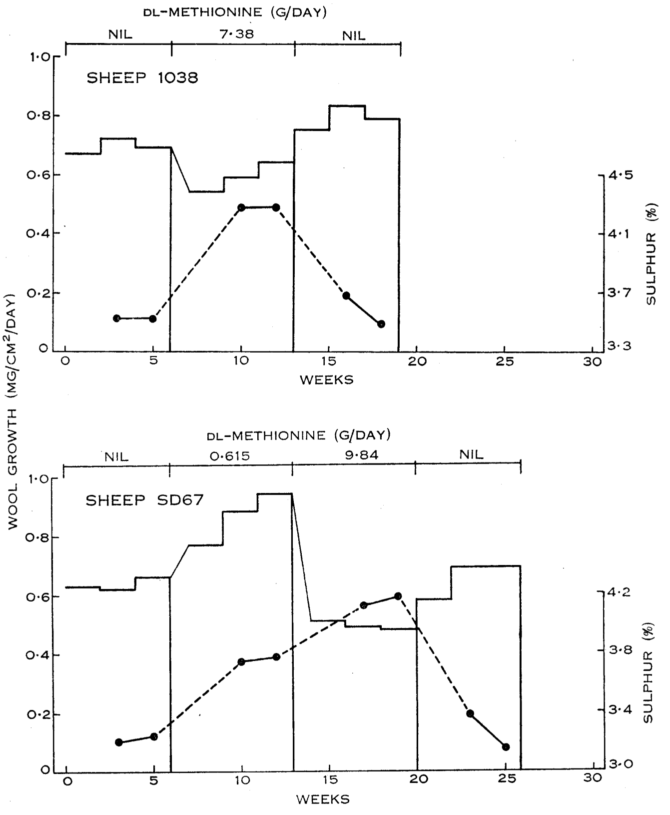

Fig. 4.—Effect of DL-methionine per abomasum on growth and sulphur content of wool. Sheep SD67 and 1038, experiment 3, diet 800 g/day chopped hay. Broken lines joining sulphur values indicate that some wool samples, collected between these points, were not analysed. Wool growth and sulphur are expressed on the basis of clean dry wool.

DL-methionine (Table 2, experiments 1 and 3). Experiment 3 also demonstrated that there was no significant stimulation of wool growth following a high level of supplementation with DL-methionine. With sheep SD67 wool growth quickly returned to about the pretreatment level when supplementation stopped, while with sheep 1038 wool growth returned to a level slightly above the pretreatment value.

There are insufficient data to make a precise estimate of the optimum level of DL-methionine for stimulation of wool growth on this diet. Sheep SD67 grew most wool when given 1·23 g methionine (Fig. 2), but wool growth was only slightly lower with 2·46 g (Fig. 2) and 0·615 g (Fig. 4). Also, sheep 1024 and 1038 responded

Table 2

EFFECT OF S-AMINO ACIDS GIVEN PER ABOMASUM ON GROWTH AND SULPHUR CONTENT OF WOOL

Details of the experiments are given in Table 1 and in Figures 2–5

Expt. No.	Sheep No.	Abomasal Supplement (g/day)	Growth of Clean Dry Wool (g/day)	Increase in Wool Growth (%)	Sulphur Content of Wool (%)	Recovery of Supplementary Sulphur in Wool (%)*
1	SD67	DL-Methionine				
		0	4·1	—	3·08	—
		2·46	5·9	44	4·20	23
		4·92	5·0	22	4·14	7
		9·84	4·1	0	4·18	2
		2·46	6·3	54	4·22	26
		1·23	7·2	76	4·04	62
2	1024	DL-Methionine				
		0	4·8	—	3·20	—
		2·46	9·2	92	3·94	39
		7·38	7·3	52	3·94	8
		0·615	9·1	90	3·45	120
	1038	DL-Methionine				
		0	5·0	—	3·37	—
		7·38	6·7	34	4·18	7
		2·46	10·2	104	4·22	49
		0·615	9·6	92	3·82	149
3	SD67	DL-Methionine				
		0	4·5	—	3·22	—
		0·615	6·4	42	3·76	72
		9·84	3·4	−24	4·15	0
	1038	DL-Methionine				
		0	6·2	—	3·52	—
		7·38	5·5	−11	4·27	1
4	SD67	L-Cysteine				
		0	4·4	—	3·17	—
		0·5	5·8	32	3·60	52
		2·0	7·4	68	4·18	32
		8·0	6·6	50	4·29	7
5	1392	L-Cysteine				
		0	5·0	—	3·31	—
		2·0	8·1	62	4·10	31
		6·0	7·3	46	4·18	9
6	1038	D-Methionine				
		0	4·6	—	3·42	—
		1·0	7·0	52	4·18	63
7	1024	Methionine hydroxy analogue				
		0	5·4	—	2·99	—
		3·0	9·6	78	3·77	37

* i.e. percentage of the sulphur in the supplement accounted for in the wool grown during supplementation.

similarly to 2·46 and 0·615 g methionine (Fig. 3). Thus, it can be suggested tentatively that maximal stimulation of wool growth on this diet with methionine per abomasum was obtained with *c.* 1·0–2·0 g/day.

In contrast to effects on wool growth, all amounts of DL-methionine above 1·23 g/day produced a "maximal" increase in sulphur content of wool (Figs. 2, 3, and 4; Table 2). Sulphur contents were only slightly reduced with 1·23 g methionine (Fig. 2) and were substantially reduced, but still above basal levels, with 0·615 g methionine (Figs. 3 and 4).

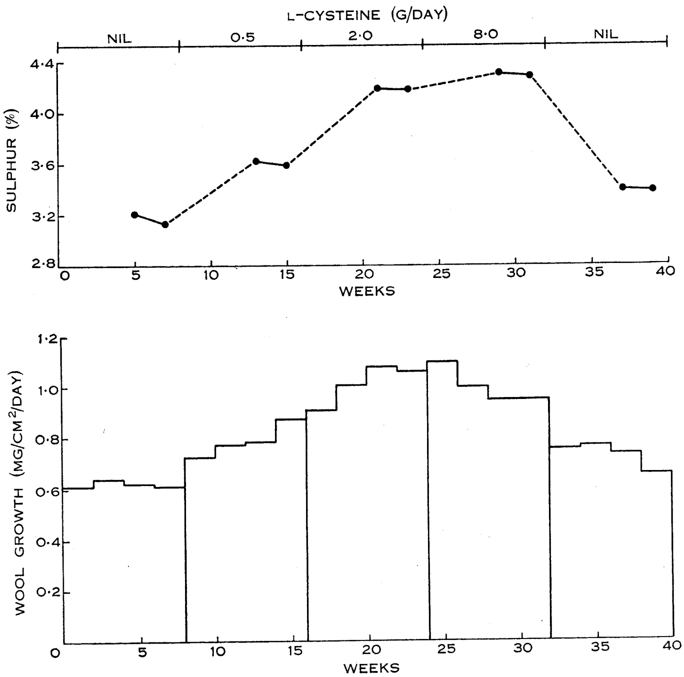

Fig. 5.—Effect of L-cysteine per abomasum on growth and sulphur content of wool. Sheep SD67, experiment 4, diet 800 g/day chopped hay. Broken lines joining sulphur values indicate that some wool samples, collected between these points, were not analysed. Wool growth and sulphur are expressed on the basis of clean dry wool.

In experiments 1, 2 and 3, amounts of DL-methionine above 2·46 g/day reduced the rate of eating. Administration of the highest level of methionine to sheep SD67 in experiment 1 (9·84 g/day) was terminated after 6 weeks, as the sheep had left feed residues (400–500 g/day) for 3 days, and had also developed mild scouring. Appetite returned and scouring stopped two days after the methionine level was reduced to 2·46 g/day. However, 9·84 g/day DL-methionine were given to the same sheep for 7 weeks in experiment 3 without any adverse effects.

(ii) L-*Cysteine*.—The effects of the abomasal administration of amounts of L-cysteine ranging from 0·5–8·0 g/day are shown in experiments 4 and 5 (Fig. 5 and

Table 2). All levels of cysteine stimulated wool growth. In contrast to methionine, there was only a small reduction in wool growth (relative to the maximum) with 6·0 or 8·0 g of L-cysteine (Table 2). While the maximum wool growth response obtained with both cysteine and methionine was of the same order (Table 2), no estimate can be made of the optimum amount of cysteine for wool growth. A comparison of the wool growth of sheep SD67 in experiments 1, 3, and 4 (Table 2), suggests that at low levels of supplementation there are no substantial differences in the wool growth response to equivalent amounts of cysteine or methionine.

Effects on sulphur content of wool were similar to those obtained with methionine. A level of 0·5 g cysteine produced a moderate increase in sulphur, while levels above this produced "maximal" values (Fig. 5 and Table 2). A similar "maximal" sulphur content in wool from sheep SD67 was obtained with both methionine and cysteine (Table 2).

No adverse effects were observed with high levels of cysteine.

(c) *Effect of* D-*Methionine on Growth and Sulphur Content of Wool*

The D-isomer of methionine is also effective in stimulating wool growth. However, the increase obtained with 1·0 g/day was only about half that expected from a similar amount of DL-methionine (Table 2), which would be near the suggested optimum level of 1·0–2·0 g/day.

D-Methionine is at least as effective as DL-methionine in increasing the sulphur content of wool; "maximal" values for the sulphur content of wool grown by sheep 1038 were *c.* 4·2% with each supplement (Table 2).

(d) *Effect of MHA on Growth and Sulphur Content of Wool*

MHA (3·0 g/day) stimulated wool growth to about the same extent as an approximately equivalent amount (2·46 g) of DL-methionine. Maximum daily wool growth by sheep 1024 with both MHA and DL-methionine was *c.* 1·0 mg/cm^2 or 9–10 g (Table 2, expts. 2 and 7).

The sulphur content of wool was also increased substantially with MHA. Although the sulphur content of the wool grown during MHA supplementation was slightly less than for wool grown by the same sheep during methionine supplementation, the increase over basal values was similar with both supplements (Table 2, expts. 2 and 7).

(e) *Efficiency of Conversion of Supplementary Sulphur into Wool*

The proportion of the supplementary sulphur accounted for in the wool grown during supplementation is influenced considerably by the amount of S-amino acid given (Table 2). If all supplements are considered as cystine equivalents, at levels of 4 g or above the "incorporation" was 9% or less, while with 2 g, values ranged from 23–49%. With lower amounts very high values were obtained: 52–72% (expts. 1, 3, 4, and 6), and 120 and 149% (expt. 2).

(*f*) *Effect of Sulphur-containing Amino Acids on Body Weight*

Figure 6 shows the body weights of three sheep over a period of 50 weeks during which the sheep were given the chopped hay diet alone. Small decreases in body weight were associated with shearing. Apart from this effect, the body weights of two sheep remained relatively stable while that of the third sheep decreased slightly. The administration of DL-methionine or L-cysteine into the abomasum resulted

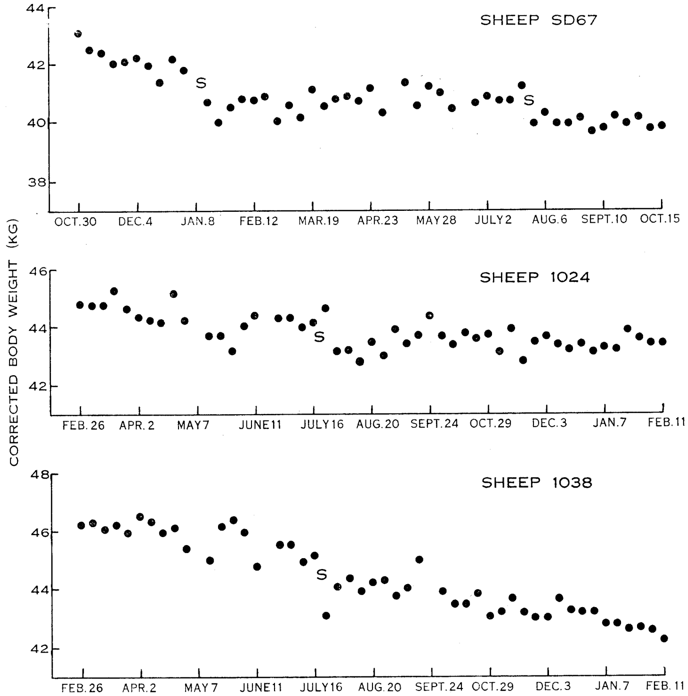

Fig. 6.—Seasonal pattern of body weight for three sheep receiving 800 g/day chopped hay for 50 weeks. The body weights were corrected for fleece growth. The sheep were shorn at times indicated by S.

in small but consistent increases in body weight (Fig. 7). The drop in body weight during the last week on 9·84 g DL-methionine (sheep SD67, expt. 1) occurred after a body weight response was observed and was probably associated with feed residues and scouring. In contrast to effects on wool growth, there was a progressive increase in body weight with each increment of S-amino acid. The lowest level of DL-methionine (0·615 g/day) did not increase body weight significantly. D-Methionine and MHA also produced a small increase in body weight.

IV. Discussion

(*a*) *General*

While the environmental temperature and diet were controlled during these experiments to minimize variation due to effects other than imposed treatment, the possibility of some seasonal rhythm in wool growth must be considered. However, sheep SD67 showed no seasonal trend in wool growth during a control period (Fig. 1). The seasonal rhythm shown by sheep 1024 and 1038 resulted in a maximum wool growth of only about 0·6 mg/cm²/day, which was well below the maximum wool growth obtained during S-amino acid supplementation (1·0–1·1 mg/cm²/day). In experiments 3, 6, and 7 any seasonal rhythm in the wool growth of sheep 1024 and

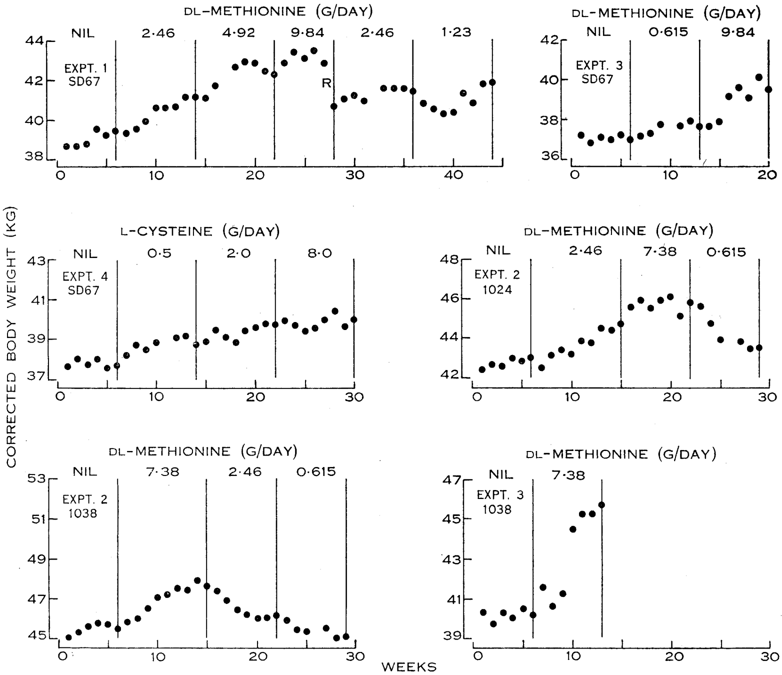

Fig. 7.—Effect of S-amino acids per abomasum on body weight. Details of the experiments are given in Table 1 and in Figures 2–5. The body weights were corrected for fleece growth. A period of feed residues in experiment 1 is indicated by R.

1038 would have tended to reduce the response obtained. The fact that reversing the order of the first two treatment levels between sheep in experiment 2 (Fig. 3) did not alter the response also suggests that seasonal effects were not important. Wool growth on the lowest level of methionine (expt. 2) may have been elevated slightly due to seasonal effects.

The responses to the different levels of methionine and cysteine should be considered in relation to a probable intake of 2–3 g/day cyst(e)ine [i.e. cyst(e)ine plus methionine expressed as cystine] in the basal diet (Reis and Schinckel 1963). Thus, while the amounts of S-amino acid infused in these experiments cover a range several times greater than the intake on the basal diet, the lowest amounts infused represent only a small increase over the basal level. However, the amount of S-amino acids available for absorption from the small intestine would presumably be considerably less than 2–3 g/day. Thus, the amount of nitrogen reaching the duodenum on this diet is similar to the nitrogen intake and is probably mainly microbial nitrogen (Hogan, unpublished data). When allowances are made for non-amino nitrogen (Hogan 1965), for the digestibility of rumen microbial protein (McNaught *et al.* 1950, 1954; Reed, Moir, and Underwood 1949) and for the amino acid composition of this protein (Hungate 1966) only about 1·0–1·5 g/day S-amino acids would be available for absorption.

(*b*) *Effects on Wool Growth*

These experiments confirm previous observations that S-amino acids can substantially increase wool growth (Reis and Schinckel 1963, 1964). Further, it is now apparent that the amount of S-amino acid given into the abomasum is important and there appear to be differences in the response to cysteine and methionine. Thus, while there was an optimum level for each amino acid, excessively high levels of DL-methionine substantially depressed wool growth relative to the maximum response, whereas high levels of L-cysteine appeared to depress wool growth only slightly.

As the different levels of S-amino acid were given sequentially, the possibility of residual effects of high levels of supplementation on subsequent treatment levels must be considered. In particular, the effectiveness of 0·615 g methionine (Fig. 3) may be due to such a phenomenon. However, it seems likely that the wool growth responses to 0·615 g and 2·46 g methionine (Fig. 3) are in fact similar as the wool growth of sheep SD67 was increased similarly by infusions of 0·615 and 2·46 g methionine given as the first level of supplementation in separate experiments (Figs. 2 and 4; Table 2). Also, there was no indication of a significant stimulation of wool growth following a high level of DL-methionine in experiment 3. The slightly higher post-treatment wool growth of sheep 1038 could be due to seasonal rhythm. The drop in the sulphur content of the wool grown during administration of 0·615 g DL-methionine (Fig. 3), or following a high level of DL-methionine (Fig. 4), also suggests that there was no significant residual effect due to previous levels of methionine supplementation.

There is no direct evidence regarding the mechanism of action of S-amino acids in stimulating wool growth, but various possibilities have been discussed by Reis and Schinckel (1963). The probable general anabolic effect suggested by these authors is supported by the small, but consistent, increases in body weight obtained with all supplements. However, the effects on body weight do not parallel those on wool growth as the highest levels of S-amino acid resulted in the greatest increase in body weight. Hormones known to be anabolic for protein metabolism, e.g. growth hormone, insulin, may be involved in any general anabolic effect. The increase in the sulphur content (and hence cystine) of wool during administration of S-amino acids is evidence that at least part of the effect of these supplements is obtained by augmenting the supply of cyst(e)ine to the follicles. However, as pointed out by Reis and Schinckel (1963), this is not necessarily the primary mechanism of action. Protein synthetic steps in the wool follicles may well be influenced by the amount of S-amino acid absorbed from the abomasum, since both Hanking and Roberts (1964) and Fleck, Shepherd, and Munro (1965) have shown that *in vitro* protein synthesis in rat liver is influenced by amino acids in the diet.

The very high "recoveries" of supplementary sulphur in wool with low levels of S-amino acid supplementation (especially methionine) may be due to the stimulation of incorporation of dietary or endogenous S-amino acids into wool. A specific stimulatory effect of methonine would not be surprising as methionine could stimulate wool growth in several ways other than as a source of cysteine. Thus, methionine appears to have a special role as a chain initiator in protein synthesis (Noll 1966; Clark and Marcker 1966), and is also effective in stimulating the synthesis of RNA (Munro 1965) and of adenine nucleotides (Stekol, Bulba, and Holowecky 1966) in liver, both of which are important for active protein synthesis. Further, methionine has a key role in amino acid transport and can enhance accumulation of other amino acids in cells, required for protein synthesis (Christensen 1963, 1964).

The adverse effects of high levels of methionine, and to a lesser extent cysteine, on wool growth may be associated with an amino acid imbalance or toxicity as defined by Harper (1964) and Harper and Rogers (1965). However, in contrast to the usual finding in these situations, the adverse effects on wool growth were obtained without any reduction in food intake or body weight. The administration of the highest level of methionine in experiment 1 (*c.* 1·2% of the diet) was associated with a depressed food intake and mild scouring after 6 weeks, but the inhibitory effect on wool growth was observed prior to this effect. In addition, toxic effects of methionine have usually been reported when methionine was added in amounts constituting from 2 to 5% of the diet (Brown and Allison 1948; Klavins, Kinney, and Kaufman 1963*a*, 1963*b*; Klavins and Peacocke 1964; Klavins and Johansen 1965). Another possible mechanism by which high levels of methionine could depress wool growth would be through effects on amino acid transport. While moderate amounts of methionine may be beneficial, excessive levels could adversely affect cellular levels of other amino acids (Christensen 1963, 1964).

The effects of varying levels of methionine and cysteine on wool growth may also be associated with effects on secretion of adrenal steroid hormones. Thus, it has been shown that increasing amounts of some amino acids (including methionine) in the diet of rats cause increased transfer of amino acids from muscle to liver; with methionine and leucine these effects are due to an increase in secretion of corticosterone by the adrenal glands (Munro 1964, 1965). If the administration of methionine to sheep has a similar effect on cortisol secretion by the adrenal glands, high levels of methionine might be expected to cause removal of amino acids from skin and a reduction in the rate of wool growth. The known effects of adrenal steroids on wool growth are consistent with this hypothesis. Thus, levels of plasma cortisol are normally low in sheep and administration of increasing doses of cortisone acetate to adrenalectomized sheep causes stepwise inhibition of wool growth (Ferguson, Wallace, and Lindner 1965). There is also limited evidence that a very low dose of cortisol given intradermally stimulates wool growth slightly while higher levels are inhibitory (Downes and Wallace 1965).

The effectiveness of D-methionine in stimulating wool growth is not surprising as the D-isomer can be converted to L-methionine in several mammalian species (Meister 1965). Also, both D- and L-methionine can be converted to cysteine, via homocysteine and cystathionine (Berg 1953). The extent to which methionine affects wool growth simply by conversion to cysteine cannot be assessed. The stimulation of wool growth obtained with MHA is presumably also due to its conversion to L-cysteine via homocysteine and cystathionine.

(c) *Effects on Sulphur Content of Wool*

The increases in sulphur content of wool obtained with these sulphur supplements can all be considered to reflect an increased synthesis of certain high sulphur proteins in wool (Gillespie, Reis, and Schinckel 1964; Gillespie and Reis 1966). This has been specifically demonstrated for wool grown during methionine infusion in experiment 1 (Gillespie and Reis 1966). The increase in sulphur content of wool obtained during methionine infusions can be explained by conversion of at least some of the methionine to cysteine, as all the extra sulphur in wool grown during methionine infusions has been shown to be in the form of cyst(e)ine (Gillespie, Reis, and Schinckel 1964; Gillespie and Reis 1966). The conversion of methionine to cysteine and incorporation of the cysteine into fibre keratin has been demonstrated in the guinea pig (De Bersaques 1964) and the sheep (Downes, Sharry, and Till 1964). In the latter study the conversion of L-methionine to cyst(e)ine was shown to occur in the skin. MHA could also be converted to cysteine by the above reactions.

The effect of the amount of S-amino acids given on the sulphur content of wool was different to the effect on the overall synthesis of wool proteins. There were no marked differences in the response obtained from different sulphur supplements, indicating that conversion of methionine or MHA to cysteine was not a limiting factor. Further, there was no inhibition by higher amounts of supplement, and the lowest level of cysteine or methionine increased the sulphur content to only a moderate extent. The fact that it is possible to produce a substantial stimulation of synthesis of specific sulphur-rich proteins virtually in the absence of a wool growth response (see highest level of methionine, Figs. 2 and 4) suggests that synthesis of these proteins may be a special case. The only limitation to their synthesis may be an adequate supply of S-amino acids. This supports the two-stage theory of keratin synthesis in wool follicles, in which the high-sulphur proteins are the last formed (Downes, Sharry, and Rogers 1963; Downes *et al.* 1966), and is consistent with the suggestion of Gillespie (1965) that the high-sulphur proteins of α-keratins may be synthesized by the stepwise addition of sulphur-rich peptides to precursors. The results of this study do not support our earlier hypothesis (Reis and Schinckel 1964), namely that the synthesis of high-sulphur proteins may be the rate-limiting step in keratin synthesis.

It is apparent that only 1–2 g/day cysteine (or equivalent methionine) are needed on this diet to produce maximum stimulation of synthesis of high-sulphur proteins in wool; 0·5 g/day causes a measurable increase. Gillespie and Reis (1966) postulated that stimulation of synthesis of specific sulphur-rich proteins required a minimum amount of S-amino acid. It would appear that this amount is not more than 0·5 g/day.

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