THE INFLUENCE OF ABOMASAL AND INTRAVENOUS SUPPLEMENTS OF SULPHUR-CONTAINING AMINO ACIDS ON WOOL GROWTH RATE

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Abstract

Various amounts (0·6–10·0 g/day) of L-cystine, L-cysteine, and L- and DL-methionine were given as abomasal or intravenous infusions to 10 sheep, in three experiments. The sheep received a daily ration of 800 g of a mixture of equal parts of chopped wheaten and lucerne hays. Responses in length growth rate and diamete oi wool fibres were measured by autoradiography and fibre volume output was calculated from these values.

Four amounts of L-methionine (0·62, 1·23, 2·46, and 4·92 g/day; treatments 1–4 respectively) were given as abomasal supplements to four sheep. All amounts significantly increased length growth rate, fibre diameter, and fibre volume. The largest increases were obtained with treatment 2; these increases were significantly greater than the increases obtained with treatments 1 and 4. These results indicate that the optimum amount of methionine for stimulation of wool growth on this diet is about 1–2 g/day.

L-Cystine and L-cysteine (2 g/day) produced equivalent effects on all indices of wool growth. The abomasal and intravenous routes of supplementation were equally effective for L-cyst(e)ine (2 g/day) and for L-methionine (2·5 g/day).

Abomasal supplements of DL-methionine and L-cystine (2·5 and 2·0 g/day respectively) increased fibre volume by 40–50%; both length growth rate and fibre diameter increased in each of four sheep. An infusion of 10 g/day methionine reduced wool growth to the basal rate, whereas equimolar amounts of cystine (8 g/day) sustained approximately the same rate of wool growth as did 2 g/day. Changes in length growth rate and fibre diameter were parallel and were virtually completed by 8 days.

I. Introduction

Dietary supplementation with sulphur-containing amino acids (*S*-amino acids) has usually failed to stimulate the wool growth rate of sheep receiving roughage based diets (see Reis and Schinckel 1963; Colebrook *et al.* 1968; Wright 1971). This lack of response to dietary supplementation with *S*-amino acids may be attributed to the degradation of these amino acids by rumen microorganisms (Lewis and Emery 1962; Zikakis and Salsbury 1969; Nader and Walker 1970).

In contrast, the effectiveness, as wool growth supplements, of *S*-amino acids that are not exposed to degradation in the rumen is now well documented. Reis and

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Schinckel (1963) and Reis (1967) showed that daily abomasal supplements of 0·5–2·0 g L-cysteine, or equimolar amounts of DL-methionine, stimulated wool growth markedly. As wool proteins are rich in cystine but contain very little methionine (Crewther *et al.* 1965), methionine may be effective because its sulphur is efficiently converted to cyst(e)ine sulphur (Meister 1965). However, as suggested by Reis (1967), specific effects of methionine are also possible. Recent work confirmed the effectiveness of the abomasal route for supplements of DL-methionine (Graceva 1969; Langlands 1970; Robards 1971). Parenteral routes of administration were also effective for supplements of L-cysteine (Dryden *et al.* 1969; Downes *et al.* 1970), L-methionine (Downes *et al.* 1970) and DL-methionine (Graceva 1969; Wickham 1970; Barry 1971; Wright 1971). The abomasal infusion of larger amounts of DL-methionine (5–10 g/day) resulted in a reduced wool growth response or even depressed wool growth below pre-treatment values (Reis 1967, 1970). In contrast, limited data (Reis 1967) suggested that equivalent, large amounts of L-cysteine did not produce these adverse effects on wool growth.

This paper reports additional information on the utilization of *S*-amino acids for wool growth. Direct comparisons were made of the effectiveness of intravenous and abomasal routes of administration of cyst(e)ine and methionine. The dose–response curve of L-methionine given per abomasum was studied, and the effectiveness of cystine and cysteine was compared. Finally, more data were obtained on the effects of large doses of cystine and methionine. The technique of autoradiography was employed in order to study the time course of the effects in some experiments, and to measure effects on length growth rate and diameter of fibres. Changes in plasma amino acid patterns in response to the *S*-amino acid infusions in these experiments are reported by Reis *et al.* (1973).

II. Experimental

(*a*) *Sheep and Diet*

Mature Merino wethers were fitted with abomasal cannulae near the pylorus and were kept in metabolism cages in a room maintained at a temperature of 23±3°C. The daily ration was 800 g of a mixture of equal parts of chopped wheaten and lucerne hays, fed either as a loose mix (experiments 1 and 2) or ground and pelleted (experiment 3). The ration was offered once daily at about 10 a.m. (experiments 1 and 2) or eight times daily (100 g every 3 hr; experiment 3). Water was available *ad libitum*.

(*b*) *Plan of Experiments*

There were three experiments in which amino acids were administered as either abomasal or intravenous infusions. The details of these experiments are listed below:

Experiment 1

1(*a*): Four sheep received L-methionine per abomasum at four dose levels (0·62, 1·23, 2·46, and 4·92 g/day; treatments 1–4 respectively) in a latin square design. Each infusion period was 14 days.

1(*b*): Four weeks after the completion of experiment 1(*a*) the same sheep were given L-methionine intravenously (2·46 g/day) for 14 days.

In both experiments wool growth was measured over 4-day intervals pre-experimentally and at the end of each treatment period.

Experiment 2

Two sheep were given 2 g/day L-cystine or L-cysteine (2·6 g L-cysteine.HCl) either abomasally or intravenously, during 14-day periods, as indicated in the following tabulation:

Period	Sheep 5962	Sheep 5850
1	Control	Control
2	Cystine, intravenous	Cystine, abomasal
3	Cystine, abomasal	Cystine, intravenous
4	Recovery	Recovery
5	Cysteine, intravenous	Cysteine, abomasal
6	Cysteine, abomasal	Cysteine, intravenous

Wool growth was measured during the last 4 days of each period, except for period 4.

Experiment 3

Four sheep received L-cystine or DL-methionine per abomasum during two infusion periods, each of 20 days, as indicated in Table 3. Wool growth was measured at intervals of 4 days during 8 days immediately prior to the infusions and throughout the treatment periods.

(*c*) *Administration of Amino Acids*

The amino acids were obtained from various sources: DL-methionine, Koch–Light Laboratories, England; L-methionine, Scientific and Research Equipment Co., Sydney, Australia; L-cysteine, Koch–Light Laboratories, England; supplied as the hydrochloride; and L-cystine, Carlo Erba, Milan, Italy and Sigma Chemical Co., U.S.A.

Aqueous solutions of the amino acids were prepared for abomasal infusion. L-Cystine was first dissolved in 2N HCl (*c.* 10 ml per gram cystine) and the infusion volume was made up with water. For intravenous infusions, the amino acids were first dissolved in about 100 ml of sterile water (HCl was added with cystine as above); sterile isotonic saline was then added to make up the volume. Methionine solutions for abomasal infusions were prepared from a refrigerated stock solution; other amino acid solutions were prepared freshly each day. Before commencing the intravenous infusions, tubing (Silastic medical grade, Dow Corning Corporation, Michigan, U.S.A.) was implanted in the jugular vein of each sheep.

The solutions were infused continuously at a steady rate, by means of a peristaltic pump. The individual infusion volumes varied slightly with the pump tubing and were about 200 ml per 24 hr for intravenous infusions and 500–600 ml per 24 hr for abomasal infusions.

(*d*) *Wool Growth*

Wool growth responses were assessed by the autoradiographic technique of Downes *et al.* (1967). Both length growth rate and diameter of wool fibres were measured, and fibre volume was calculated from these values assuming that the fibres were cylindrical. As the fibres were not medullated, these volumes can be regarded as proportional to the mass of wool. Intravenous injections of tracer doses of L-[^{35}S]cystine were given 4 days apart during control periods and at the end of each treatment period (expts. 1 and 2) or at intervals of 4 days throughout the experiment (expt. 3). Fibre diameter was measured at the front of each radioactive zone. This position on the fibre corresponded approximately to the time of injection. In experiments 1 and 2, fibres were taken from three sites (shoulder, midside, and thigh) on each sheep and 20 fibres per site were measured; in experiment 3, fibres were taken from 11 sites on one side of each sheep and 10 fibres per site were measured.

(*e*) *Statistical Analysis*

For experiment 1, the mean results for six groups of 10 fibres were taken to give composite values of length growth rate and fibre diameter for each sheep in each period. The data were analysed by a statistical procedure based on that of Williams (1949) for determining direct effects and residual effects of treatments.

III. Results

(a) *Wool Growth Response to Various Levels of* L-*Methionine given per Abomasum*

In experiment 1, all amounts of methionine infused significantly increased length growth rate, fibre diameter, and fibre volume above the pretreatment values (direct effects, Table 1). Although treatment 2 consistently gave the greatest increases in these indices of wool growth, the responses in length growth rate and fibre diameter were not significantly greater than those obtained with treatment 3. All the responses obtained with treatment 2 were significantly greater than those obtained with treatments 1 and 4. The only significant residual effect observed was on fibre diameter with treatment 2 (Table 1). Expressed as a percentage of the pretreatment value, the treatment volume responses were 118, 130, 125, and 114 for treatments 1–4 respectively. The methionine infusions did not cause any appreciable change in the ratio of mean fibre length per day to mean diameter; the values for the pretreatment period and for treatments 1–4 respectively were 17·1, 16·3, 16·4, 16·5, and 17·1.

Table 1

INFLUENCE OF ABOMASAL SUPPLEMENTS OF L-METHIONINE ON WOOL GROWTH

Four sheep each received the four amounts of L-methionine as abomasal supplements during 14-day periods (see experiment 1*a*). Measurements were made during the last 4 days of each period, and the values given are adjusted treatment means and estimates of residual effects. Mean pretreatment values were: fibre length growth rate, 400 μm/day; fibre diameter, 23·6 μm; $10^{-3}\times$ fibre volume growth rate, 176 μm^3/day

Treatment	L-Methionine (g/day)	Change in length growth rate (μm/day)		Change in diameter (μm)		$10^{-3}\times$ Change in volume growth rate (μm^3/day)	
		Direct effect	Residual effect	Direct effect	Residual effect	Direct effect	Residual effect
1	0·62	13·6	4·5	1·54	−0·25	30·8	−0·9
2	1·23	26·6	0·6	2·40	0·54	51·6	9·0
3	2·46	21·9	−1·7	2·00	0·01	42·0	−0·9
4	4·92	19·6	−3·5	0·93	−0·30	23·9	−7·2
Difference for significance at 5% level		6·1	7·3	0·43	0·52	8·4	10·1

(b) *Relative Effectiveness of* L-*Cystine and* L-*Cysteine as Supplements for Wool Growth*

In experiment 2, L-cystine and L-cysteine (2 g/day) had similar effects in both sheep; they increased length growth rate and fibre diameter, and hence fibre volume, when given by either the abomasal or intravenous route (Table 2). The ratio of mean length of wool grown per day to the mean diameter was a characteristic of each sheep and was not altered by the infusions (Table 2). When the results for each amino acid were pooled regardless of route of administration and expressed relative to the pre-treatment values [Figs. 1(*a*) and 1(*b*)] it can be seen that, overall, the effects of the two amino acids on wool growth were similar.

(c) Comparison of Intravenous and Abomasal Administration of S-Amino Acids as Supplements for Wool Growth

The results for both amino acids were also pooled for a comparison of the intravenous and abomasal routes of administration [Figs. 1(c) and 1(d)]. Overall,

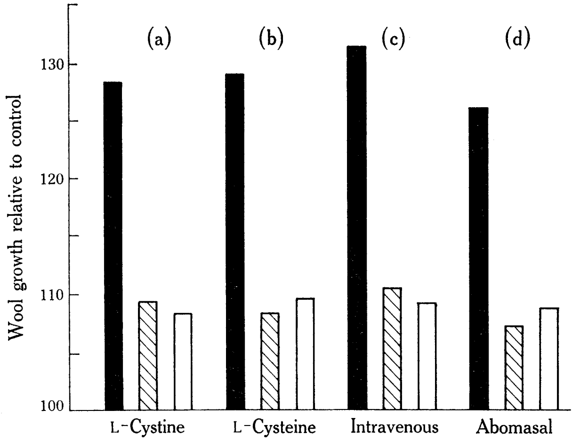

Fig. 1.—Effect of supplements of cystine and cysteine on wool growth in volume (solid bars), length (hatched), and diameter (open bars). Mean responses are shown to L-cystine (*a*) and L-cysteine (*b*) given by both intravenous and abomasal routes, and to intravenous (*c*) and abomasal (*d*) supplements of both cystine and cysteine. See experiment 2 and Table 2 for details.

it can be concluded that the two routes were approximately equally effective for cystine, but there may be a small advantage of intravenous over abomasal administration for cysteine (Table 2). However, more extensive data would be needed to establish the extent of any difference that may exist.

TABLE 2

EFFECT OF CYSTINE AND CYSTEINE SUPPLEMENTS ON WOOL GROWTH

Supplements of 2 g/day L-cystine or L-cysteine (given as 2·6 g L-cysteine.HCl) were given either abomasally or intravenously during 14-day periods. See experiment 2 for details. Wool growth was measured during the last 4 days of each period

Treatment	Length growth rate of wool (μm/day)		Wool fibre diameter (μm)		Length/diameter*		$10^{-3}\times$ Wool fibre volume growth rate (μm^3/day)	
	Sheep 5962	Sheep 5850	Sheep 5962	Sheep 5850	Sheep 5962	Sheep 5850	Sheep 5962	Sheep 5850
Nil	309	356	23·0	22·8	13·4	15·6	130	148
L-Cystine (intravenous)	347	386	24·0	25·4	14·4	15·2	157	199
L-Cystine (abomasal)	329	390	24·5	25·4	13·4	15·4	156	202
L-Cysteine (intravenous)	339	396	25·1	25·6	13·5	15·5	169	206
L-Cysteine (abomasal)	326	376	24·8	24·9	13·1	15·1	159	184

* i.e. ratio of mean fibre length per day to mean diameter.

In experiment 1(*b*) the wool growth response to an intravenous infusion of 2·46 g/day of L-methionine for 2 weeks was compared with the response to the same amount of L-methionine given via the abomasum in experiment 1(*a*). Data are available for three sheep only, as one sheep failed to eat satisfactorily for the last 4 days of the intravenous infusion. Fibre volume outputs for the three sheep were similar in the basal periods prior to experiments 1(*a*) and 1(*b*); respective wool fibre volume growth rates $\times 10^{-3}$ were 186 and 180 μm^3/day. Taking the basal rate as the mean of these two values, the relative volumes were: basal, 100; abomasal infusion, 123; and intravenous infusion, 124. Thus, the two routes of administration were about equally effective for wool growth.

(*d*) *Comparison of Cystine and Methionine as Abomasal Supplements for Wool Growth*

In experiment 3, the effects of L-cystine and DL-methionine on length growth rate, fibre diameter, and volume of wool produced were compared at two dose levels. The results are summarized in Table 3. The data were calculated from the last 8 days

TABLE 3

COMPARISON OF CYSTINE AND METHIONINE SUPPLEMENTS FOR WOOL GROWTH

Four sheep received L-cystine or DL-methionine during 20-day infusion periods as indicated. Sheep 9057 failed to complete period 1 on 10 g/day methionine and a measurement of wool growth was not obtained. The values given are means for 8 days prior to supplementation and for the last 8 days of each supplementation period, except for sheep 5842 in period 1, when data for days 8–16 were used

Abomasal supplement (g/day)	Infusion period	Length growth rate of wool (μm/day)	Wool fibre diam. (μm)	Length/ diam.*	$10^{-3}\times$ Wool fibre volume growth rate (μm^3/day)	Increase in fibre volume (%)
Sheep 5842						
Nil		302	24·2	12·5	141	—
L-Cystine, 2·0	1	332	27·4	12·1	199	41
L-Cystine, 8·0	2	334	27·7	12·1	204	45
Sheep 9055						
Nil		377	23·7	15·9	167	—
L-Cystine, 2·0	2	410	26·8	15·3	232	39
L-Cystine, 8·0	1	415	25·3	16·4	210	26
Sheep 6003						
Nil		314	20·0	15·7	98	—
DL-Methionine, 2·5	1	366	23·1	15·8	153	56
DL-Methionine, 10·0	2	324	20·0	16·2	102	4
Sheep 9057						
Nil		286	21·6	13·2	106	—
DL-Methionine, 2·5	2	324	25·0	13·0	160	51

* i.e. ratio of mean fibre length per day to mean diameter.

in each period, except for sheep 5842 in period 1, when data for days 8–16 were used. Sheep 9057 failed to complete period 1 on 10 g methionine due to feed refusals and no data are available. Appetite was restored when the infusion was stopped.

The results confirm that 2 g cystine and 2·5 g methionine are effective supplements for wool growth; fibre volume was increased 40–50% and both length growth rate and fibre diameter increased in every sheep. Methionine infused at the rate of 10 g/day substantially reduced wool growth relative to 2·5 g/day. In contrast, the two levels of cystine infused sustained approximately the same rates of wool growth. As in experiment 2, the ratio of mean length of wool grown per day to the mean diameter was a characteristic of each sheep and was not altered by supplements of either amino acid (Table 3).

The pattern of response to cystine and methionine supplements for two sheep is shown in Figure 2. Length, diameter, and volume responses were parallel and the changes were virtually completed by 8 days. The adverse effect of 10 g methionine compared with an equimolar amount of cystine is readily apparent.

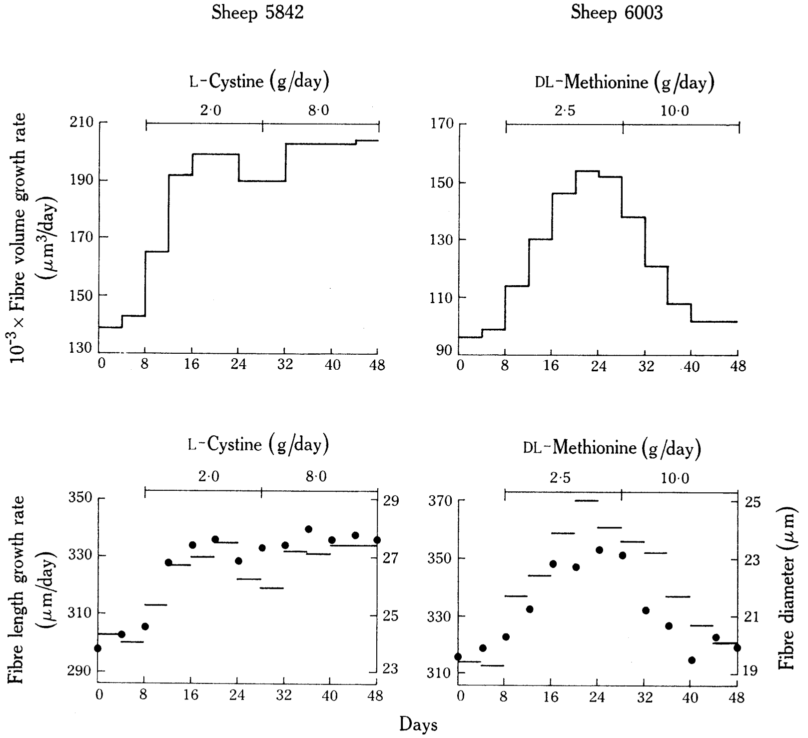

Fig. 2.—Comparison of the effects of abomasal supplements of cystine and methionine on wool growth. Abomasal supplements were given for 20 days as indicated; see experiment 3 and Table 3 for details. Length growth rates (—) and diameters (●) were measured during successive 4-day periods; volumes were calculated using the mean of the diameters at the beginning and end of each 4-day period.

IV. Discussion

The present experiments, as well as confirming that the S-amino acids stimulate wool growth, show that there are no appreciable differences in the response obtained to effective dose levels of methionine, cystine, or cysteine, given by either the abomasal or intravenous route.

This direct evidence for the near equivalence of the abomasal and intravenous routes is consistent with the conclusion of Downes *et al.* (1970) that "the alimentary hormones, such as gastrin, secretin, and pancreozymin, which may influence metabolism through effects on insulin secretion (Dupre *et al.* 1969) as well as through effects on the digestive tract, do not play a major part in bringing about the response to the S-amino acids". Abomasal supplementation is thus essentially increasing the supply of the particular S-amino acid available to the wool follicles.

The equivalence of cysteine and cystine as supplements for wool growth is not surprising as these amino acids are readily interconvertible by oxidation and reduction or through thiol–disulphide exchange. Accordingly, although most of the sulphur in wool is present as cystine (Crewther *et al.* 1965), either cystine or cysteine may be used as a supplement for wool growth.

While the present results and much earlier data (see Introduction) indicate that cyst(e)ine and methionine have a similar value as supplements for wool growth, the relative value of these two amino acids is not definitely established. Firstly, no direct comparisons have been made in the same sheep, and secondly, the dose–response curve for each amino acid is not established. While the optimum amount of methionine appears to be 1–2 g/day (Reis 1967; experiment 1, this paper), there are insufficient data available for cyst(e)ine. It should be noted that the dose–response curve may depend on the composition and amount of the diet offered, as these factors will influence the amounts and proportions of the amino acids available for absorption from the small intestine. It is not known whether the effectiveness of methionine as a supplement for wool growth is due solely to its conversion to cysteine via cystathionine, either in organs such as the liver (Meister 1965) or in the skin or wool follicles (Downes *et al.* 1964), or whether it also has direct effects as suggested by Reis (1967).

The results obtained in experiment 3 indicate a marked difference between the effects of cystine and methionine when large amounts are given. Whereas 10 g/day of methionine depressed wool growth relative to 2·5 g/day of methionine, in accordance with previous results (Reis 1967, 1970), an equivalent amount of cystine (8 g/day) was about as effective as 2 g/day cystine. Thus, it appears that the adverse effects on wool growth of high levels of methionine are due specifically to the inability of the animal to metabolize this amino acid rapidly enough or to store it in tissues by a mechanism such as that possible with cyst(e)ine, namely thiol–disulphide reactions with proteins. This conclusion is supported by the data on plasma amino acids (Reis *et al.* 1973) from the present experiments, which show that plasma methionine concentration starts to rise steeply when amounts of methionine above 3–4 g/day are infused. Very high concentrations of methionine are present in the plasma of sheep receiving 10 g/day of methionine as abomasal infusions (Reis and Tunks 1971; Reis *et al.* 1973). In contrast, infusion of an equimolar amount of cystine (8 g/day) causes only a slight rise in plasma cystine (Reis *et al.* 1973).

The present results (experiment 3) confirm the previous evidence that changes in wool growth rate in response to abomasal infusions of casein or *S*-amino acids (Downes *et al.* 1970; Reis and Downes 1971), or to changes in nutrition (Downes and Sharry 1971), can be measured satisfactorily in short experiments, using the autoradiographic technique. The responses in both length growth rate and fibre diameter were virtually completed by 8 days, so that measurements made with the autoradiographic technique between days 8 and 12 of a 12-day treatment period would be adequate to assess the effect of a nutritional treatment.

Supplements of *S*-amino acids altered length growth rate and fibre diameter in about the same proportions, with the result that the ratio of mean fibre length per day to mean diameter remained unchanged. These effects are the same as those produced by changes in the level of nutrition (Downes and Sharry 1971). In contrast, differential effects on length growth rate and fibre diameter are produced by large amounts of some hormones such as thyroxine (Downes and Wallace 1965), exposure of the skin to low temperatures (Downes and Hutchinson 1969; Jolly and Lyne 1970; Lyne *et al.* 1970) and abomasal infusion of the protein zein (Reis and Colebrook 1972). Thus, it would appear that *S*-amino acids influence wool growth in the same manner as normal changes in nutrition, rather than by causing large alterations in the balance of hormones such as thyroxine.

The wool growth rate of sheep consuming moderate amounts of roughage diets or pasture is usually well below the sheep's potential. Under these conditions, the wool growth rate can be increased appreciably by parenteral or abomasal supplementation with 1–2 g/day of cyst(e)ine or methionine. With such diets, rumen microbial protein and any undegraded dietary protein reaching the abomasum should be of similar amino acid composition (McDonald 1969). However, this may not be so with all diets and, if appreciable amounts of dietary protein escape degradation in the rumen, the response to supplementary *S*-amino acids may be altered because different proportions of amino acids would be already available for absorption from the small intestine.

V. Acknowledgments

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