

# METABOLIC FATE OF PARENTERALLY ADMINISTERED SULPHUR-CONTAINING AMINO ACIDS IN SHEEP AND EFFECTS ON GROWTH AND COMPOSITION OF WOOL

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## Summary

Intravenous or intraperitoneal infusions of either L-cysteine (2.0 g/day) or L-methionine (2.5 g/day) for 20 days increased wool diameter and its length growth rate, the main increases occurring during the first 8 days of infusion. Thus parenteral administration of these amino acids is effective in stimulating wool growth.

The metabolic fate of small and large doses of L-[<sup>35</sup>S]cystine, administered either intravenously or intraperitoneally was studied. Experiments using [<sup>35</sup>S]cystine as a tracer showed that intraperitoneal depots of about 50 g cystine were absorbed at a fast enough rate to stimulate wool growth. A similar dose of L-[<sup>35</sup>S]cystine was also administered subcutaneously, but the administration of this amount caused obvious discomfort to the sheep and subcutaneous injection was not studied further.

Doses of 25–100 g of L-cystine, injected intraperitoneally, produced significant increases in wool growth and in sulphur content of the wool for a period of about 10 weeks. The results for individual sheep were variable, but the mean maximum wool growth rate, 27% above the basal rate, occurred during the second 2-week period.

Intraperitoneally administered DL-methionine (25–100 g) was absorbed and metabolized too rapidly to provide a useful supplement for wool growth.

## I. INTRODUCTION

Wool proteins are rich in the sulphur-containing amino acid, cystine, so an adequate supply of cystine for protein synthesis in the wool follicles is essential to maintain a high rate of wool growth. The rumen microorganisms can synthesize cystine from various precursors supplied by plant tissues, including inorganic sulphate (Block, Stekol, and Loosli 1951). However, cystine is also catabolized in the rumen, and the addition of cystine to the diet has little effect on wool growth rate (Marston 1932; Du Toit *et al.* 1935). Marston and Robertson (1928) suggested that cystine was likely to be the primary nutritional factor limiting wool growth. Subsequently, Marston (1935) observed a 34% increase in wool growth in a sheep which was given daily subcutaneous injections of 1 g of L-cysteine. More recently, Reis and Schinckel (1963) and Reis (1967) have shown that daily supplements of 1–2 g of L-cysteine or DL-methionine given per abomasum stimulate wool growth markedly. These supplements also substantially increased the cystine, and hence sulphur, content of wool (Reis and Schinckel 1963; Gillespie and Reis 1966; Reis 1967). Methionine is presumably effective because its sulphur is efficiently converted to cyst(e)ine sulphur.

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Dryden, Wickham, and Cockrem (1969) have infused L-cysteine intravenously into sheep and reduced winter depression in wool growth. Gracheva (1969) found methionine (3 g/day; 90 days) given abomasally or subcutaneously to sheep increased the length growth rate of wool 40 and 20% respectively.

When L- $^{35}\text{S}$  cysteine is administered intravenously to sheep about 30% of the  $^{35}\text{S}$  is incorporated into wool proteins as cystine (Downes 1961). Other evidence (Downes, Sharry, and Till 1964) shows that the skin can use the sulphur-containing amino acids (*S*-amino acids) directly and can convert the methionine-S to cystine-S. Parenteral supplementation with *S*-amino acids should therefore increase wool growth. Since the rate of wool growth of grazing sheep is below maximum for most of the year in many Australian environments (Williams and Schinckel 1962) this seemed a possible way of increasing wool growth. Accordingly the method has been investigated in some detail.

## II. EXPERIMENTAL

### (a) *Sheep and Diet*

The sheep used in the experiments were mature Merino wethers, except for two (4740 and 4755) which were English Leicester  $\times$  Merino wethers. The sheep were kept indoors in individual pens at an uncontrolled ambient temperature, or in metabolism cages in a room maintained at a temperature of  $23 \pm 3^\circ\text{C}$ . 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 11 a.m.; details of the diet are given with each experiment.

### (b) *Administration of Amino Acids*

The amino acids were obtained from L. Light and Co. Ltd., England.

For intravenous or intraperitoneal infusion of *S*-amino acids, tubing (Silastic medical-grade tubing, Dow Corning Corporation, Michigan, U.S.A.) was implanted in the jugular vein or into the peritoneal cavity of sheep kept in metabolism cages. Solutions of L-cysteine hydrochloride (2 g) or L-methionine (2.5 g) (dissolved in 80 ml of sterile water) were infused using a peristaltic pump, over a period of 6 hr each day, commencing when the sheep were fed. One ml of concentrated hydrochloric acid was added to keep in solution any cystine formed during the infusion.

For intravenous injection experiments 2 g L-cysteine was dissolved in 30 ml 1N HCl and administered via the jugular vein over a period of 10 min.

Intraperitoneal injections were given with the sheep in a standing position. A syringe attached to a 12- or 13-gauge, 2-in. needle was used to inject the amino acids at a site 2 in. lateral to the sixth lumbar vertebra and 2 in. anterior to the tuber coxae. The amino acids were mixed with sterile water (2 ml/g of amino acid); methionine was mixed by stirring and cystine was homogenized with the water to form a "cream", using a Servall Omni-Mixer. L-Cystine suspensions of the same consistency were injected subcutaneously.

### (c) *Growth and Sulphur Content of Wool*

Estimates of wool growth rate were made either by the tattooed patch method (Reis 1967) or by the radioautographic technique of Downes, Clarke, and Dagg (1967). Results obtained by the first method were multiplied by previously determined factors to give rates of wool growth in grams per day. With the radioautographic technique, intravenous injections of L- $^{35}\text{S}$  cysteine were given at intervals of 4 days; both length growth rate and diameter of fibres (at the front of each radioactive zone) were measured. Sulphur content of wool (expressed as percentage in clean dry wool) was determined in duplicate samples by the procedure of Reis and Schinckel (1963).

*(d) Procedures with [<sup>35</sup>S]Amino Acids**(i) Labelled Amino Acids*

L-[<sup>35</sup>S]Cystine was obtained from the Radiochemical Centre, Amersham, England. Cystine of low specific radioactivity (2  $\mu$ Ci/g) was prepared in order to study the metabolic fate of large parenteral doses. For this purpose L-[<sup>35</sup>S]cystine (500  $\mu$ Ci) was added to 1 litre of a solution of L-cystine (1M) in 3N HCl, and the pH adjusted to 5.0 with NH<sub>4</sub>OH. The precipitated cystine was filtered off, washed with water and ethanol, and dried.

DL-[<sup>35</sup>S]Methionine (Commissariat à l'Énergie Atomique, France) was diluted to the desired specific radioactivity by mixing it with DL-methionine in hot aqueous solution. After adding ethanol the solution was cooled rapidly to produce small crystals of methionine. These were filtered off and dried.

*(ii) Radioassay*

Two liquid scintillation solutions were used in the preparation of samples for radioassay. Solution A consisted of toluene with 2,5-diphenyloxazole (0.4% w/v) and 1,4-bis(5-phenyloxazol-2-yl)benzene (0.01% w/v). Solution B contained 7 parts of solution A by volume mixed with 6 parts of Triton X-100 (supplied by Robert Bryce and Co. Ltd., Sydney) (Patterson and Greene 1965).

In each experiment standards were prepared by dissolving weighed amounts of the labelled amino acid (c. 20 mg) in 7.5 ml water and adding 10 ml of solution B. The samples were shaken vigorously and allowed to stand at 5°C for at least 2 hr to form a clear gel before counting. The standards were assayed with each batch of unknowns to enable recoveries to be expressed as the percentage of the dose.

All samples were assayed at the "balance-point" (Packard instrument manual) in a Packard Tri-Carb liquid scintillation spectrometer (model 3375). At least 2000 counts were obtained for each sample and the counting rates were corrected for background and decay. The counting efficiency of each plasma and urine sample was determined by using the automatic external standard supplied with the spectrometer after calibration against standards containing known amounts of <sup>35</sup>S.

*(iii) Sampling*

Blood samples (5 ml) were taken from the jugular vein, transferred to tubes containing heparin (0.05 ml, 1% w/v in 0.9% NaCl), and centrifuged (6500 g; 15 min; 2°C). Plasma samples (1 ml) were added to 6.5 ml water in 20-ml glass vials. Scintillation solution B (10 ml) was then added and the samples shaken vigorously and assayed as described above for the standards. Samples (1 ml) of urine were similarly prepared for assay.

Duplicate samples (100 g) of each collection of faeces were macerated, dried overnight at 105°C, and weighed. Subsamples (100 mg dry wt.) were combusted by a modification of the oxygen-flask method of Kalberer and Rutschmann (1961). After absorption of the combustion products in 12 ml of a solution of ethanolamine (1 part) in 2-methoxyethanol (9 parts), a 10-ml aliquot was mixed with scintillator (10 ml solution A) in a 20-ml glass vial.

After the sheep had become accustomed to their cages and their wool growth rates, as measured by clipping tattooed areas, had become reasonably steady, several regions of skin on each sheep were prepared as plucking sites. The wool was uniformly clipped from the sites several weeks before administering the labelled doses. At various intervals afterwards, samples of wool were plucked, washed several times with light petroleum (Shell X4; b.p. 60–80°C), dried at 100°C for 1 hr, weighed into glass vials, and 10 ml solution A was added. Since the counting rates obtained by this method depend on the average fibre diameter (Downes and Till 1965), the counting efficiencies were determined subsequently by combusting representative samples from each sheep, as described above for faeces. The total amount of wool grown by the sheep during the period of growth of each plucked sample was estimated from the measured wool growth rates. This value and the specific radioactivity of the plucked samples were used to calculate the total amount of <sup>35</sup>S incorporated into the wool.

## III. RESULTS

*(a) Comparison of Intravenous and Intraperitoneal Infusion of L-[<sup>35</sup>S]Cystine*

L-[<sup>35</sup>S]Cystine (12  $\mu$ Ci; 0.1 mg in 80 ml 0.9% w/v NaCl) was infused intraperitoneally over a 6-hr period into two sheep. Two weeks later the same amounts of L-[<sup>35</sup>S]cystine were infused intravenously over a 6-hr period into the same sheep.

The results obtained with each sheep were very similar. During the infusion periods the amount of <sup>35</sup>S in the plasma increased at a steady rate, the rate during the intraperitoneal infusions being about two-thirds of the rate during the intravenous infusions (Fig. 1). There was a further increase in plasma <sup>35</sup>S during the

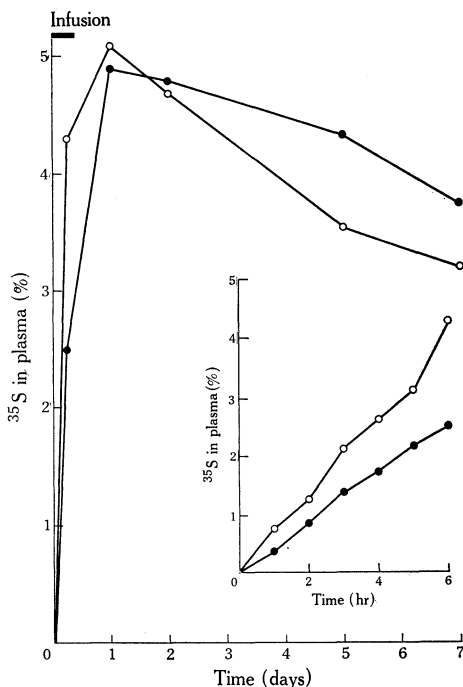


Fig. 1.—Amount of <sup>35</sup>S per litre of plasma (as a percentage of total dose administered) during (inset) and after 6-hr intravenous (O) and intraperitoneal (●) infusions of L-[<sup>35</sup>S]cystine (12  $\mu$ Ci; 0.1 mg in 80 ml 0.9% w/v NaCl). Results shown are means for two sheep which each received an intraperitoneal infusion followed 2 weeks later by an intravenous infusion. The results for the latter experiment were corrected by subtracting the amounts of <sup>35</sup>S remaining from the first experiment. These were calculated from the results for samples taken just before starting the second experiment and from estimates from the extrapolated plasma <sup>35</sup>S versus time curve.

first 16 hr after the intraperitoneal infusion was stopped, presumably because of continuing absorption and incorporation of [<sup>35</sup>S]cystine into plasma proteins (Downes 1961). Thus, 16 hr after ending either the intravenous or intraperitoneal infusions the amount of <sup>35</sup>S in the plasma was approximately the same.

In each experiment the <sup>35</sup>S was incorporated into the wool with high efficiency, 30–40% of the dose appearing in the wool during the first 14 days. Approximately 20% of the <sup>35</sup>S from each dose appeared in the urine and 30% in the faeces during the first 14 days.

*(b) Effect of Parenteral Infusions of L-Cysteine and L-Methionine on Growth and Sulphur Content of Wool*

Parenteral supplementation of four sheep with either L-cysteine (2.0 g/day) or L-methionine (2.5 g/day) (given intravenously or intraperitoneally) increased

both the length growth rate and diameter of the wool; the mean volume of fibre produced during the last 8 days of a 20-day supplementation period was 33–83% higher than the presupplement value (Table 1). The response of wool growth to

TABLE 1

EFFECT OF PARENTERAL INFUSIONS OF L-CYSTEINE AND L-METHIONINE ON GROWTH AND SULPHUR CONTENT OF WOOL

The sheep received 800 g/day of a diet of equal parts chopped wheaten and lucerne hays. A supplement of L-cysteine (2.0 g/day) or L-methionine (2.5 g/day) was given intravenously (i.v.) or intraperitoneally (i.p.) as indicated, for 20 days. The values given are means for 8 days prior to supplementation and for the last 8 days of supplementation. Either 40 or 60 fibres (10 fibres per site) were measured from each sheep

Sheep	Supplement	Length Growth Rate of Wool ( $\mu\text{m}/\text{day}$ )	Wool Fibre Diameter ( $\mu\text{m}$ )	$10^{-3} \times \text{Wool}$ Fibre Volume Growth Rate ( $\mu\text{m}^3/\text{day}$ )	Increase in Fibre Volume (%)	Wool Sulphur Content (%)
5832	Nil	414	30.8	309	—	2.44
	L-Cysteine (i.v.)	485	32.9	412	34	3.45
5925	Nil	369	22.7	149	—	2.58
	L-Methionine (i.v.)	414	24.7	198	33	3.55
4755	Nil	361	23.9	162	—	2.50
	L-Cysteine (i.p.)	446	29.1	297	83	3.60
4740	Nil	368	30.5	269	—	2.66
	L-Methionine (i.p.)	468	33.8	420	56	3.61

supplementation was rapid, most of the change in both length and diameter occurring in the first 8 days of supplementation (Fig. 2). All forms of supplementation also increased the sulphur content of the wool markedly (Table 1).

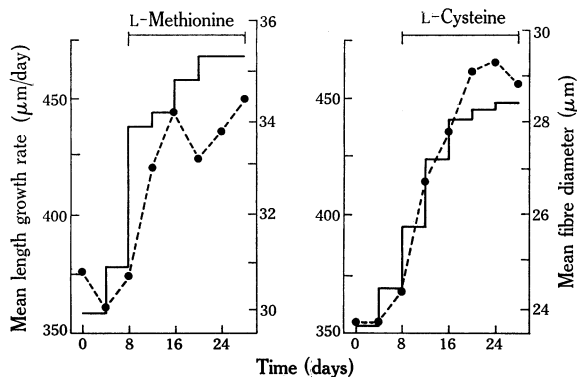


Fig. 2.—Effect on wool growth of intraperitoneal supplementation with L-methionine (2.5 g/day) or L-cysteine (2.0 g/day) for 20 days. Each amino acid was infused during a 6-hr period each day. Length growth rates (—) and diameters (●---●) were measured during successive 4-day periods.

(c) *Metabolic Fate of Large Amounts of L-[<sup>35</sup>S]Cysteine and DL-[<sup>35</sup>S]Methionine*

In the first experiment of this series the metabolic fate of L-[<sup>35</sup>S]cysteine after intravenous, subcutaneous, and intraperitoneal administration was studied in three sheep. The L-[<sup>35</sup>S]cysteine had the same specific radioactivity (2  $\mu\text{Ci/g}$ ) in the three tests. The fate of DL-[<sup>35</sup>S]methionine given intraperitoneally was studied in a fourth sheep.

The first sheep was given daily intravenous doses (2 g each) of L-[ $^{35}\text{S}$ ]cystine for 11 days. From measurements of  $^{35}\text{S}$  in the blood plasma it was estimated that more than 90% of each dose was cleared from the circulation within an hour. Twenty-four hours after giving each dose there was an increase in concentration of  $^{35}\text{S}$  in the plasma over the pre-injection value, the increase being equivalent to about 3% of the dose per litre.

A second sheep was given a single intraperitoneal dose (40 g) of L-[ $^{35}\text{S}$ ]cystine while a third sheep received the same amount of L-[ $^{35}\text{S}$ ]cystine subcutaneously. The latter dose was divided into 5-g lots and injected during a 30-min period into eight separate sites on one side of the animal. Within 10 min of completing the injections small amounts of  $^{35}\text{S}$  appeared in the blood of both sheep. The average rate of increase in plasma  $^{35}\text{S}$  during the first 10 days was about the same as that observed in the sheep which received the 11 intravenous doses (Fig. 3). The amount

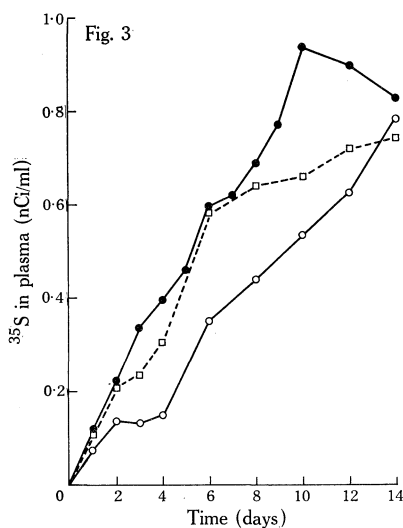


Fig. 3.—Concentration of  $^{35}\text{S}$  in plasma of three sheep dosed large amounts of L-[ $^{35}\text{S}$ ]cystine [first experiment, Section III(c)]. The subcutaneous ( $\square$ ) and intraperitoneal ( $\circ$ ) doses were given on day 0, and the third sheep was given 2 g L-[ $^{35}\text{S}$ ]cystine per day intravenously ( $\bullet$ ) during days 0–10.

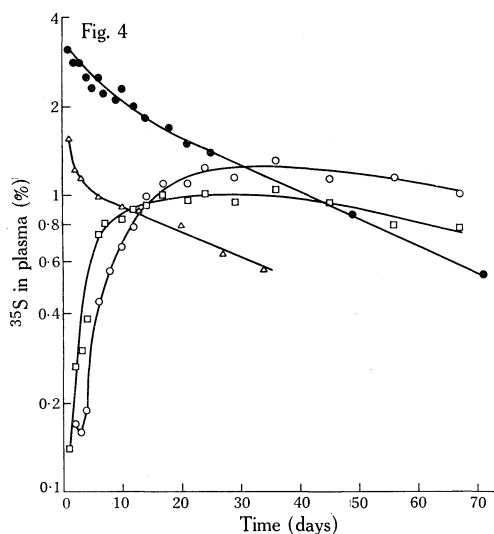


Fig. 4.—Amount of  $^{35}\text{S}$  per litre of plasma at various times after administering large doses of [ $^{35}\text{S}$ ] amino acids to four sheep [first experiment, Section III(c)]. L-[ $^{35}\text{S}$ ]Cystine (2  $\mu\text{Ci/g}$ ) administered as follows: 40 g intraperitoneally ( $\circ$ ); 40 g subcutaneously (divided between eight sites) ( $\square$ ); 2 g/day intravenously during days 0–10 ( $\bullet$ ). DL-[ $^{35}\text{S}$ ]Methionine (1  $\mu\text{Ci/g}$ ) administered as follows: 40 g intraperitoneally ( $\Delta$ ). Each result for the sheep dosed intravenously was calculated as a percentage of total dose administered prior to sampling.

of  $^{35}\text{S}$  in the plasma continued to increase more slowly for a further 20–30 days (Fig. 4). Even 60 days after giving the subcutaneous and intraperitoneal doses the rate of decrease in plasma  $^{35}\text{S}$  was still slower than the exponential rate observed after giving the intravenous doses (Fig. 4).

After administering DL-[ $^{35}\text{S}$ ]methionine (40 g; 1  $\mu\text{Ci/g}$ ) intraperitoneally the maximum amount of  $^{35}\text{S}$  in the plasma was attained within 3 days. From then on the amount fell continuously and the rate of decline became exponential after 6

days (Fig. 4). Thus, compared with cystine, methionine was absorbed much more rapidly from the intraperitoneal depot.

Approximately 17% of the  $^{35}\text{S}$  from the first intravenous dose of L- $^{35}\text{S}$  cystine was excreted in the urine during the first day. During the 10-day period of intravenous dosing and the following 6 days 43% of the total dose of  $^{35}\text{S}$  was excreted in the urine and 8% in the faeces. Similarly a large proportion (*c.* 50%) of the  $^{35}\text{S}$  from the 40-g intraperitoneal dose of DL- $^{35}\text{S}$  methionine was excreted in the urine in the first 6 days. In contrast to these results, less than 1% of the  $^{35}\text{S}$  from either the intraperitoneal or subcutaneous doses of L- $^{35}\text{S}$  cystine appeared in the urine during the first day and a total of less than 15% was excreted in the urine and faeces during the first 16 days. The contrast between the results with intraperitoneal or subcutaneous administration of L- $^{35}\text{S}$  cystine and those with intravenous administration is illustrated by the cumulative excretion patterns (Fig. 5).

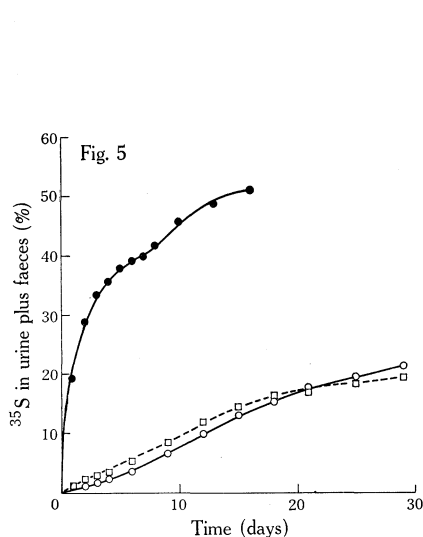


Fig. 5.—Cumulative excretion of  $^{35}\text{S}$  (as a percentage of total dose administered) by sheep after intraperitoneal (○), subcutaneous (□), and intravenous (●) administration of large amounts of L- $^{35}\text{S}$  cystine (see legend to Fig. 4).

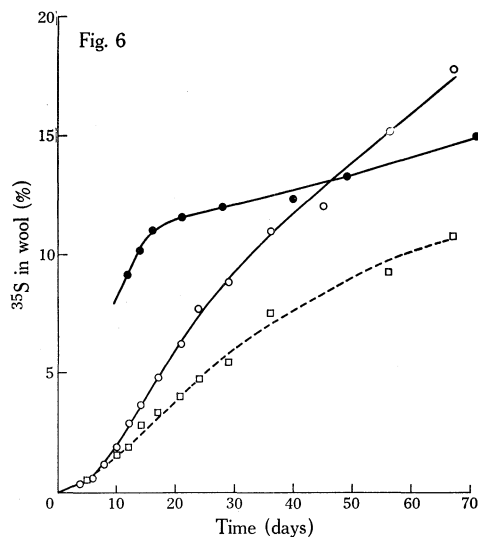


Fig. 6.—Cumulative incorporation of  $^{35}\text{S}$  into wool (as a percentage of the dose administered) of three sheep after intraperitoneal (○), subcutaneous (□), and intravenous (●) administration of large amounts of L- $^{35}\text{S}$  cystine (see legend, Fig. 3). The results for the intravenous route are expressed as a percentage of total dose given during the first 11 days; during this period the specific radioactivity of wool samples plucked from this animal increased at a steady rate.

During the period of intravenous dosing and the following 4 days  $^{35}\text{S}$  was incorporated into the wool at an approximately constant rate. Incorporation of more  $^{35}\text{S}$  into wool continued during the next 60 days (Fig. 6). At the end of this period about 15% of the dose was present in the wool. During the same time, a total of 18 and 11% respectively of the  $^{35}\text{S}$  from the intraperitoneal and subcutaneous doses was incorporated into the wool. The greatest rate of incorporation occurred during the second and third weeks but even at the end of the first 60 days the rate of incorporation had only decreased to about half the maximum (Fig. 6).

During the second and third weeks after injecting the intraperitoneal and subcutaneous doses the rate of wool growth increased by about 20% in both of these sheep. The intravenous doses produced an increase of about 40% in the rate of wool growth during the following 2-week period, but no change in wool growth rate was detected after administering the intraperitoneal dose of DL-[ $^{35}\text{S}$ ]methionine even though about 17% of the  $^{35}\text{S}$  from this dose was incorporated into the wool during the first 70 days.

These results indicated that the subcutaneous and intraperitoneal depots of cystine were slowly absorbed and incorporated into wool with good efficiency. However, the subcutaneous doses caused obvious discomfort to the sheep. Because of this and the fact that only relatively small amounts of material could be injected subcutaneously, it was decided to examine the results obtained with the intraperitoneal route of administration in a second experiment in which four sheep each received L-[ $^{35}\text{S}$ ]cystine (50 g; 2  $\mu\text{Ci/g}$ ) as a single dose. In these sheep the proportion of the dose excreted in the urine during the first 40 days ranged from 8 to 50% (Fig. 7). A comparison of Figures 7(a) and 7(b) shows that the higher rates of excretion

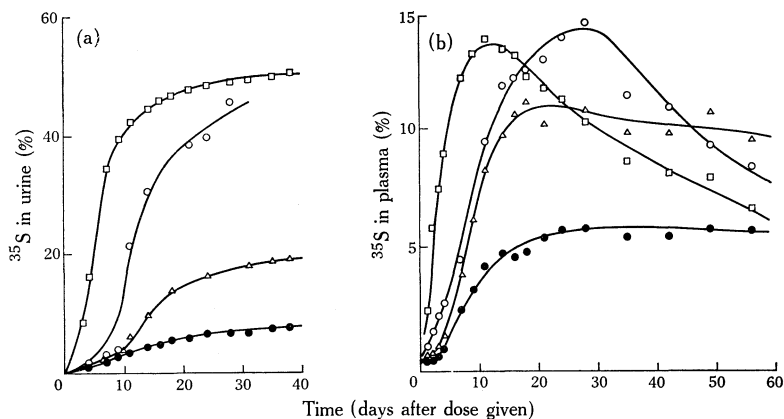


Fig. 7.—Cumulative excretion of  $^{35}\text{S}$  in urine (a) and amount per litre of plasma (b) of four sheep which each received 50 g L-[ $^{35}\text{S}$ ]cystine as a single intraperitoneal dose. Results expressed as a percentage of the total dose of label administered.

occurred in the sheep whose plasma  $^{35}\text{S}$  concentration increased and then decreased more rapidly. The proportion of the dose incorporated into the wool during the first 70 days ranged from 13 to 25%. Wool grown on tattooed areas was measured at weekly intervals. On average, there was an increase in wool growth of about 25% 3–4 weeks after the doses were given and these higher rates were maintained for several more weeks.

(d) *Effects of Large Intraperitoneal Doses of L-Cystine or DL-Methionine on Growth and Sulphur Content of Wool*

In view of the above results a wider range of amounts of L-cystine (25–100 g) were given intraperitoneally to another eight sheep to measure effects on growth rate and sulphur content of wool. For comparison, similar amounts of DL-methionine

were also injected at the same time into eight sheep. The mean wool growth rate for each group of sheep is shown in Figure 8. The intraperitoneal doses of L-cystine significantly increased the wool growth rate for about 10 weeks. The mean maximum

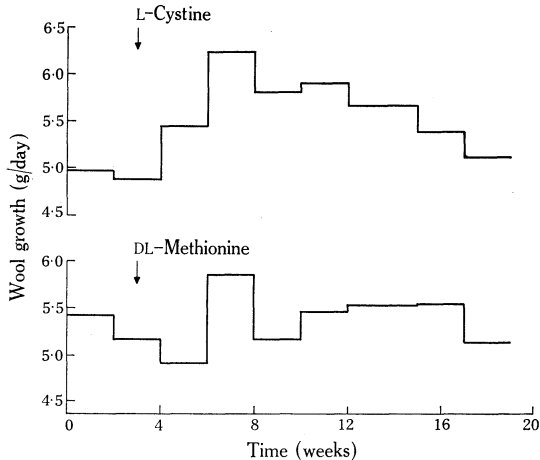


Fig. 8.—Mean wool growth in sheep (eight/group) given large intraperitoneal doses (25–100 g) of L-cystine or of DL-methionine.

wool growth (weeks 6–8, Fig. 8) was 1.3 g/day (27%) above the basal rate; this increase was significant ( $P < 0.01$ ). Allowing for changes in diameter and the time

TABLE 2

EFFECT OF INTRAPERITONEAL DOSES OF L-CYSTINE AND  
DL-METHIONINE ON WOOL GROWTH

The sheep received 800 g/day of a diet of equal parts chopped wheaten and lucerne hays. A single intraperitoneal injection of L-cystine or DL-methionine was given to pairs of sheep as indicated. Wool growth was calculated as clean dry wool on the basis of tattooed patch clippings

Dose of L-Cystine (g)	Post-injection Wool Growth of Individual Sheep (%)*	Dose of DL-Methionine (g)	Post-injection Wool Growth of Individual Sheep (%)*
25	116, 114	25	107, 96
50	127, 131	50	100, 110
75	112, 112	75	112, 88
100	145, 101	100	120, 80

\* Values given as a percentage of basal rate of wool growth. Basal rate calculated as mean wool growth for weeks 1–4 inclusive, and post-injection rate as mean wool growth for weeks 6–12 inclusive (see Fig. 8). Intraperitoneal doses were given at the end of week 3.

of about a week for newly grown wool to emerge from the skin (Downes and Sharry, unpublished data) the maximum wool growth occurred during the second 2-week period after giving the dose. Data for individual sheep (Table 2) indicate consistent

responses to all dose levels of cystine except 100 g; further work is needed to determine the optimum dose level. Overall, there was no effect of intraperitoneal doses of methionine on wool growth, although there was a transient increase during weeks 6–8 (Fig. 8); individual responses were variable (Table 2).

Both cystine and methionine injections increased the sulphur content of the wool grown; cystine produced larger and more sustained increases (Fig. 9).

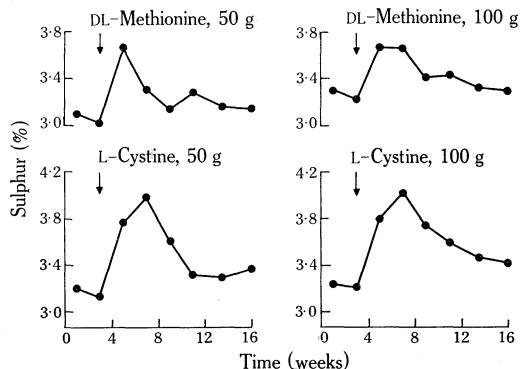


Fig. 9.—Sulphur content of wool grown by sheep which were dosed intraperitoneally with 50 or 100 g of L-cystine or of DL-methionine.

During the week following injection, nine sheep, mainly those that received the larger doses of cystine and methionine, left significant feed residues for periods of up to 5 days. One sheep that received 100 g methionine, continued to refuse some food for 2 weeks. In general, cystine was easier than methionine to inject. Most sheep that received methionine developed temporary lameness during the first week and one sheep subsequently developed an abscess over the injection site. Lameness was also observed in the sheep that received 100 g cystine.

#### IV. DISCUSSION

The main conclusions to be drawn from the results are firstly, that parenteral administration of either L-cyst(e)ine or DL-methionine is effective in stimulating wool growth; and secondly, that large doses of L-cystine injected intraperitoneally act as depots from which the cystine is released at a rate sufficient to increase wool growth, while similar depots of DL-methionine are absorbed too rapidly to be useful. Here, presumably, the methionine concentration is raised to abnormally high levels, already known to cause a suppression in wool growth (Reis and Tunks, unpublished data).

A comparison of the results of intraperitoneal and intravenous infusions of tracer amounts of L-[ $^{35}\text{S}$ ]cystine shows that the small amounts of cystine were absorbed from the peritoneal cavity rapidly and were incorporated into the plasma proteins and wool with about the same efficiency as the intravenous doses. Also, when L-cysteine (2 g/day) or L-methionine (2.5 g/day) was infused intravenously or intraperitoneally there was a rapid increase in wool growth, the main increase occurring during the first 8 days. Although there may be differences in the metabolism of cysteine and cystine in the sheep, their overall effects on wool growth have been found to be similar (Downes *et al.* 1970). A 12-day supplementation period with measurements made between days 8 and 12 should therefore be sufficient to evaluate the wool growth response to an amino acid supplement.

The changes in the growth rate and sulphur content of the wool and the results of the  $^{35}\text{S}$  tracer studies all show that the *S*-amino acids were effective in stimulating wool growth when given parenterally. No direct comparisons of the results with those obtained with abomasal supplements were made but the changes observed were of the same order as those previously described (Reis and Schinckel 1963; Reis 1967). This indicates that the alimentary hormones, such as gastrin, secretin, and pancreaticozym, 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.

The 11 daily intravenous doses of 2 g L- $^{35}\text{S}$ ]cystine produced a gradual increase in the specific radioactivity of the plasma proteins, whose subsequent turnover rate was similar to that previously observed with single 2-g doses (Downes 1961). The steady increase in plasma  $^{35}\text{S}$  after administering 40 g of L- $^{35}\text{S}$ ]cystine subcutaneously or intraperitoneally shows that the cystine was absorbed slowly from these depots. The rate of absorption during the first 2–3 weeks was nevertheless approximately equivalent to a daily intravenous dose of 2 g (Fig. 3). Such a rate of absorption would be expected to stimulate wool growth (Fig. 2) and this expectation was confirmed (Fig. 8).

The effect of a subcutaneous injection of L- $^{35}\text{S}$ ]cystine divided between eight sites was similar to that of a single large intraperitoneal injection, but the subcutaneous administration of large doses was more difficult. It should be emphasized that these results were obtained by injecting the relatively insoluble cystine, not cysteine, as an aqueous suspension because the aim was to produce a depot that would be released slowly. No attempt to dissolve more of the cystine, for example by adding acid, was made. Marston (1935) injected cysteine hydrochloride solutions after neutralizing with sodium carbonate, but in our experience this procedure leads to the production of large necrotic areas at the injected sites.

These results obtained after intraperitoneal administration of methionine show that this amino acid is absorbed too rapidly and so does not provide a satisfactory intraperitoneal depot. The depression in wool growth and transient increase in the sulphur content of the wool during the first 2 weeks after methionine administration were probably due to the presence in the blood of abnormally high concentrations of methionine which are already known to produce the observed effects (Reis 1967). Furthermore, in the sheep which received 40 g of DL- $^{35}\text{S}$ ]methionine intraperitoneally, no change in the rate of wool growth was detected even though 17% of the dose of  $^{35}\text{S}$  was incorporated into the wool, an amount in the range (15–30%) observed with 2 g doses administered abomasally (Downes *et al.* 1970). This also fits in with the previous results (Reis 1967) showing that large supplements of methionine are associated with enhanced production of high sulphur proteins in wool without any overall effect on the rate of wool growth (Gillespie and Reis 1966). In addition to the negligible effects on wool growth there were obvious ill effects on the sheep when large amounts of methionine were injected.

A rate of wool growth of 20 g clean wool per day is about the maximum ever observed, and would represent an output of about 3 g cystine per day in wool. Since no more than 30–40% of the cystine injected into the blood is incorporated into wool, an intraperitoneal depot of 100 g cystine would therefore be expected to provide the equivalent of about 2 weeks wool growth at the maximum rate. If the

depot were released at a steady rate of, say, 2 g/day, an increase in wool growth would be expected to last about 2 months. In practice these ideal conditions were not attained: the rate of release was not constant (Fig. 7) and the increases in wool growth were quite variable. Further research would be needed to explain the cause of such variations. In the present experiments the mean maximum wool growth, representing approximately a 30% increase, occurred during the second 2-week period after giving the dose. Thus, in order to maintain and possibly to enhance the increase in wool growth intraperitoneal doses would have to be given at intervals of about 4-6 weeks.

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