Metabolism of Sulfur-containing Amino Acids by Pregnant Merino Ewes

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Abstract

The availability and utilization of cystine and methionine were measured in single-bearing Merino ewes on three occasions, approximately 90, 110 and 130 days after mating, and the effects on these traits of sulfur amino acids (SAA) infused into the abomasum were also measured. Two levels of SAA were infused containing 0·5 or 1·0 g day\(^{-1}\) organic sulfur with DL-methionine contributing two-thirds and L-cystine one-third of the supplementary sulfur. The quantity of the diet offered was increased at each occasion so as to maintain maternal liveweight.

The rates of irreversible loss of both cystine and methionine from plasma increased as pregnancy advanced, but the ratios between the rates of irreversible loss and intake of digestible organic matter (DOMI) did not vary with stage of pregnancy. The average daily rates of irreversible loss of cystine and methionine by the ewes consuming the diet alone were 13·6 and 119 mmol kg\(^{-1}\) DOMI respectively. The average rates of irreversible loss of methionine (I\textsubscript{m}, mmol h\(^{-1}\)) and of cystine (I\textsubscript{c}, mmol h\(^{-1}\)) were both linearly (P < 0·05) related to the rate of infusion of organic sulfur into the abomasum (s, g day\(^{-1}\)): I\textsubscript{m} = 2·44 (±0·33) s + 1·28 (±0·13); and I\textsubscript{c} = 0·16 (±0·02) s + 0·30 (±0·01). Five per cent of the rate of irreversible loss of cystine arose from trans-sulfuration of methionine by ewes consuming the ration only, but greater percentages (14 and 22\%) were observed when the ration was supplemented with SAA (P < 0·05). These transfer quotients were not influenced by stage of pregnancy. The stage of pregnancy did not influence the concentration of cystine or methionine in the plasma, but the abomasal infusions of SAA significantly increased the concentration of both SAA.

The ewes consuming the basal diet were in positive balance for both nitrogen and sulfur. The retention of nitrogen did not vary with stage of pregnancy (average (s.e.), 5·8 (0·9) g day\(^{-1}\)), but that of sulfur increased from 0·6 to 1·0 and 1·3 g day\(^{-1}\) in periods 1, 2 and 3, respectively (P < 0·05). The retentions of nitrogen (N, g day\(^{-1}\)) and of sulfur (S, g day\(^{-1}\)) were linearly and significantly related to the rate of infusion of organic sulfur into the abomasum (s, g day\(^{-1}\)): N = 2·7 (±0·7)s + 4·4 (±0·3); and S = 0·49 (±0·03)s + 0·72 (±0·01). Significantly decreased concentrations of leucine, serine and threonine in the plasma of SAA-supplemented ewes also indicated that increased availability of SAA improves the efficiency of usage of nitrogen by pregnant ewes.

The growth in length of the wool fibres and their average diameter were similar in the three periods. Average diameter (D, µm) was linearly related to s (P < 0·05). D = 1·1 (±0·1)s + 20·4 (±0·1). The sulfur content of the wool was significantly increased by SAA treatment (34 and 36 mg sulfur g\(^{-1}\) wool in the control and supplemented ewes respectively).

Introduction

The annual clean fleece production of grazing Merino ewes which bear a lamb may be 6–15\% less than that of ewes in the flock which do not become pregnant (Corbett 1979). The reduced annual production results from markedly depressed rates of wool production during late pregnancy and early lactation, but the rate of wool production may commence to decline as early as day 70 of pregnancy (Corbett 1966). As the quantity of amino acids, particularly the sulfur-containing amino acids (SAA), available for absorption from the intestines effectively limits the rate of wool growth by non-mated sheep consuming roughage diets (Reis 1979),
Williams et al. (1978) attempted to increase the wool production of pregnant ewes by increasing the post-ruminal supply of SAA to single-bearing ewes. This treatment did not increase the rate of wool growth of the ewes during late pregnancy and early lactation, but the sulfur content of the wool grown did increase.

The interpretation of these results in terms of the availability and utilization of the SAA for wool growth is difficult, given the limited biokinetic data for the SAA, particularly in pregnant and lactating ewes.

The present study measured the concentrations and rates of irreversible loss of both cystine and methionine from plasma during the second half of pregnancy in Merino ewes, and the sensitivity of these and other traits to supplementary SAA administered into the abomasum. Although both sulfur-containing amino acids, cyst(e)ine and methionine, can increase the rate of wool production, the optimum quantities and ratios of the two amino acids for this purpose are unknown. In an attempt to alleviate deficiencies in both SAA, both methionine and cystine were administered, with the former providing twice as much sulfur as the cystine. This ratio was chosen because Byington et al. (1972) had shown that the growth of rats was superior with diets in which the ratio between methionine-S and cystine-S was 2:1.

Materials and Methods

A small flock of mature medium-wool Merino ewes was joined with Dorset Horn rams after synchronization of oestrus in late spring. Thirty-three days after mating, a laparotomy was performed on the ewes which did not return to service, to identify those pregnant ewes with a single fetus. Abomasal fistulae were established and cannulae inserted into six ewes (Hart and Williams 1972) about 47 days after mating.

Following surgery, the ewes were housed indoors in metabolism cages, and offered 700 g day⁻¹ of the experimental diet (lucerne chaff and oat grain; 1:1 by weight) until the commencement of the experimental treatments 76 days after mating.

The study was conducted in three periods: 76–97, 98–119 and 120–141 days after mating. The quantity of the diet offered to each ewe remained constant during each period, but was increased at the beginning of each period. The ewes were offered 700, 850 and 1000 g day⁻¹ in periods 1, 2 and 3 respectively. The increments were intended to supply sufficient additional metabolizable energy to maintain maternal liveweight as pregnancy advanced (Anon. 1965). A supplement of coarse salt and limestone (1:1) was added to the daily ration at a rate of 20 g kg⁻¹ diet. The ewes were drenched with a broad-spectrum anthelmintic before the experiment commenced.

The experimental treatments were imposed by varying the quantities of l-cystine and DL-methionine contained in 750 ml of solution infused continuously into the abomasum each day. The quantities were 0, 0.5 and 1.0 g day⁻¹ organic sulfur(s), with methionine-sulfur providing two-thirds of the total infused sulfur in each treatment. The cystine and methionine solutions were pumped through separate lines from a peristaltic pump, acidified water and water being the respective solvents.

Experimental Design and Procedure

The three SAA treatments were imposed on six pregnant ewes during three periods within two 3 × 3 latin squares using a cross-over design (p. 43 of Patterson and Lucas 1962). The design was balanced for estimation of the direct and residual effects of treatments. There were 4 degrees of freedom for residual.

As the treatments involved increasing levels of sulfur, the regression coefficient of each variable on the quantity of sulfur infused was calculated, after adjustment for any residual effects of treatments. The coefficients then demonstrate the change in the variable for a 1 g day⁻¹ increase in organic sulfur. Levels of probability less than 5% were deemed significant.

In each period, each ewe received an intravenous infusion of radioactively labelled cystine and methionine 14 days after the treatments commenced. Faeces and urine were quantitatively collected from the commencement of the infusions of the labelled SAA. Seven days after the isotopic infusions, wool fibres growing at the flank were plucked to assess the rate of wool growth and the uptake of isotope into wool.

Infusion of Isotope

L-[³⁵S]methionine (SJ123) and L-(3-3'H)[³H]cystine (TRA 307) were purchased from Amersham (Australia); the specific radioactivities of the purchased radiochemicals were quoted as 150–220 GBq mmol⁻¹ and 60 GBq mmol⁻¹ respectively. These isotopes were diluted with sterile saline before infusion into a
jugular vein through a polyvinyl catheter which had been inserted the previous day. The solutions were infusing using a syringe pump; the (3H)cystine and [35S]methionine solutions were contained in separate syringes. The rate of administration of each solution was estimated from the difference in weights of the syringes at the start and end of each infusion which continued for 6 h. Weighed aliquots of the infusion solutions were assayed for 3H and 35S radioactivity by liquid scintillation spectrometry. During the final 3 h of infusion, four samples of blood were collected.

From each of these, the specific radioactivities of cystine and methionine in the plasma were assayed. The average specific activity (A) of each amino acid was used to compute the rate of irreversible loss of the amino acid (I) from the plasma from the formula (after Steele 1964) \( I = F/A \), where \( F \) is the rate of infusion of radioactive amino acid.

In previous papers (Williams et al. 1972a; Williams et al. 1979), the solution of this equation was termed the entry rate of the substance. However, the rate of irreversible loss, equivalent to the entry rate, is now the preferred designation (White et al. 1969).

**Balance Studies**

Faeces and urine were quantitatively collected for 7 days from the start of the isotope infusions. The urine was collected into vessels containing the mercuric chloride/acetic acid preservative described by Rocks (1979). The volume of urine excreted was measured each day and an aliquot stored at -20°C until required for analysis. The faeces collected daily were dried at 70°C to constant weight in a forced draught oven. Samples of the dry faeces were stored for analysis.

When bulked samples were required for assays, urine samples from different daily collections were mixed in proportion to the volume excreted. Similarly, a 5% sample of each day's weight of dry faeces was used to form a composite sample before grinding to pass a 1-mm sieve. The nitrogen and sulfur were assayed in the bulked samples and in samples of the diet. The contents of organic matter in the feed and faeces were also assayed.

**Chemical Assays**

The nitrogen content was assayed by Kjeldahl analysis (Anon. 1970). Sulfur content was assayed by perchloric/nitric acid digestion followed by turbidometric determination of sulfate-sulfur (Mottershead 1971). The organic matter in feed and faeces was determined from the loss of dry weight following ashing at 600°C for 2 h.

Plasma urea and sulfate were assayed by the methods of Marsh et al. (1965) and Hogan and Breen (1960) respectively.

The blood samples collected during the isotope infusions were drawn into cold syringes, plasma separated and deproteinized with solid sulfoosalicylic acid (30 mg ml^-1) as quickly as possible after blood collection (c. 15 min). The protein-free filtrates were stored at -20°C until chromatography. An aliquot of the protein-free filtrate, containing norleucine as an internal standard, was introduced into an Amino Acid Analyser, having a column of Durrum 4A resin. The effluent from the column was split, one stream proceeding to interact with ninhydrin and the other to a fraction collector. The lithium-based buffer system was adjusted to achieve optimum separation and isolation of cystine and methionine. The collected fractions containing these two compounds were assayed for 35S and 3H by liquid scintillation with the spectrometer settings adjusted for dual-isotope counting. The emulsion-counting system of Patterson and Greene (1965) was used for incorporation of the aqueous samples. This chromatographic procedure provided data on both the concentrations of the two sulfur-containing amino acids (\( \mu \text{mol l}^{-1} \)) and their specific radioactivities (Bq \( \mu \text{mol}^{-1} \)), as well as the concentrations of several other amino acids in the plasma. The method devised by Hendler (1964) was used to separate the radioactivity due to 35S and 3H.

The acid-insoluble material from the plasma was washed twice with 2% sulfoosalicylic acid, then sequentially with water, ethanol, ethanol : ether (1:1), and finally ether. This lipid-free acid-insoluble material was reacted with mercapto-ethanol at 37°C in phosphate buffer (0.1 mol l^-1; pH: 7.5). The 35S and 3H (i.e. disulfide-bound cystine, Downes 1961) released by this extraction were measured.

The radioactivity in the clean dry plucked fibres was measured after combustion of the samples in an oxygen-flask and incorporation of the liquid containing the absorbed gaseous products of combustion into a gel with toluene : Triton X-100 at 7:6 v/v (Patterson and Greene 1965).

**Wool Growth**

The wool was removed from a small area of skin at the mid-side of each ewe at the start of each period. Fibres were plucked from this clipped area, 7 days after the infusion of radioisotopes.

The plucked fibres were cleaned by washing in petroleum spirit, ethanol and water, before being placed
on slides for autoradiography (Downes et al. 1967) and the remainder of the dried wool fibres combusted in a flask containing oxygen with 1% of hydrogen peroxide as the absorbing solution. The $^{35}$S and $^3$H were measured in this solution, and the radioactivities per unit weight of fibre were calculated.

The autoradiographed fibres were examined microscopically. Fibres ($n = 100$) were measured for diameter and length. The diameter was measured at approximately half the distance from bulb end to $^{35}$S darkening ($D_b$), and at a third and two-thirds of distance from the darkening to the tip of the fibre ($D_t$ and $D_d$). Total fibre length ($L$) and length from bulb to darkened area ($L_b$) were also measured. $D_b$ and $L_b$ were analysed as the traits responding to the experimental treatments. The radioactivities ($^3$H and $^{35}$S) per unit weight of plucked fibre were corrected by multiplication with the factor $[0-33(D_b + D_t + D_d)]^2 L/D_b L_b$. These corrected radioactivities then represented the $^3$H and $^{35}$S in unit weight of fibre grown in the 7 days following the infusion of radioactive amino acids.

The wool was clipped from another small area of skin at the midside 7 days after the treatments commenced and reclipped 7 days after the treatments ceased. The sulfur content of the dry fibre from this clipped wool was assayed by the method of Mottershead (1971).

Results

*Liveweight and General*

The appetite of the ewes recovered quickly after surgery and remained satisfactory throughout the experiment, as did their general health. Gestation length varied from 146 to 149 days. The ewes gave birth to live single lambs whose weights were between 4-0 and 6-1 kg.

The pregnant ewes weighed 36-5 ± 1-5 kg 71 days after mating. The lambs were taken from the ewes at birth, and the ewes were then offered 700 g day$^{-1}$ of the experimental diet, as at the initial weighing. After consuming this ration for 2 weeks, the ewes weighed 36-6 ± 1-7 kg. Allowing for the fleece growth during the intervening 3 months, and assuming equal gut fill, the ewes would have lost only 1–2 kg of liveweight during the latter half of pregnancy.

*Digestibility of Diet and Retention of Nitrogen and Sulfur*

The average digestibility of the organic matter in the diet was 0.71 kg kg$^{-1}$ OM. Period of measurement and SAA treatment did not significantly influence the digestibility of organic matter. The ewes consumed on average 430, 510 and 570 g day$^{-1}$ of digestible organic matter in periods 1, 2 and 3.

The nitrogen content of the dietary dry matter was 24 g kg$^{-1}$. The retention of nitrogen ($N$, g day$^{-1}$) was linearly ($P < 0.05$) related to the daily rate of abomasal infusion of organic sulfur ($s$, g day$^{-1}$). The relationship, with standard errors in brackets, was $N = 2.7 (+0.7)s + 4.4 (+0.3)$. Nitrogen retention did not vary among periods (5-3, 6-6 and 5.4 g N day$^{-1}$) retained in periods 1, 2 and 3 respectively; s.e., 0.9). There was a residual effect due to the treatments ($P < 0.05$), with nitrogen retention being greater in a period following one in which the sheep received 0-5 or 1.0 g day$^{-1}$ organic sulfur (+0.7 and +1.8 g N day$^{-1}$) than following one in which the sheep received no additional organic sulfur (+1.1 g N day$^{-1}$). The effects of treatments on the nitrogen balance were exerted through their influence on urinary nitrogen excretion rather than by any changes in faecal nitrogen excretion, since this did not vary with treatment (Fig. 1).

The dietary dry matter contained 3.4 g S kg$^{-1}$. The retention of this sulfur ($S$, g day$^{-1}$) was linearly related ($P < 0.05$) to the quantity of organic sulfur infused ($s$, g day$^{-1}$): $S = 0.49 (+0.03)s + 0.72 (+0.01)$. There was a significant linear trend for the quantity of sulfur excreted in urine to increase as the supplementary levels of organic sulfur increased (595, 850 and 1085 mg day$^{-1}$), but faecal output of sulfur was not influenced by treatments. The increased sulfur retention observed with the advance of pregnancy ($P < 0.05$) was associated with decreasing quantities of sulfur excreted in the urine, but with increasing amounts in the faeces (Fig. 1).

The treatments did not influence the ratio of nitrogen to sulfur retained by the ewes, the average ratio being 6.6. This ratio significantly decreased as pregnancy advanced in the ewes, changing from 8-8 in period 1 to 6-9 and 4-2 in periods 2 and 3 respectively (s.e., 0.9).
**Metabolism of Cystine and Methionine**

The concentrations and rates of irreversible loss of the two sulfur amino acids are shown in Table 1. The concentrations of both 'free' cystine ($C_f$, $\mu$mol l$^{-1}$) and methionine ($M$, $\mu$mol l$^{-1}$) did not vary significantly throughout the experiment, but both traits responded linearly ($P < 0.05$) to the treatments ($s$, g sulfur infused daily). The relationships were: $C_f = 7.4 \pm 1.3s + 13.0 \pm 0.5$; and $M = 52.5 \pm 7.2s + 17.9 \pm 2.9$. The concentration of disulfide-bound cyst(e)ine was not influenced by either treatments or periods, but the ratio of bound to free cystine was significantly decreased by the SAA supplements (0.55, 0.41 and 0.32 for 0, 0.5 and 1.0 g day$^{-1}$ organic sulfur respectively).

**Fig. 1.** Excretion and retention of (a) nitrogen and (b) sulfur by pregnant ewes during each measurement period and the effects of supplementary organic sulfur on these measurements.
Table 1. Direct effects of supplementary SAA on the concentrations of cystine and methionine in plasma, and the rates of irreversible loss of these two sulfur-containing amino acids

<table>
<thead>
<tr>
<th>Supplementary sulfur (g day⁻¹)</th>
<th>0</th>
<th>0.5</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma concentration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free cystine (µmol l⁻¹)</td>
<td>12.9</td>
<td>17.0</td>
<td>20.3</td>
</tr>
<tr>
<td>Bound cystine (µmol l⁻¹)</td>
<td>6.3</td>
<td>6.8</td>
<td>5.4</td>
</tr>
<tr>
<td>Methionine (µmol l⁻¹)</td>
<td>20.2</td>
<td>39.6</td>
<td>72.7</td>
</tr>
<tr>
<td>Ratio of ³⁵S to ³H</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in 'free' cystine</td>
<td>0.027</td>
<td>0.047</td>
<td>0.060</td>
</tr>
<tr>
<td>in disulfide-bound cystine</td>
<td>0.12</td>
<td>0.17</td>
<td>0.26</td>
</tr>
<tr>
<td>Rate of irreversible loss</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystine (mmol h⁻¹)</td>
<td>0.295</td>
<td>0.395</td>
<td>0.457</td>
</tr>
<tr>
<td>Methionine (mmol h⁻¹)</td>
<td>1.29</td>
<td>2.48</td>
<td>3.73</td>
</tr>
<tr>
<td>Methionine — cystine (%)</td>
<td>2.5</td>
<td>4.5</td>
<td>5.3</td>
</tr>
<tr>
<td>Cystine — methionine (%)</td>
<td>5.5</td>
<td>13.9</td>
<td>21.8</td>
</tr>
</tbody>
</table>

⁺Standard errors of mean value given in parentheses. BPercentage of the rate of irreversible loss of methionine being converted to cystine. CPercentage of the rate of irreversible loss of cystine being derived from methionine.

The rates of irreversible loss for both amino acids tended to increase as pregnancy advanced, but this effect was only significant for cystine (0.33, 0.37 and 0.45 mmol h⁻¹ in periods 1,

![Supplementary sulfur](image)

Fig. 2. Daily rates of irreversible loss of cystine and methionine per kg DOMI for pregnant ewes during each measurement period and the effects of supplementary organic sulfur on the rates of irreversible loss.
2 and 3, respectively). When the rates of irreversible loss were expressed relative to the weight of digestible organic matter consumed, the analyses then showed that the rates of loss of both cystine and methionine were independent of period (Fig. 2). The relationships between the concentrations and the rates of irreversible loss of the two sulfur amino acids are demonstrated in Figs 3a and 3b.

![Graph](image)

**Fig. 3.** Relationships between the concentration and the rate of irreversible loss for (a) cystine and (b) methionine. Individual pairs of values are shown for ewes in periods 1 (△), 2 (○) and 3 (□), with the values for control ewes having open symbols and for SAA-supplemented ewes having closed symbols.

The rates of irreversible loss (mmol h⁻¹) of both cystine ($I_c$) and methionine ($I_m$) responded linearly ($P < 0.05$) to the abomasal infusions of organic sulfur (s, g day⁻¹), the relationships being: $I_c = 0.16 (±0.02)s + 0.30 (±0.01)$; and $I_m = 2.44 (±0.33)s + 1.28 (±0.13)$. For cystine, the increased rates of irreversible loss accounted for 94 and 80% of the abomasally infused cystine included in the low and high level of SAA supplementation, respectively. The 'recoveries' of infused methionine were greater, being 180 and 248%.

The ratio of $^{35}$S to $^3$H in the cystine isolated by the chromatographic separation responded linearly to the treatments ($P < 0.05$). The percentage of the rate of irreversible loss of methionine
being converted to cystine ($M_\text{c}$) and the percentage of the rate of irreversible loss of cystine derived from methionine $C_m$ were calculated from the ratio of $^{35}$S to $^3$H in the ‘free’ cystine from plasma (Benevenga and Egan 1983). Stage of pregnancy did not influence either trait, but both were linearly related ($P < 0.05$) to the quantity of organic sulfur infused (s, g day$^{-1}$). The relationships were: $M_\text{c} = 2.8 (±0.4)s + 2.7 (±0.2)$ and $C_m = 16.3 (±1.7)s + 5.6 (±0.7)$.

The ratio of $^{35}$S/$^3$H in the disulfide-bound cystine extracted from the plasma proteins with mercaptoethanol also showed an increase ($P < 0.05$) with increasing SAA supplementation, but the ratio was 4-5 times greater than that measured in the cystine isolated from the column (Table 1).

**Plasma Amino Acid and Urea Concentrations**

The chromatographic procedure was optimized for separation and isolation of cystine and methionine, but several other amino acids in plasma were isolated and their concentration measured (Table 2). The concentrations of threonine, serine and leucine were significantly reduced by SAA supplementation. The regression coefficients (μmol l$^{-1}$ g$^{-1}$ S day$^{-1}$) were −80 (±17), −65 (±14.3) and −44 (±5.3) for threonine, serine and leucine respectively. Leucine was the only amino acid significantly influenced by periods. The concentration was greater in period 3 (108 μmol l$^{-1}$) than in periods 1 and 2 (71 and 67 μmol l$^{-1}$; s.e., 14).

The concentration of urea-nitrogen and sulfate-sulfur in plasma were similar in each period and were not influenced by the SAA treatments (Table 2).

**Growth of Wool, its Sulfur Content and Uptake of $^{35}$S and $^3$H**

The sulfur content of the wool was lower in the first measurement period (34.1 mg g$^{-1}$) than in the two final periods (36.5 and 36.3), and responded to the SAA supplements, being 34.1 in the control ewes and 36.4 in those receiving the SAA supplement (s.e., 0.3; $P < 0.05$).

The average diameter of the fibres produced and the average daily increment in fibre length were similar in each of the periods, averaging 20.9 μm (s.e., 0.3) and 313 μm day$^{-1}$ (s.e., 16) respectively. The SAA supplements did not increase the daily increment of fibre length, but did have a significant linear effect on the average diameter of the fibres produced (Table 3).

### Table 2. Concentrations of serine, threonine, and leucine and of urea nitrogen and sulfate sulfur in plasma of ewes receiving 0, 0.5, or 1.0 g day$^{-1}$ supplementary sulfur

<table>
<thead>
<tr>
<th></th>
<th>Supplementary sulfur (g day$^{-1}$)</th>
<th>s.e. of means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine (μmol l$^{-1}$)</td>
<td>118, 62, 53</td>
<td>(8)</td>
</tr>
<tr>
<td>Threonine (μmol l$^{-1}$)</td>
<td>129, 58, 49</td>
<td>(12)</td>
</tr>
<tr>
<td>Leucine (μmol l$^{-1}$)</td>
<td>109, 72, 65</td>
<td>(6)</td>
</tr>
<tr>
<td>Urea nitrogen (μmol l$^{-1}$)</td>
<td>4.8, 5.2, 4.7</td>
<td>(0.2)</td>
</tr>
<tr>
<td>Sulfate sulfur (mEq l$^{-1}$)</td>
<td>3.25, 3.47, 3.22</td>
<td>(0.35)</td>
</tr>
</tbody>
</table>

### Table 3. Growth in fibre length, the average diameter of fibres grown and the content of sulfur in wool for ewes supplemented with 0, 0.5 or 1.0 g day$^{-1}$ organic sulfur

<table>
<thead>
<tr>
<th></th>
<th>Supplementary sulfur (g day$^{-1}$)</th>
<th>s.e. of means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre length growth (μm day$^{-1}$)</td>
<td>315, 298, 328</td>
<td>(15)</td>
</tr>
<tr>
<td>Fibre diameter (μm)</td>
<td>20.5, 20.6, 21.6</td>
<td>(0.1)</td>
</tr>
<tr>
<td>Sulfur content (%)</td>
<td>3.4, 3.6, 3.6</td>
<td>(0.03)</td>
</tr>
<tr>
<td>Specific radioactivity (% dose/g S)</td>
<td>18.6, 16.0, 9.4</td>
<td>(1.4)</td>
</tr>
<tr>
<td>$^3$H</td>
<td>7.4, 8.3, 4.9</td>
<td>(0.7)</td>
</tr>
<tr>
<td>$^{35}$S</td>
<td>0.42, 0.54, 0.54</td>
<td>(0.01)</td>
</tr>
<tr>
<td>ratio $^{35}$S/$^3$H</td>
<td></td>
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</tr>
</tbody>
</table>

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The relationship between average fibre diameter \( (D) \) and organic sulfur infused \( (s, \text{ g day}^{-1}) \) was 
\[
D = 1.1 \times (\pm 0.1)s + 20.4 \times (\pm 0.1).
\]
There was a significant residual effect of the higher level of SAA supplementation. Fibres produced by sheep that received the higher level of supplementation in a previous period were 0.6 \( \mu \text{m} \) thicker than average, whereas those from sheep which received 0 or 0.5 g day\(^{-1} \) of organic sulfur during the previous period were 0.3 \( \mu \text{m} \) finer than average.

The specific radioactivity of \( ^{35}\text{S} \) in the wool fibres grown during the 7 days following the infusions of the isotopically labelled SAA did not differ between periods \( (\bar{x}, 68 \text{ Bq} \ ^{35}\text{S} \text{ g}^{-1} \text{ sulfur}; \text{s.e., } 11) \) but the specific radioactivity of \( ^{3}\text{H} \) in this wool sulfur was greater \( (P < 0.05) \) in the second \( (190 \text{ Bq} \ ^{3}\text{H} \text{ g}^{-1} \text{ sulfur}) \) than in the first \( (136) \) or third \( (112) \) period of measurement \( (\text{s.e., } 27) \), with infusions of \( 10^{5} \text{ Bq} \) of both \( ^{35}\text{S} \) and \( ^{3}\text{H} \). For both isotopes, the specific radioactivities were lower in those sheep which received the SAA supplementations (Table 3). With equal quantities of the two radioisotopes infused, the ratio of \( ^{35}\text{S}/^{3}\text{H} \) in the plucked clean fibre was significantly greater in the third period \( (0.610) \) than in the first or second periods \( (0.468 \text{ and } 0.411; \text{s.e., } 0.038) \). This ratio \( (R) \) was linearly \( (P < 0.05) \) related to the quantity of organic sulfur infused \( (s) \): 
\[
R = 0.12 \times (\pm 0.01)s + 0.44 \times (\pm 0.01).
\]

**Discussion**

These studies were undertaken primarily to examine the changes in availability and utilization of the two sulfur-containing amino acids, cystine and methionine, which occur with the advance of pregnancy in the ewe, and the sensitivity of these traits to a supplementary supply of the two SAA when delivered into the abomasum. The rates of irreversible loss of the two amino acids increased as the ewes advanced in pregnancy (this result being significant for cystine). The lack of significant variation due to periods for the concentrations of the two SAA, and for the rate of irreversible loss of both cystine and methionine when expressed per unit intake of DOM, suggest that the physiological changes associated with the advance of pregnancy did not influence the availability of SAA to the ewes. The published rates of irreversible loss of the two SAA (Fennessy et al. 1978; Kobayashi et al. 1978; Gill and Ulyatt 1979; Williams et al. 1979; Benevenga and Egan 1983) have been measured in non-pregnant sheep. When these were expressed per unit DOM (estimated), most were about 30\% greater than the values obtained in this experiment with pregnant ewes. However, the rates for both methionine and cystine obtained by Pisulewski and Butterly (1985) with non-pregnant sheep were similar to the present results. More direct comparisons would be required to confirm that the rates of irreversible loss of the two SAA are not altered by pregnancy. The results of Egan et al. (1983) and MacRae and Egan (1983) suggest that pregnancy did not alter the rate of irreversible loss of another essential amino acid, threonine.

When cystine was infused into the abomasum, the measured rate of irreversible loss of cystine accounted for 94\% of the infused cystine. Although this ‘recovery’ is greater than that measured previously (Williams et al. 1979), the inclusion of methionine in the SAA supplement precludes any direct comparison, given the increased conversion of methionine to cystine. Both the concentration of methionine in plasma and its rate of irreversible loss from the plasma were more responsive to the supplementary SAA than were the concentration and the rate of irreversible loss of cystine. Reis et al. (1973) demonstrated that the concentration of methionine in plasma was very sensitive to the quantity of this amino acid infused into the abomasum. This sensitivity has been used to estimate the sheep's requirement for methionine, utilizing the very large increases in concentration that occur when the quantity infused exceeds the animal’s requirement (Wakeling et al. 1970; Mercer and Miller 1973; Strath and Shelford 1978). In this experiment, both the concentration of methionine in plasma and the retention of nitrogen showed linear responses to the supplementary SAA. Consequently, the requirements of the pregnant ewe for methionine could not be assessed from these data. The large apparent sensitivity of the rate of irreversible loss of methionine resulted from the difference between the rates of irreversible loss of methionine for the control ewes and those receiving the supplementary SAA being greater than the rate of infusion of methionine infused into the abomasum. The
results of Fennessy et al. (1978) and of Kobayashi et al. (1978) indicate 'recoveries' greater than 100%. Pisulewski and Buttery (1985) obtained 'recoveries' varying from 34 to 114% infused methionine in animals receiving between 0 and 5 g day\(^{-1}\) of \(\text{L}-\text{methionine}\). On the other hand, Benevenga and Egan (1983) obtained a quantitative recovery when the labelled methionine was infused into the abomasum in contrast to the intravenous infusions used by other authors. As Katz (1982) has demonstrated, the site of entry of the labelled compound can influence the estimated rates of irreversible loss of metabolites which have rapid rates of turnover.

The responses of the ewes to the abomasal infusions were also measured in terms of the balances of nitrogen and sulfur and of the growth of fibre. The significant responses in both nitrogen and sulfur balances indicated that the availability of SAA from the basal diet was limiting the overall efficient retention of both elements by the ewes. The basal diet was consumed in sufficient quantities to provide a gross positive nitrogen balance. Approximately 1, 2 and 3 g nitrogen would have been accumulating daily in the gravid uterus during the three periods (Langlands and Sutherland 1968). Thus, despite positive net maternal balances of nitrogen, the provision of additional SAA for absorption resulted in further nitrogen being retained by the ewes. These responses were due to both direct and residual effects of the treatments. The results for the retention of sulfur were generally similar to those for nitrogen, except that the retention of sulfur increased in each period, leading to a reduction in the ratio of the nitrogen to sulfur retained. Sulfur accumulates in the gravid uterus at a more rapid rate than does nitrogen (Langlands and Sutherland 1973). This is probably a reflection of the increasing concentration of cystine in the fetus (Meir et al. 1981), presumably due to the growth of the fibres comprising the birthcoat. While changes in the retentions of nitrogen and sulfur are generally related, the ratios observed in this experiment (6–8) were less than those quoted by Bray and Till (1975) for sheep. The authors do not know of any published evidence which indicates the ratio in pregnant ewes, but Oddy (personal communication) has recorded similarly low ratios in pregnant ewes.

The reduced concentrations in plasma of the essential amino acids, threonine and leucine, associated with the treatments were consistent with a more effective utilization of nitrogen by ewes supplemented with SAA. Naismith (1980) has observed that rats pass through an anabolic/catabolic sequence during pregnancy, with the transmission between these two states being controlled by the hormonal balance of progesterone and placental lactogen. If the pregnant ewe passes through a similar sequence, it is probable that the additional nitrogen retained because of the treatments may have been stored by the ewe in the first period, but by the gravid uterus during the two later periods. The significant residual effect of treatment on the nitrogen balance would be consistent with this dynamic sequence.

The provision of additional SAA for absorption from the intestines of non-mated sheep has generally resulted in increased rates of fibre production, but has not invariably led to greater retention of nitrogen; compare Robards (1971) and Dove and Robards (1974). However, the demands of the pregnant ewes for amino acids generally are undoubtedly greater than those of non-mated animals. Hence, the pregnant ewes may have a greater capacity to respond to any alleviation of a deficiency by increased conservation of protein, either by increased synthetic rates or decreased catabolic rates. This capacity for response may be a manifestation of an altered partitioning of amino acids preferentially towards maternal tissues (early in pregnancy) or the gravid uterus and its fetus (later in pregnancy), and away from the skin and follicles, with resultant effects on the rate of wool growth. Further research is needed to ascertain whether the increased retentions of nitrogen and sulfur due to supplementation with SAA influence the productivity of the ewe and/or her offspring in the longer term.

A significant response to the supplementary SAA was observed only in fibre diameter; none in the growth rate in length of the fibre. If all follicles were producing fibres (see later), the values observed for the lengths and diameters of the fibres would indicate that the mass of wool produced per unit area of skin (\(w/a\)) would have been approximately 10% greater in the ewes supplemented with SAA. Williams et al. (1978) observed a quantitatively similar response in \(w/a\) during late pregnancy by ewes given twice daily injections of SAA into the abomasum.
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(0.37 g organic S day\(^{-1}\)), but the response was not significant, nor was any significant response in average diameter of fibre observed. However, in both experiments the responses were small compared with those published for non-mated sheep (Reis 1979). There was no indication from the results in this experiment that the response in fibre growth declined with the advance of pregnancy. Thus, the point at which pregnancy is associated with a reduced sensitivity of the follicles to respond to SAA remains uncertain.

Two further points concerning the effects of pregnancy on wool growth arise from the results and will be discussed. Firstly, the volumes of the fibres produced did not decline with advancing pregnancy, under the experimental conditions of increasing intakes of feed to maintain maternal liveweight. The conclusion that the reduction in wool production due to pregnancy could be prevented by additional feed was also drawn by Williams et al. (1978). This has been demonstrated more directly by Williams and Butt (unpublished data), and shown to apply to both single- and twin-bearing ewes. Oddy (1985) concluded that pregnant ewes incur an obligate loss of wool production, regardless of the nutrient supply; however, in his experiment, the intakes of feed did not differ between non-mated and pregnant ewes, with resultant greater losses of liveweight by the pregnant ewes relative to the non-pregnant animals. A combination of the two experimental approaches (e.g. non-pregnant animals pair-fed with the pregnant ewes) is needed to resolve these apparently contradictory conclusions. Secondly, during the examination of the auto-radiographed fibres, plucked fibres with bulb ends were always observed to have incorporated the radioactive label distant from the bulb. Thus all follicles were presumably growing actively. At present, the relative contributions of the components to any reduced rate of wool growth during pregnancy are poorly defined. The extensive field data of Brown et al. (1966) indicated that reduced fibre density was associated with lower production of wool in pregnancy, but Oddy (1985) found no evidence for loss of productive follicles during pregnancy.

The increased content of sulfur in the wool of the ewes supplemented with SAA confirmed previous results (Williams et al. 1978). Thus, the treatments made additional SAA available to the follicles, with consequent increased synthesis of the high-sulfur proteins (Gillespie et al. 1969).

In adult mammals, methionine can be converted to cysteine, via the trans-sulfuration pathway (Finkelstein and Mudd 1967). Both Benevenga and Egan (1983) and Pisulewski and Butterly (1985) have demonstrated in non-pregnant sheep that trans-sulfuration is not the major pathway for catabolism of methionine in sheep, and consequently a relatively small proportion of the cysteine available to tissues is derived from methionine. However, the supply of additional methionine does stimulate trans-sulfuration. The present results were similar, with 2-5% of the methionine being converted to cysteine in the control ewes, increasing to 4.5-5.3% in the SAA supplemented ewes. The similarity of these values to those obtained with non-pregnant sheep (Benevenga and Egan 1983; Pisulewski and Butterly 1985) indicates that pregnancy has little effect on this pathway in sheep. These transfer quotients, derived from the specific radioactivities of cystine and methionine in plasma, were reflected in the ratios between \(^{35}\)S and \(^{3}H\) in the plasma proteins (results not presented) and in the wool fibres. Furthermore, the transfer quotients did not vary with stage of pregnancy, although the ratio of \(^{35}\)S to \(^{3}H\) in fibre was greater in the third period near parturition.

These studies lead to the conclusion that pregnancy does not greatly influence the availabilities of the two sulfur-containing amino acids, cystine and methionine, as assessed by their irreversible loss rates and concentrations in plasma. Furthermore, the effects of supplementary SAA, supplied at the abomasum, on these traits appear to be consistent with the effects previously observed in non-mated sheep. Thus, the data do not serve to explain the much lower responses in the rate of wool growth to SAA by pregnant ewes. Certainly, the efficiency of wool growth, as measured by wool growth per unit intake of feed, is lower during pregnancy (Oddy 1985). Williams et al. (1972b) showed that sheep with genetically determined lower efficiency have a limited capacity to respond to abomasal SAA in terms of increased rates of wool growth. If the low efficiency of production is the common factor in these two situations, the changes in biological function responsible for the effect still require elucidation, as does the function.
itself in governing the rate of wool growth.

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