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Sulfur and Methionine Metabolism in Sheep. V. Utilization of Methionine Isomers

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Abstract

Merino wethers were fed 600 g dry matter per day of a ground and pelleted 50 : 50 oaten chaff : lucerne chaff diet. The ration was fed in 12 equal protions at 2-hourly intervals. The diet of some sheep was supplemented with infusions of 2·5 g/day of either L-, DL- or D-methionine into either the rumen or duodenum. Ruminal sulfide-sulfur pools, the amount of sulfur flowing at the duodenum and excreted in faeces and urine, and the amount of sulfur incorporated into wool were measured. [^{35}S]-L-Methionine injections were given to each sheep and the excretion of [^{35}S]-sulfur and its incorporation into wool were measured.

Infusion of amino acid into the rumen or duodenum did not affect the ruminal sulfide-sulfur pool. Neither ruminal nor duodenal supplements of methionine affected the flow of protein sulfur from the duodenum. However, the flows of total and neutral sulfur from the duodenum were greater ($P < 0{\cdot}01$) when the amino acid was infused into the duodenum rather than into the rumen; form of the supplement had no significant effect.

Faecal sulfur excretion was not affected by either the form of the supplement or the site at which it was infused. However, ruminal infusions of methionine resulted in large increases ($P < 0{\cdot}001$) in urinary excretion of inorganic sulfate-sulfur (*c.* 841 *v.* 402 mg); infusions into the duodenum also increased, but to a lesser extent, the excretion of inorganic sulfate-sulfur (*c.* 565 *v.* 402 mg) in urine.

Supplementation post-ruminally increased ($P < 0{\cdot}001$) sulfur and nitrogen retention, wool growth, and fibre diameter of and sulfur incorporation into wool; ruminal supplements had no effect although sulfur retention tended to increase.

Urinary excretion of sulfur was lower ($P < 0{\cdot}01$) and sulfur retention was greater ($P < 0{\cdot}05$) with L-methionine than with D-methionine. These differences were largely associated with differences due to supplementation into the duodenum. Although there were no differences between the isomers in their effect on wool growth rate, the natural isomer resulted in a higher ($P < 0{\cdot}01$) sulfur content of wool and tended to increase sulfur incorporation into wool.

The possible reasons for differences are discussed, as are reasons for discrepancies between supplementary sulfur excretion and incorporation into wool estimated by either a radioactive tracer technique or a method of comparing supplemented sheep with control animals.

Introduction

The relative nutritional effectiveness of different isomeric forms of methionine has, for some time, been a matter of conjecture. Most of the information available on this subject is in the nature of growth or production responses (e.g. Wretlind and Rose 1950; Bishop 1964; Almquist 1970).

There is a lack of information on the relative rates of utilization of L- and D-methionine by rumen micro-organisms. Further, much of the published work (e.g. Gil and Shirley 1972; Bull and Vandersall 1973; Gil *et al.* 1973*a*, 1973*b*, 1973*c*; Kahlon

et al. 1975) has been conducted under non-physiological conditions, often employing high substrate concentrations, washed cell suspensions, and long-term incubations. Extrapolations from these to *in vivo* situations may not be valid. No *in vivo* comparisons were found in the literature.

The utilization of the isomers of methionine for growth has been studied in rats (e.g. Wretlind and Rose 1950; Wretlind 1952) and chicks (e.g. Brueggemann *et al.* 1962; Bauriedel 1963; Bishop 1964; Marret *et al.* 1964). In a review of sulfur nutrition of nonruminant species, Almquist (1970) interpreted some of the available data as meaning that, depending upon the levels fed, D-methionine might appear inferior to, equal to, or superior to the L-isomer. This interpretation is in accord with the data reviewed, but does not explain them. More recent work with chickens (Katz and Baker 1975), humans (Kies *et al.* 1975; Zezulka and Calloway 1976) and cats (Teeter *et al.* 1978) indicates less than 100% availability from D-methionine as compared with L-methionine.

In sheep, Reis (1967) found that D-methionine infused into the abomasum was effective in stimulating wool growth, but states 'the increase obtained with 1·0 g day^{-1} was only about half that expected from a similar amount of DL-methionine'. However, Reis *et al.* (1978) concluded that abomasal doses of radioactive L-, DL- and D-methionine were utilized with similar efficiency for wool growth. Doyle and Moir (1979*c*) have suggested possible differences in the metabolism of L- and D-methionine within the ruminant system.

In order to establish whether there are differences in the metabolism of methionine isomers by the ruminal micro-organisms, an experiment was carried out to study the effects of ruminal and duodenal infusions of L-, DL- and D-methionine on ruminal sulfide pools, and 'marker-corrected' sulfur flows at the duodenum. The excretion, retention and utilization of sulfur from these infusions and from injections of [^{35}S]-L-methionine into the rumen and duodenum were also measured to indicate whether differences occurred in metabolism of the isomers within the tissues.

Materials and Methods

Experimental Animals and Diets

Eleven Merino wethers each weighing *c.* 36 kg were fitted with a permanent ruminal cannula (Jarrett 1948) and a simple T-piece cannula in the proximal duodenum.

The basal diet comprised chaffed oaten hay (49%), chaffed lucerne hay (49%) and a low-sulfur mineral mix (Hume and Bird 1970) (2%). It was fed in a ground and pelleted form, each animal receiving 50 g dry matter every 2 h.

Tap water was available *ad libitum* in stainless steel troughs.

Experimental Design

The experiment consisted of a preliminary period (period I, 42 days duration), two treatment periods (periods II and IV, each 35 days) and two recovery periods (periods III and V, each of 28 days). Each treatment was received by three sheep; the allocation of sheep to treatments in periods II and IV is outlined in Table 1.

Urine and faeces were collected during the last 7 days of periods I and III and between days 17 and 23 of periods II and IV. Excreta were also collected during periods of infusion of ^{51}Cr-EDTA and [^{103}Ru]tris(1,10-phenanthroline)ruthenium(II)chloride (^{103}Ru–phen) (days 10–13 of the treatment periods) and in subsequent days to estimate the recovery of the markers.

The data were analysed as a non-orthogonal balanced incomplete block design using a contrast matrix. Where there were statistically significant relationships between parameters measured in periods I and II and periods III and IV analysis of covariance was used to adjust the treatment period

means (Steel and Torrie 1960). Treatment comparisons made were: L-methionine *v.* D-methionine; DL-methionine *v.* D-methionine; control *v.* ruminal infusion; and ruminal infusion *v.* duodenal infusion.

Infusion and Injection Solutions

Solutions of L-, DL- and D-methionine (Sigma grade) were prepared daily to supply *c.* 2·5 g of amino acid in about 300 ml of water when infused continuously into the rumen or duodenum.

A dual marker system using ^{51}Cr-EDTA and ^{103}Ru–phen (see Faichney 1975) was used to estimate 'marker-corrected' flow, and sulfur composition of digesta at the duodenum. The ^{51}Cr-EDTA was also used to estimate ruminal fluid volume.

Each sheep in the control and the group receiving ruminal methionine supplements was given a single intraruminal injection of [^{35}S]-L-methionine (supplied by the Radiochemical Centre, Amersham, England). Approximately 160 μCi of radioactivity, along with 600 μg of non-radioactive L-methionine, was given to each sheep. Animals receiving post-ruminal amino-acid infusions were given radioactive methionine injections into the duodenum.

Table 1. Design of the experiment, showing tag numbers of sheep

All sheep were fed the basal ration during periods I, III and V

Treatment	Sheep used in period: II	IV
Control[A]	17, 38	17, 38
L-Methionine per rumen (L-Met R)	31, 34	37
L-Methionine per duodenum (L-Met D)	37	31, 34
DL-Methionine per rumen (DL-Met R)	32, 35	39
DL-Methionine per duodenum (DL-Met D)	39	32, 35
D-Methionine per rumen (D-Met R)	33, 36	40
D-Methionine per duodenum (D-Met D)	40	33, 36

[A] Data from sheep 38 were discarded as it died during period II prior to the completion of digesta and excreta collections.

Sampling Methods

Ruminal liquor was obtained through a tube extending through the cannula bung into the rumen contents as described by Bird (1972).

Infusions of amino acids into the duodenum were stopped 7 min prior to sampling, and the T-piece cannula closed with another rubber bung. This bung was removed and 25 g duodenal digesta was collected into a polythene cup clamped onto the cannula. The infusion line was replaced and the infusion recommenced. Four samples were taken each day for 3 days.

Wool production was estimated by the tattoo patch technique described by Reis and Schinckel (1964). Patches were clipped at the commencement, after 14 days, and after 42 days of period I, after 10 and 35 days in periods II and IV, and after 14 and 28 days in periods III and V.

Analytical Methods

Dry matter, organic matter and total nitrogen in feed and excreta samples were determined by the procedures described by Hume *et al.* (1970). Sulfur determinations on feed, digesta, excreta and wool samples were made by the methods described by Bird and Fountain (1970). [^{35}S]-Sulfur was determined in daily faecal and urine samples for 6 days after injection of the isotope and in wool samples clipped after each injection. Scintillation counting was carried out under refrigerated conditions as described by Bird and Fountain (1970) using the toluene–triton (2+1)-based scintillant of Patterson and Greene (1965).

^{51}Cr-EDTA and ^{103}Ru–phen were determined in rumen liquor, duodenal digesta and excreta as described by Tan *et al.* (1971). Duodenal digesta were fractionated as described by Doyle and Moir (1979*b*). Ruminal sulfide-sulfur pools and 'marker-corrected' flows of sulfur at the duodenum were estimated as described by Doyle and Moir (1979*a*).

Table 2. Ruminal fluid volume, ruminal sulfide-sulfur pool, and 'marker-corrected' digesta and sulfur flows from the duodenum

Values are treatment means ± s.e.

	Treatment						
	Control	L-Met R	L-Met D	DL-Met R	DL-Met D	D-Met R	D-Met D
Rumen fluid volume[A] (litre)	2·65±0·635	2·96±0·318	3·99±0·830	3·13±0·231	3·35±0·274	2·81±0·574	3·15±0·603
Rumen sulfide-sulfur pool (mg)	11±3·2	16±1·4	15±0·5	19±2·6	14±2·0	17±2·9	12±2·6
Duodenal digesta flow (kg day^{-1})	6·46±0·767	7·04±0·396	7·71±1·048	6·71±0·585	8·08±1·294	7·50±0·509	8·20±0·946
Duodenal sulfur flow (mg day^{-1})							
Total	1280±105·0	1369±33·9	2387±329·2	1561±122·6	2249±352·5	1932±596·6	2940±664·1
Protein	773±9·0	887±97·2	918±81·8	897±15·0	982±78·2	993±215·3	1026±170·4
Reducible	39±16·0	29±3·3	28±1·2	42±14·9	32±7·1	25±0·9	29±4·2

[A] Ruminal fluid volume of sheep 37 in period II was not estimated due to infusion problems. Hence the mean was calculated using a missing value.

Results

Ruminal Sulfide-sulfur Pool and 'Marker-Corrected' Flow of Sulfur at the Duodenum (see Table 2)

Neither ruminal fluid volume nor ruminal sulfide-sulfur pool size was affected by treatment.

'Marker-corrected' flows of total and neutral sulfur at the duodenum were greater ($P < 0{\cdot}01$) when supplements were given into the duodenum compared with into the rumen. This was due partly to increased ($P < 0{\cdot}05$) duodenal digesta flows with infusions at the duodenum. The isomeric form of the amino acid did not influence total or neutral sulfur flows at the duodenum and there was no difference between ruminal infusion and the control treatments in this regard. Within the neutral sulfur fraction, protein sulfur flow was not influenced by the form or site of the amino acid infusion.

The control sheep had a greater ($P < 0{\cdot}05$) flow of reducible sulfur from the duodenum than sheep receiving ruminal supplements of methionine. However, the amount of sulfur flowing in this form was always less than 4% of the total sulfur flow.

Excretion and Retention of Sulfur and Nitrogen (see Table 3)

Neither the isomeric form of the supplement nor the site at which it was infused affected sulfur or nitrogen excretion in faeces.

Total and inorganic sulfate-sulfur excretion in the urine was greater ($P < 0{\cdot}001$) in sheep receiving ruminal supplements than in control sheep or sheep receiving post-ruminal supplements. In addition the excretion of total sulfur ($P < 0{\cdot}01$) and inorganic sulfate-sulfur ($P < 0{\cdot}05$) was greater in sheep given the D-isomer than in those receiving L-methionine.

There was no effect of form of the supplement or site of infusion on excretion in the urine of neutral or ester sulfate-sulfur. Urinary nitrogen output was less ($P < 0{\cdot}001$) with duodenal than with ruminal supplements, and tended to be less with L-methionine than with D-methionine infusions. As with urinary sulfur differences, the differences between isomers was largely associated with differences due to supplementation into the duodenum.

Sulfur retention was not increased by ruminal infusions of methionine; it was increased ($P < 0{\cdot}001$) by duodenal infusions. Further, sulfur retention was greater ($P < 0{\cdot}05$) when the natural isomer was provided compared with supplementation with the D-form. Similarly, nitrogen retention was improved ($P < 0{\cdot}001$) by infusions of amino acid into the duodenum, and tended to be greater with L- than with D-methionine.

Wool Growth Rate and Composition (see Table 4)

Duodenal infusions of methionine increased ($P < 0{\cdot}001$) the rate of wool growth compared with ruminal supplementation. There was no effect of the isomeric form of methionine supplements on wool growth rate.

The greater growth rate resulting from duodenal supplements of methionine was accompanied by larger ($P < 0{\cdot}001$) fibre diameter, and increased ($P < 0{\cdot}001$) sulfur content of and sulfur incorporation into wool. Wool fibre diameter of sheep given the natural isomer was less ($P < 0{\cdot}01$) than that of sheep receiving the D-amino acid; sulfur content of wool was greater ($P < 0{\cdot}01$) in sheep receiving L-methionine supplements, and sulfur incorporation into wool tended to increase.

Table 3. Dietary intake, infusion amounts, excretion and retention of sulfur and nitrogen

Values are treatment means ± s.e.

Parameter	Treatment						
	Control	L-Met R	L-Met D	DL-Met R	DL-Met D	D-Met R	D-Met D
Sulfur intake (mg day^{-1})							
Dietary	1184±27·0	1184±27·0	1184±27·0	1184±27·0	1184±27·0	1184±27·0	1184±27·0
Infusion	0	532±1·3	538±2·1	540±3·7	538±2·1	542±3·0	540±4·5
Faecal sulfur (mg day^{-1})							
Total	487±11·0	454±16·2	492±11·7	481±16·2	489±23·7	477±6·4	480±14·6
Neutral	448±14·0	420±19·2	457±12·7	444±14·7	451±25·0	444±9·6	445±13·6
Ester sulfate	20±7·0	24±1·7	27±1·8	20±2·2	28±3·5	26±0·9	27±3·2
Inorganic sulfate	19±4·0	10±4·5	8±2·2	15±4·4	10±2·2	6±4·1	8±4·5
Urinary sulfur (mg day^{-1})							
Total	662±22·5	1052±32·6	787±76·3	1110±57·9	801±13·6	1038±55·7	847±28·1
Neutral	66±6·5	53±25·3	120±11·5	51±11·6	74±31·1	69±25·5	112±20·0
Ester sulfate	154±4·0	162±20·8	145±30·0	166±18·6	130±18·5	175±20·4	160±38·0
Inorganic sulfate	402±12·0	837±72·8	522±52·9	893±47·1	597±41·1	794±70·7	575±40·3
Sulfur retention (mg day^{-1})							
Total	76±60·5	219±62·6	434±92·9	143±50·5	423±55·9	220±74·5	387±34·4
Nitrogen (g day^{-1})							
Intake[A]	12·29±0·225	12·59±0·150	12·44±0·153	12·60±0·153	12·44±0·153	12·60±0·150	12·45±0·152
Faecal	3·11±0·080	3·02±0·102	3·27±0·193	3·04±0·023	3·08±0·112	3·05±0·079	3·16±0·096
Urinary	7·25±0·060	7·01±0·199	5·54±0·637	7·61±0·616	5·90±0·147	7·38±0·202	6·31±0·322
Retention	1·93±0·205	2·56±0·050	3·63±0·590	1·95±0·501	3·46±0·382	2·18±0·113	2·98±0·183

[A] Sum of dietary and infusion intake.

Table 4. Clean dry wool growth rate and fibre diameter, sulfur content of wool and sulfur incorporation into wool

Values are means ± s.e.

Parameter	Treatment						
	Control	L-Met R	L-Met D	DL-Met R	DL-Met D	D-Met R	D-Met D
Wool growth rate							
(mg 100 cm^{-2} day^{-1})	58±9·0	68±6·6	122±9·3	75±15·6	128±6·0	70±6·4	125±15·3
(g day^{-1})	4·59±0·730	5·69±0·315	10·26±0·658	5·82±1·029	10·04±0·511	5·59±0·290	9·82±0·678
(% increase)	0	24·0	123·5	26·8	118·7	21·8	113·9
Wool fibre diameter (μm)	16·5±0·45	16·7±0·10	19·9±0·21	16·9±0·07	21·0±1·13	16·6±0·81	20·1±1·04
Sulfur content of wool (mg g^{-1})	28·8±0·80	27·3±1·36	35·9±0·71	28·7±1·10	35·9±0·22	27·8±0·61	34·8±0·82
Sulfur incorporation into wool							
(mg day^{-1})	132±17·5	155±1·8	368±20·2	165±22·5	360±16·2	155±5·1	340±16·2
(% of retention)	173·7	70·8	84·8	115·4	85·1	70·5	87·9

Table 5. Recovery of supplementary sulfur from L-, DL- and D-methionine[A] and [^{35}S]-L-methionine in faeces, urine and wool

Values are means (expressed as percentage of dose) ± s.e.

Parameter	Treatment						
	Control	L-Met R	L-Met D	DL-Met R	DL-Met D	D-Met R	D-Met D
Faeces							
Isotope ^{35}S	22·4±0·10	18·4±0·90	7·4±0·57	18·5±1·15	7·2±0·26	19·3±0·90	6·7±0·20
Cold S[A]	—	−5·4±2·23	0·2±2·36	−0·5±2·32	−0·3±3·74	−1·2±1·70	−1·9±2·70
Urine							
Isotope ^{35}S	48·3±11·90	69·6±2·54	17·5±2·05	65·4±2·73	20·9±2·90	56·4±9·02	13·0±0·35
Cold S[A]	—	82·2±4·58	29·5±14·96	91·9±14·25	32·0±1·73	78·3±10·28	40·4±6·68
Wool							
Isotope ^{35}S	9·0±1·40	7·3±1·01	39·8±7·52	8·1±3·33	36·3±6·44	8·8±0·70	39·6±7·43
Cold S[A]	—	5·5±2·82	42·8±3·96	7·3±2·53	41·5±1·87	5·4±2·00	37·6±1·34

[A] Percentage of the sulfur in the supplement, accounted for by difference, to control values either in the excreta or in wool grown during supplementation.

There was no difference in the sulfur content of wool in sheep receiving ruminal methionine supplements or in the control sheep, but the incorporation of sulfur into wool was less ($P < 0{\cdot}05$) in the unsupplemented sheep.

Recovery of [^{35}S]-sulfur (see Table 5)

Sheep receiving duodenal injections of [^{35}S]-L-methionine excreted less ($P < 0{\cdot}001$) [^{35}S]-sulfur in the faeces compared with those receiving ruminal injections. The recovery of ^{35}S in urine was also less ($P < 0{\cdot}001$) in control sheep or those receiving duodenal supplements. However the incorporation of ^{35}S into wool was greater ($P < 0{\cdot}001$) with duodenal than with ruminal injections.

The isomeric form of the cold methionine affected the amount of ^{35}S excreted in the urine; L-methionine resulted in greater ($P < 0{\cdot}05$) excretion than D-methionine, and DL-methionine also resulted in more ($P < 0{\cdot}05$) ^{35}S in the urine than D-methionine.

Discussion

There is no information comparing the relative rates of dissimilation of methionine isomers by ruminal micro-organisms, or of the resulting metabolic products and their utilization. Doyle and Moir (1979*b*) found that ruminal supplements of DL-methionine were almost completely degraded by the ruminal micro-organisms, and the small ruminal sulfide-sulfur pools (see Doyle and Moir 1979*a*) indicated that the rate of breakdown to sulfides and the subsequent absorption of these was extremely rapid. Sheep receiving ruminal infusions of the methionine isomers had similar sulfide-sulfur pools, and as the 'marker-corrected' sulfur flow at the duodenum with these treatments was similar to the control, there appears to be no difference in the extent of metabolism of the isomers in the reticulorumen.

As reported by Bird and Moir (1972), there was no effect of the site of methionine infusion on the flow of protein sulfur at the duodenum; isomeric form of the amino acid also had no effect. The increased total and organic sulfur flows with supplementation into the duodenum compared with those with ruminal supplements may be partly due to the increased flow of digesta at the duodenum, but are more probably due to the inadequacy of the precautions taken to exclude infusate from the duodenal samples.

The limited amount of digestion and flow data suggest that there were no differences in extent of metabolism of the methionine isomers in the reticulorumen, but the large variability and the nature of these data do not allow firm conclusions to be drawn about the pathways of metabolism.

Previous studies have shown that faecal excretion of sulfur may be increased when methionine supplements are infused into the rumen compared with the abomasum (Bird and Moir 1972; Reis *et al.* 1978). In the present experiment, there were no differences in faecal total or neutral sulfur excretion due to supplementation with L-, DL- or D-methionine given into the rumen or duodenum. However, faecal excretion of ^{35}S from [^{35}S]-L-methionine was greater when injections were given into the rumen compared with the duodenum. Doyle and Moir (1979*c*) found that ruminal infusions of DL-methionine increased faecal sulfur excretion compared with that in control sheep, but only at low levels of dry matter intake. From the information available it can only be concluded that microbial modification of ruminal supplements (see Bird and Moir 1972) or incorporation of sulfur from these into the micro-organisms

may result in increased excretion under certain conditions. As there was no additional sulfur flow at the duodenum of sheep receiving ruminal supplements compared with the control, faecal sulfur excretion would not be expected to increase. Duodenal supplements of methionine were presumably absorbed intact from the small intestine, although the possibility of some fermentation in the caecum and colon cannot be discounted.

The large increase in urinary sulfur excretion due to ruminal supplements accounted for between 67 (D-methionine) and 92% (DL-methionine) of the sulfur infused. The discrepancies between these estimates and those from [^{35}S]-L-methionine injections (see Table 5) will be discussed later.

Total and inorganic sulfate-sulfur excretion in urine also increased with duodenal supplements, but the magnitude of the increase was dependent on the isomeric form of the amino acid. Only 29% of the supplementary sulfur from L-methionine was accounted for by the increase compared with 32% with DL- and 40% with D-methionine. In contrast, Reis *et al.* (1978) recovered only 11 (L-methionine)–14% (D-methionine) of radioactive doses infused into the abomasum in the urine.

The D-isomer can be converted to L-methionine in several mammalian species (Meister 1965) through oxidative deamination to the corresponding ketonic acid and subsequent asymmetric synthesis of the natural amino acid (Jackson and Block 1937–38). Consequently, both L- and D-methionine can be converted to L-cysteine, via S-adenosylmethionine (S-AM), homocysteine and cystathionine (Berg 1953; Moir 1979). Reis *et al.* (1978) suggested that both L- and D-methionine are efficiently converted to L-cyst(e)ine. However, recently Case and Benevenga (1976) presented evidence for an S-AM-independent catabolism of methionine in the rat, and Steele and Benevenga (1978) suggested that the transamination pathway resulting in the formation of α-keto-γ-methiolbutyrate, 3-methylthiopropionate and mercaptan (CH_3SH) is of importance.

Methionine may be degraded to α-keto-γ-methiolbutyrate (Meister 1965), methyl cysteine which is not used as a source of cysteine or methyl groups (see Thompson 1967), and methionine sulfoxide (Black 1963). The ketonic acid (Jackson and Block 1937–38) and the sulfoxide (Meister 1965) can be used to resynthesize methionine. However, both methyl cysteine and α-keto-γ-methiolbutyrate may be degraded to CH_3SH (Canellakis and Tarver 1953*a*) and further degraded to sulfate (Canellakis and Tarver 1953*b*). Both CH_3SH (Challenger and Walshe 1955) and sulfate may be excreted in urine.

The metabolism of methionine isomers as measured by urinary excretory products in dogs and humans has been found to be different in some experiments (Kinsell *et al.* 1948; Kies *et al.* 1975) and similar in others (Stekol 1935; Albanese *et al.* 1944). Zezulka and Calloway (1976) suggested that utilization of D-methionine is mainly through contribution of sulfur to the sulfate pool rather than by conversion to L-methionine. Such a pathway may explain the increased inorganic sulfate excretion by sheep receiving the D-isomer. However, Reis *et al.* (1978) found no increase in urinary excretion of ^{35}S from abomasal infusion of [^{35}S]-D-methionine compared with that from radioactive L-methionine. It must be remembered that the metabolism and utilization of sulfur-containing amino acids will depend on the sulfur status of the animal, and also on the species of animal as evidenced by differences in specific activity of enzymes, between both species and tissues (e.g. Finkelstein 1967; Radcliffe and Egan 1974).

Post-ruminal supplements of methionine improved both nitrogen and sulfur retention. The general anabolic effect obtained with abomasal infusions has been reported previously (Reis 1967; Robards 1971), and is presumably due to the limiting effect of methionine at the tissue level (see Nimrick *et al.* 1970). However, duodenal infusions of L-methionine improved both nitrogen and sulfur retention relative to similar supplements of D-methionine. Kies *et al.* (1975) found that L-methionine improved nitrogen retention in humans while D-methionine had no effect. Of the improved sulfur retention by sheep, 66% was incorporated into wool with L-methionine, 66% with DL-methionine and 67% with D-methionine. Assuming a concentration of 16·2% (w/w) nitrogen in clean dry wool (see Reis and Schinckel 1964), wool growth accounted for 54% of the extra nitrogen retained with L-methionine, 58% with DL-methionine and 81% with D-methionine. These data indicate that D-methionine was as efficient as L-methionine in promoting wool growth, but not in improving tissue incorporation or storage of nitrogen.

In absolute terms more sulfur was retained and there was a tendency for more sulfur to be incorporated into wool when the natural form was given into the duodenum than when the unnatural form was given. However, there was no effect of the isomeric form of methionine on wool growth rate. It is possible to produce a substantial stimulation of synthesis of specific sulfur-rich proteins in the absence of a wool-growth response (Reis 1967). The present work indicates that L-methionine may be more effective in this regard than D-methionine, although both isomers were equal in their ability to increase wool growth. Reis (1967) found that D-methionine given abomasally was less effective than DL-methionine in promoting wool growth rate. The results obtained by Reis and those from the present experiment are difficult to interpret due to the small number of animals used and the different experimental conditions.

With ruminal supplements, sulfur incorporated into wool did not exceed sulfur retention; the reverse situation occurred with the control animal. This indicates that ruminal supplements of L-, DL- or D-methionine have a sparing effect on drain of sulfur by catabolism from the tissues for wool growth at low levels of dry matter intake (see Doyle and Moir 1979*c*), but the metabolic mechanism involved cannot be delineated.

Discrepancies in the estimated excretion of sulfur from [^{35}S]-L-methionine and the cold methionine supplements were found to occur (see Table 5). These differences may have been due to the method of administration of the isotope, which was injected with 600 μg of carrier L-methionine while the infusion rate of the cold supplement was 1730 $\mu g\ s^{-1}$. This may have affected the metabolism of the isotope. In addition, if the sulfur metabolism of sheep on the control diet was different from that of sheep receiving the supplements, then estimates of the amount of sulfur excreted from cold supplements might be incorrect. The use of isotopes to trace the metabolism of supplements is also complicated by factors such as differential dilution of radioactive sulfur with cold sulfur as discussed by Williams (1973). In the present experiment, dilution effects could be due to the presence of methionine which was not in isotopic equilibrium with the injected radioactive material, that is feed methionine, methionine from endogenous inputs into the gastrointestinal tract, infused methionine (as mentioned above) and methionine in the sheep's tissues.

Variation in urinary excretion of ^{35}S from [^{35}S]-L-methionine when injected into sheep given L- compared with D-supplements, and the increased disparity between

radioactive and cold sulfur excretion in the urine in sheep receiving the D-amino acid compared with similar estimates for sheep receiving the natural isomer, indicate further differences in metabolism of the two isomers.

The data presented illustrate clear differences between L- and D-methionine as supplements for sheep at the tissue level under these experimental conditions. This finding indicates the need for better information on the pathways of methionine metabolism in the tissues.

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