SULPHUR METABOLISM AND EXCRETION STUDIES IN RUMINANTS

VIII.* METHIONINE DEGRADATION AND UTILIZATION IN SHEEP WHEN INFUSED INTO THE RUMEN OR ABOMASUM

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Abstract

Methionine (2 g/day) was continuously infused either abomasally or ruminally into two groups of sheep (four per group) over a 6-week period. After a further 6 weeks on the basal diet, which supplied daily 900 g dry matter, 18·3 g nitrogen, and 1·45 g sulphur, the infusion treatments were reversed. A control group of four sheep did not receive infusions. After 13 days on each treatment L-[^{35}S]-methionine was continuously infused, along with the carrier methionine, for 5 days during which the sheep were fed at 2-hourly intervals.

Of the [^{35}S]methionine infused ruminally 74% of the ^{35}S flowed to the omasum; 6·4% of this was inorganic ^{35}S. A large proportion of the organic ^{35}S was associated with microbial or particulate material. It was estimated that less than a quarter of the ^{35}S could have been as free methionine. Partial degradation of methionine may account for portion of the organic ^{35}S found in omasal digesta. Low concentrations of [^{35}S]H_2S, [^{35}S]CH_3SH, or other sulphides were found in ruminal or omasal digesta.

The distribution of ^{35}S (as % of dose) in faeces, urine, and wool after ruminal or abomasal infusions of [^{35}S]methionine was 30 *v.* 11%, 39% *v.* 20%, and 5·6% *v.* 22·8%, respectively.

Wool growth and sulphur content of the wool was increased by 28 *v.* 68% and 4·5% *v.* 19·7% above the controls for ruminal or abomasal infusions, respectively. Body weight gains also improved with methionine infusions: control, ruminal, and abomasal treatment mean gains were 0·09, 0·41, and 0·66 kg/week, respectively. Larger nitrogen and sulphur balances were associated with the greater responses to abomasal infusions. Ruminal infusions did not alter the flow of protein to the omasum or the digestibility of organic matter.

The responses obtained with ruminal infusions of methionine suggest that increasing the dietary intake of methionine, or the rate of fluid flow from the rumen, may enable dietary supplements of methionine to effect improvements in wool production and body weight gains.

I. Introduction

Methionine given parenterally, subcutaneously, or abomasally to sheep can, in some circumstances, stimulate wool growth (e.g. Reis and Schinckel 1963; Reis 1967; Langlands 1970; Downes *et al.* 1970*a*) but dietary supplements of methionine, or hydroxy analogue of methionine (MHA), are generally considered to be ineffective (e.g. Reis 1970). However, there appears to have been only one comparison made

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between these methods of methionine supplementation, that of Graceva (1969), where 3 g methionine was given orally or subcutaneously or infused into the abomasum daily. The wool growth responses observed were 17·9, 33·7, and 42·2% above basal, respectively.

The fate of methionine in the rumen has been largely inferred from that of cyst(e)ine. The ruminal microorganisms rapidly degrade cyst(e)ine to H_2S (e.g. Nader and Walker 1970; Bird and Hume 1971; Bird 1972*a*) and, by analogy, methionine is thought to behave similarly (e.g. McDonald 1968). Recently studies of the metabolism of methionine by ruminal microorganisms have been initiated (Zikakis and Salsbury 1969; Salsbury *et al.* 1971; Nader and Walker 1970; Bird 1972*a*) and it is now evident that there are differences in the rates of degradation and end products resulting from the decomposition of methionine and cystine. Bird (1972*a*) found that the concentrations of CH_3SH, or of H_2S, in the rumen following ruminal infusions of methionine were extremely low compared with the concentrations of sulphide arising from the infusion of cyst(e)ine. This suggested that the methionine molecule may have been only partly degraded in the rumen (e.g. to α-keto-γ-methylmercaptobutyrate) or that the rate of methionine degradation was slow, in which case increasing the intake of methionine or increasing the rate of fluid flow from the rumen might enable sufficient methionine to flow from the rumen and ultimately stimulate wool production. Downes *et al.* (1970*b*), found a substantially greater proportion of inorganic ^{35}S in the plasma and greater urinary excretion of ^{35}S when [^{35}S]methionine was fed compared with abomasal infusions, indicating extensive ruminal degradation of methionine. However, the data of Johnson, Goodrich, and Meiske (1970) do not support that conclusion.

In order to clarify and extend those results an experiment was devised to determine the proportion of ^{35}S from ruminally infused [^{35}S]methionine passing to the omasum and the composition of ^{35}S in the omasal digesta. The distribution of ^{35}S in wool, urine, and faeces resulting from the continuous ruminal or abomasal infusions of [^{35}S]methionine (with 2 g methionine/day) was also followed. Effects on wool production, body weight gain, nitrogen and sulphur balance, and ruminal metabolism of organic matter and nitrogen were assessed.

II. Experimental Methods

(*a*) *Experimental Design*

Twelve mature Merino wethers, each fitted with omasal and ruminal cannulae (see Hume, Moir, and Somers 1970) were used. Four (W27, G186, O59, G184) were used as a control group for changes in body weight and wool growth and received the basal ration without methionine infusions. Of the remainder, after 6 weeks on the basal ration four sheep (W24, B77, W29, B97) received 2 g of DL-methionine per day infused continuously into the rumen for 6 weeks, while the others (O58, O62, O42, W21) were given the infusions per abomasum. Following a further 6 weeks on the basal diet the infusion groups were reversed.

The sheep were confined in metabolism cages in a constantly lit room for the entire experiment.

(*b*) *Basal Diet*

The ration fed (900 g dry matter/day) comprised 46·0% lucerne chaff, 46·0% oaten chaff, 5% starch, 2% minerals, and 1% urea. The composition of the mineral mix has been described by Hume, and Bird (1970). The urea was dissolved in a little water and then mixed into the

ration during its preparation. The ration was sufficiently moist (84% dry matter) to prevent sifting of the added starch, minerals, or lucerne and was fed without drying. It supplied, on average, 1·45 g sulphur and 18·28 g nitrogen daily. Including the sulphur and nitrogen of the infused methionine the average daily intakes of sulphur and nitrogen were 1·88 and 18·47 g, respectively. The organic matter content of the diet was 91–92% of the total dry matter.

From the start of each period through to the end of each collection phase (22 days) the daily ration was fed in 12 equal portions, at 2-hourly intervals, in order to approach steady-state flow conditions in the digestive tract. During the remainder of each treatment period, and the preliminary and interim periods, the animals were fed once daily. The control group was fed once daily throughout the experiment. No feed refusals occurred during treatment periods in any group of animals.

The sheep were allowed to drink tap water *ad libitum*.

(*c*) *Infusion of Solutions*

Methionine was continuously infused by a peristaltic pump into the rumen or abomasum at the rate of 2 g/day. The volume of fluid infused daily was between 700 and 800 ml. Infusions into the abomasum was made via the omasal cannula, as described by Hume, Moir, and Somers (1970) and Bird (1972*b*). Evidence has been given (Bird 1972*b*) that little, if any, back-flow of the material to the rumen would have occurred. Methionine solution sufficient to last 2 days was made freshly every alternate day.

L-[^{35}S]Methionine (supplied by the Radiochemical Centre, Amersham, England) was infused into the rumen or abomasum, commencing 2 weeks after starting each period. From 160 to 280 μCi of radioactivity was given to each sheep in each period. The [^{35}S]methionine was infused into the abomasum, along with the non-radioactive methionine, at a constant rate over a period of 5 days. When infused into the rumen a priming dose of [^{35}S]methionine and of chromium ethylenediaminetetraacetic acid (Cr-EDTA) were given prior to commencing the continuous infusions of isotope and Cr-EDTA, in order to facilitate the attainment of a relatively high, stable concentration of these compounds in the rumen (see Weston and Hogan 1967). The ruminal infusion of [^{35}S]methionine, along with non-radioactive methionine, was continued for 5¼ days. The daily rate of [^{35}S]methionine infusion was kept constant and these values were later used to determine the proportion of the ^{35}S dose flowing daily to the omasum under these steady-state conditions.

Cr-EDTA solutions were prepared as described by Binnerts, Vant Klooster, and Frens (1968). The neutralized solution (Cr concentration 2000 p.p.m.) was infused into the rumen at the rate of 70 g/day. These infusions were given to all eight sheep in each period.

(*d*) *Sampling Methods*

Samples of omasal and rumen digesta were obtained between 9–10 a.m. and 4–5 p.m. on each of 5 days, starting 24 hr after giving the priming dose of [^{35}S]methionine or Cr-EDTA or both. The method used to collect, without contamination, omasal digesta samples from sheep receiving the abomasal infusion of methionine was as follows. The pump was turned off for 30 min, in which time the omasal bung and associated abomasal catheter was withdrawn, the cannula area washed clean, another bung used to close the cannula for 15 min, and finally this was removed and approximately 50 g digesta was collected into a polythene cup clamped onto the cannula. The abomasal catheter and omasal bung were replaced and the infusion recommenced. Omasal digesta from sheep receiving methionine ruminally was collected in a similar fashion, but without stopping the infusion. Any omasal or rumen fluid collected, but not required for analytical purposes, was returned to the sheep via the rumen cannula. Approximately 20 g of each collected sample was strained through fine bolting silk and the resulting fluid and the unstrained digesta samples were frozen and stored.

Wool production by all sheep during each period of treatment was estimated by the tattoo patch technique described by Reis and Schinkel (1964). Two patches, each approximately 10 cm square, were used, one on the left shoulder and the other on the right mid-side. Wool was clipped from these areas 13 days after starting each treatment and again 7 days after completion of the

period. These samples represented 5 weeks growth on each treatment, or about 3–4 weeks wool grown subsequent to the infusion of the isotope.

Body weights of all sheep were recorded at the beginning and end of each period.

Urine and faeces were collected from the sheep receiving methionine infusions, the collection periods starting the first day of [^{35}S]methionine infusion and finishing 3 days after the completion of that infusion (i.e. 8 days total). The method of collection and treatment of samples were as described by Hume, Moir, and Somers (1970).

(*e*) *Analytical Methods*

Chromium concentrations in strained omasal fluid and urine were determined by atomic absorption spectroscopy, using an air–acetylene flame (20 and 6 lb/in^2, respectively). 5 ml of each sample plus 5 ml of a $CaCl_2$ solution (50 g $CaCO_3$ dissolved in HCl and made to 1 litre) were diluted to 25 ml with deionized water, thoroughly mixed, and allowed to stand overnight prior to analysis of the supernatant. The presence of calcium (final concentration 1600 p.p.m.) prevented interference and improved sensitivity. Standards prepared from the infusion solutions were similarly treated. The sensitivity for 1% absorption was 0·073 p.p.m. Cr. The calculation of fluid flow rate was as described by Weston and Hogan (1967). No correction was applied for absorption of chromium, since less than 2·6% of the infused chromium was recovered in the urine and there is no evidence that Cr-EDTA is absorbed from the rumen. It was assumed in this experiment that the rate of fluid flow from the rumen would approximate that of the flow of digesta. Estimates of digesta flow rate using [^{51}Cr]EDTA and lignin (Hogan 1964) or polyethyleneglycol and lignin (Hume, Moir, and Somers 1970) were comparable, although the soluble markers tended to give higher estimates of digesta flow rate.

In the present experiment estimates of the daily flow of digesta to the omasum were based upon the mean concentration of chromium in 10 samples of omasal digesta collected over the 5-day interval. Likewise, all data relating to the concentration of ^{35}S, sulphur, nitrogen, or organic matter in omasal digesta were based on the mean of all 10 samples.

Total nitrogen in feeds, faeces, and urine, dry matter and organic matter in feeds, faeces, and omasal digesta, and protein nitrogen in omasal digesta were determined by the procedures given by Hume, Moir, and Somers (1970).

Wool samples were scoured with light petroleum (Shell X4), dried, and weighed. Wool growth was expressed as grams clean dry wool per day.

Analyses of total ^{35}S in wool, faeces, rumen fluid, and omasal digesta, and of reducible sulphur, ester sulphate sulphur, and reducible ^{35}S in these samples, and in urine, were made by the methods described by Bird and Fountain (1970) and Bird (1972*b*).

Free [^{35}S]methionine, plus other soluble ^{35}S-labelled compounds, was extracted from 10 ml of strained omasal fluid with four successive 10-ml amounts of a solution of 20 g methionine per litre. After each addition of methionine solution the omasal digesta was left standing for about 8 hr, or overnight, and then centrifuged at 35,000 *g* for 30 min and the supernatants drawn off and combined. The ^{35}S content of these solutions, and of urine, was determined by balance point liquid scintillation counting of 1-ml aliquots with the scintillant described by Bird and Fountain (1970). An internal standard technique was used to ascertain counting efficiency.

Volatile sulphides were collected from rumen and omasal digesta samples on each of the five collection days during each period. The method of sample collection and separation of H_2S and CH_3SH has been described by Bird (1972*a*). [^{35}S]$(CH_3)_2S_2$, and [^{35}S]$(CH_3)_2S$ if present, were separated from other ^{35}S sulphides by their insolubility in 4% $Hg(CN)_2$ solution. These sulphides, and also [^{35}S]CH_3SH, were collected directly into vials containing 10 ml of benzene as the absorbent. 10 ml of the toluene–methanol-based scintillant used by Bird (1972*c*) was added to each of the vials prior to counting ^{35}S activity. Also, [^{35}S]H_2S plus [^{35}S]CH_3SH were collected together in NaOH solution, as described by Bird (1972*a*), and 1-ml aliquots counted in the scintillant described by Bird and Fountain (1970). The contribution from [^{35}S]H_2S was obtained by difference.

All isotope data were corrected for decay to the corresponding half-infusion dates. Also, when calculating the distribution of the ruminally infused ^{35}S into wool and excreta, allowance was made for ^{35}S removed with omasal and rumen fluid samples.

(*f*) *Statistical Methods*

Statistical comparisons between control group means and treatment group means were made by Students' *t*-test. Comparisons between the treatment groups (infusion routes) were made using three-way analysis of variance.

III. Results

(*a*) *Flow of ^{35}S to the Omasum (Table 1)*

Of the [^{35}S]methionine infused daily, 74·1±3·39% of the infused ^{35}S flowed to the omasum and 6·35±0·39% of this ^{35}S was in a reducible form. The daily flow of ^{35}S to the omasum was estimated from the specific radioactivity of ^{35}S in the omasal

Table 1

Flow of ^{35}S to the omasum and the distribution of ^{35}S during continuous ruminal infusion of [^{35}S] methionine

Values are means±standard error. Significant differences between periods are indicated thus: n.s., not significant; ***$P < 0·001$; **$P < 0·01$; *$P < 0·05$

Parameter	Percentage recovery		Statistical difference
	Period 1†	Period 2‡	
Unstrained omasal digesta:			
Total ^{35}S (as % of daily dose)	80·9±3·26	67·2±3·39	n.s.
Reducible ^{35}S (as % of daily dose)	5·0±0·46	4·4±0·02	n.s.
Reducible ^{35}S (as % of daily dose)	6·2±0·75	6·5±0·35	n.s.
[^{35}S]H_2S (as % of daily dose)	0·82±0·10	2·53±0·29	**
[^{35}S]CH_3SH (as% of daily dose)	0·06±0·01	0·01±0·005	*
[^{35}S]$(CH_3)_2S_2$ (as % of daily dose)	0·04±0·004	0·01±0·003	*
[^{35}S]H_2S/ml (as % of [^{35}S]H_2S/ml rumen fluid)	93·7±20·2	90·6±9·4	n.s.
Strained omasal digesta:			
Total ^{35}S (as % of daily dose)	65·4±2·15	39·9±3·10	**
Total ^{35}S (as % of ^{35}S in unstrained digesta)	81·0±2·77	59·3±2·63	**
Total ^{35}S/ml (as % of ^{35}S/ml rumen fluid)	80·0±8·30	67·1±8·00	n.s.
Extractable ^{35}S (as % of daily dose)§	46·0±1·57	20·0±1·36	***
Extractable ^{35}S (as % of ^{35}S in strained digesta)§	70·4±1·65	50·5±2·77	**

† Sheep W24, B77, W29, B97.

‡ Sheep O58, O62, O41, W21.

§ ^{35}S extracted from strained digesta with a solution of methionine (concn. 20 g/l).

digesta and the daily flow of fluid to the omasum, as determined by the use of a soluble marker. Comparable estimates (mean 73·6±5·87%) of the infused ^{35}S flowing to the omasum were obtained by comparing the specific radioactivity of ^{35}S in strained omasal digesta and in strained rumen fluid. A greater degradation of the methionine in the rumen during the second period was further indicated by the greater flow of [^{35}S]H_2S to the omasum ($P < 0·01$) in that period. Substantially less ^{35}S was found in strained digesta than in unstrained digesta, but there was also a smaller proportion of the daily dose recovered in the second period ($P < 0·01$), with less extractable (soluble) ^{35}S in this strained fluid ($P < 0·01$), suggesting a greater incorporation of methionine into microbial cells in the second period.

(*b*) *Distribution of* ^{35}S *in Faeces, Urine, and Wool* (*Table 2*)

There were significant differences between periods in the proportion of the infused ^{35}S excreted in the faeces ($P < 0{\cdot}001$) or incorporated into wool ($P < 0{\cdot}001$) and differences between sheep ($P < 0{\cdot}05$) in the excretion of ^{35}S in the faeces.

More of the infused ^{35}S was recovered in the faeces ($P < 0{\cdot}001$) and urine ($P < 0{\cdot}001$) but less in the wool ($P < 0{\cdot}001$) with ruminal infusions than abomasal infusions of [^{35}S]methionine; the proportion of reducible forms of ^{35}S in faeces and urine did not differ between treatments.

TABLE 2

DISTRIBUTION OF ^{35}S IN FAECES, URINE, AND WOOL AFTER INFUSIONS OF [^{35}S]METHIONINE INTO THE RUMEN OR ABOMASUM

Period values are means, treatment values are means ± standard error. Significant treatment differences are indicated thus: *** $P < 0{\cdot}001$; n.s., not significant

Parameter	Ruminal infusion			Abomasal infusion			Statistical difference
	Period 1†	Period 2‡	Mean	Period 1‡	Period 2†	Mean	
Faeces:							
Total ^{35}S (as % of dose)	36·4	24·0	30·2±2·43	12·1	9·2	10·7±0·67	***
Reducible ^{35}S (as % of dose)	0·15	0·33	0·24±0·04	0·10	0·10	0·10±0	***
Reducible ^{35}S (as % of total)	0·4	1·4	0·9±0·19	0·8	1·1	1·0±0·06	n.s.
Urine:							
Total ^{35}S (as % of dose)	42·8	35·1	39·0±1·97	20·4	19·7	20·0±1·02	***
Reducible ^{35}S (as % of dose)	41·0	33·3	37·1±2·19	19·8	17·1	18·4±0·93	***
Reducible ^{35}S (as % of total)	95·8	94·7	95·2±2·49	97·4	88·6	93·0±5·21	n.s.
Wool:							
Total ^{35}S (as % of dose)	4·3	7·0	5·6±0·61	20·6	25·0	22·8±0·88	***

† Sheep W24, B77, W29, B97. ‡ Sheep O58, O62, O41, W21.

(*c*) *Flow of Digesta, Protein, and Sulphur to the Omasum* (*Table 3*)

The flow of digesta and of protein nitrogen or protein sulphur to the omasum did not differ with periods nor with treatments, but there were differences between sheep in the flow of digesta ($P < 0{\cdot}05$), protein ($P < 0{\cdot}05$), total sulphur ($P < 0{\cdot}05$), and neutral sulphur ($P < 0{\cdot}05$). Total sulphur flow increased with the ruminal infusion of methionine. The increased neutral sulphur output ($P < 0{\cdot}001$) was chiefly as soluble organic sulphur.

The concentration of CH_3SH in the rumen fluid and omasal digesta was below the limit of detection (0·1 μg/ml) of the analytical method used. The mean concentration of H_2S in rumen fluid of sheep receiving ruminal infusions of methionine was (3·92±0·29) μg sulphur per millilitre. This was not significantly different from that occuring during abomasal infusion periods 3·60±0·28 μg/ml. The corresponding values for omasal digesta were 3·03±0·30 and 3·09±0·35 μg/ml, respectively.

(*d*) *Excretion of Sulphur* (*Table 4*) *and Nitrogen* (*Table 5*)

Ruminal infusions of methionine resulted in greater excretion of sulphur in the faeces ($P < 0{\cdot}05$) and in the urine ($P < 0{\cdot}01$) compared with abomasal infusions.

TABLE 3

DAILY FLOW OF DIGESTA, PROTEIN, AND SULPHUR TO THE OMASUM

Period values are means, treatment values are means ± standard error. Statistical differences between treatment means are indicated thus: n.s., not significant; *** $P < 0{\cdot}001$; * $P < 0{\cdot}05$

Parameter	Ruminal infusion			Abomasal infusion			Statistical difference
	Period 1†	Period 2‡	Mean	Period 1‡	Period 2†	Mean	
Digesta (g)	11402	12538	11970±652	12771	12159	12465±374	n.s.
Nitrogen (g)§	15·0	16·6	15·8±0·63	15·1	15·3	15·2±0·51	n.s.
Protein (nitrogen × 6·25)§	93	104	99±3·9	95	95	95±3·2	n.s.
Total sulphur (mg)	1763	1802	1783±59	1525	1480	1504±37	***
Neutral sulphur (mg)	1620	1613	1616±62	1354	1306	1330±39	***
Ester sulphate sulphur (mg)	15	21	18±3	18	19	18±3	n.s.
Inorganic sulphate sulphur (mg)	128	168	148±13	157	155	156±11	n.s.
Protein sulphur (mg)¶	1152	1223	1183±52	1083	1102	1093±34	n.s.
Soluble organic sulphur (mg)	421	380	400±65	250	257	254±50	*

† Sheep W24, B77, W29, B97. ‡ Sheep O58, O62, O41, W21. § Nitrogen precipitated with tungstic acid. ¶ Precipitated with trichloroacetic acid.

TABLE 4

EXCRETION OF SULPHUR IN URINE AND FAECES

Values are expressed as mg/day. Period values are means, treatment values are means ± standard error. Statistical differences between treatment means are indicated thus: n.s., not significant; ** $P < 0{\cdot}01$; * $P < 0{\cdot}05$

Parameter	Ruminal infusion			Abomasal infusion			Statistical difference
	Period 1†	Period 2‡	Mean	Period 1‡	Period 2†	Mean	
Faeces:							
Total sulphur	712	657	685±21	653	599	626±16	*
Neutral sulphur	664	599	631±24	605	548	577±17	*
Ester sulphate sulphur	26	32	28±3	24	24	24±1	n.s.
Inorganic sulphate sulphur	24	27	25±4	26	28	27±2	n.s.
Urine:							
Total sulphur	1022	919	970±37	760	865	813±28	**
Neutral sulphur	67	78	73±15	45	57	51±10	n.s.
Ester sulphate sulphur	188	193	190±12	198	203	201±12	n.s.
Inorganic sulphate sulphur	767	648	707±36	518	605	561±26	**

† Sheep W24, B77, W29, B97. ‡ Sheep O58, O62, O41, W21.

The increased output of sulphur in the faeces was due to organic sulphur compounds ($P < 0{\cdot}05$) and the greater output in the urine was due almost entirely to increased inorganic sulphate excretion ($P < 0{\cdot}01$).

Abomasal infusions of methionine induced a decreased ($P < 0{\cdot}05$) urinary excretion of nitrogen (1 g/day) compared with ruminal infusions, despite a lesser intake of nitrogen in the latter treatment. Faecal excretion of nitrogen was unchanged.

(e) Effect of Infusions on Digestibility and on Sulphur and Nitrogen Balance (Table 5)

Ruminal infusions of methionine did not improve the apparent digestibility of dry matter or of organic matter of the diet; organic matter digestibility was actually slightly lower ($P < 0{\cdot}05$) than when abomasal infusions were given. Both

TABLE 5

INTAKE, DIGESTIBILITY, SULPHUR AND NITROGEN BALANCE

Period values are means, treatment values are means ± standard error. Statistical differences between treatment means are indicated thus: n.s., not significant; ** $P < 0{\cdot}01$; * $P < 0{\cdot}05$

Parameter	Ruminal infusion			Abomasal infusion			Statistical difference
	Period 1†	Period 2‡	Mean	Period 1‡	Period 2†	Mean	
Dry matter:							
Intake (g/day)	900	900	900±0	900	900	900±0	n.s.
Omasal digesta (%)	5·68	5·78	5·77±0·267	4·97	5·76	5·36±0·196	n.s.
Apparent digestibility	65·5	64·0	64·2±0·93	67·7	63·5	65·6±0·94	n.s.
Organic matter:							
Intake (g/day)	837	825	831±2·3	837	827	832±1·9	n.s.
Leaving rumen (g/day)§	530	583	557±13·7	509	561	535±14·6	n.s.
Output (g/day)	287	290	289±4·0	259	289	274±7·2	n.s.
Apparent digestibility	65·7	64·9	65·3±0·50	69·1	65·0	67·0±0·92	*
Digestion in rumen (% total)	55·7	45·1	50·4±2·53	56·8	49·4	53·1±2·19	n.s.
Nitrogen (g/day):							
Intake¶	18·16	18·12	18·14±0·01	19·12	18·48	18·80±0·12	**
Urinary nitrogen	10·35	10·96	10·66±0·32	9·76	9·59	9·68±0·17	*
Faecal nitrogen	4·40	4·36	4·38±0·09	4·15	4·42	4·28±0·09	n.s.
Balance	3·41	2·80	3·10±0·32	5·21	4·47	4·84±0·23	**
Sulphur (mg/day):							
Intake¶	1978	1815	1897±31	1904	1835	1870±13	n.s.
Balance	245	259	252±38	491	351	421±35	**

† Sheep W24, B77, W29, B97. ‡ Sheep O58, O62, O41, W21. § Including microbial organic matter. ¶ Including infused nitrogen (188 mg/day) and sulphur (430 mg/day) from methionine.

nitrogen balance ($P < 0{\cdot}01$) and sulphur balance ($P < 0{\cdot}01$) were improved by the abomasal infusions of methionine when compared with ruminal infusions.

There were small differences between periods in the apparent digestibility of dry matter ($P < 0{\cdot}01$) and organic matter ($P < 0{\cdot}01$), and the intake of nitrogen ($P < 0{\cdot}01$).

(f) Body Weight Gains and Wool Production Data (Table 6)

Body weight gains, wool production, and sulphur content of wool did not change between periods in the control group nor were there significant periods effects

on these variables in the treatment groups. There were, however, differences between sheep ($P < 0{\cdot}05$) in the sulphur content of the wool grown.

Body weight gains were increased by methionine infusions ($P < 0{\cdot}05$) and the gains with abomasal infusion were greater ($P < 0{\cdot}05$) than with ruminal infusions. Wool growth showed a similar pattern; both abomasal infusions ($P < 0{\cdot}001$) and ruminal infusions ($P < 0{\cdot}05$) resulted in greater wool production than the control group. In addition, the sulphur content of the wool from the abomasal infusion treatment was also higher ($P < 0{\cdot}01$). Abomasal infusions resulted in greater total wool ($P < 0{\cdot}001$) and sulphur content ($P < 0{\cdot}01$) than did ruminal infusions of methionine.

TABLE 6

BODY WEIGHT GAINS AND WOOL PRODUCTION DATA

Period values are means, treatment values are means ± standard error. Statistical differences between infusion treatment means are indicated thus: *** $P < 0{\cdot}001$; ** $P < 0{\cdot}01$; * $P < 0{\cdot}05$. Significant differences between control group and infusion treatment means are shown by similar superscripts: *a*, $P < 0{\cdot}001$; *b*, $P < 0{\cdot}01$; *c*, $P < 0{\cdot}05$

Parameter	Controls			Ruminal infusions			Abomasal infusions			Stat. diff.
	Period 1†	Period 2†	Mean	Period 1‡	Period 2§	Mean	Period 1§	Period 2‡	Mean	
Body weight gains (kg/week)	0·00	0·19	$0{\cdot}09^{c}$ ±0·103	0·44	0·38	$0{\cdot}41^{c}$ ±0·046	0·63	0·69	$0{\cdot}66^{c}$ ±0·094	*
Increase above controls (kg/week)			—			0·32			0·57	
Wool production (g/day)	6·59	6·22	$6{\cdot}41^{a,c}$ ±0·236	8·93	7·45	$8{\cdot}19^{c}$ ±0·412	10·50	11·10	$10{\cdot}80^{a}$ ±0·389	***
Increase above controls (%)			—			27·8			68·5	
Sulphur content in wool (%)	2·91	2·88	$2{\cdot}89^{b}$ ±0·046	3·04	2·99	$3{\cdot}02$ ±0·064	3·64	3·27	$3{\cdot}46^{b}$ ±0·091	**
Increase above controls (%)			—			4·5			19·7	

† Sheep W27, G186, O59, G184 (each receiving only the basal ration).
‡ Sheep W24, B77, W29, B97. § Sheep O58, O62, O41, W21.

IV. DISCUSSION

A mean 74% of the ^{35}S infused into the rumen in [^{35}S]methionine was recovered in the omasal digesta; since only 6·4% of this ^{35}S was inorganic, approximately 69% of the organic ^{35}S infused flowed to the omasum. These data were supported by the finding of a greater flow of organic sulphur to the omasum during ruminal infusions of methionine than during abomasal infusions (Table 3). The consistently low concentrations of total sulphides or of [^{35}S]sulphides in the ruminal or omasal digesta support data previously obtained (Bird 1972*a*) where very little H_2S or CH_3SH was detected after single ruminal infusions of methionine (2 g S). In the present experiment a greater proportion of the ruminal or omasal digesta [^{35}S]sulphide was found in H_2S relative to CH_3SH. There was evidence of the production of $(CH_3)_2S_2$ or $(CH_3)_2S$ (oxidation products from CH_3SH) but the concentrations of these sulphides were also small relative to H_2S. Dunham *et al.* (1968) reported that $(CH_3)_2S$ was the

sulphide produced from methionine by ruminal microorganisms *in vitro*, although Zikakis and Salsbury (1969) and Salsbury *et al.* (1971) later found that CH_3SH was the chief sulphide produced *in vitro*.

Downes *et al.* (1970*b*) found peak concentrations of inorganic ^{35}S in plasma 6 hr after orally dosing two sheep with [^{35}S]methionine, and about 40% of the infused ^{35}S was excreted in the urine in 24 hr, compared with 5% with abomasal infusions. Those results indicated a more extensive ruminal degradation of methionine than observed in the present experiment where a mean 39% of the ^{35}S from ruminally infused [^{35}S]methionine was excreted in the urine over 8 days (including 5 days during which the dose was infused) compared with 20% when the [^{35}S]methionine was infused into the abomasum. The difference, 19%, probably accounts for most of the 26% ^{35}S apparently degraded and lost from the rumen. Significantly more inorganic sulphate and total sulphur were excreted in the urine during ruminal infusions (Table 4) thus supporting the isotope data.

The significantly lower incorporation of ^{35}S into wool (5·6 *v.* 22·8%) and significantly lower wool production (8·19 *v.* 10·8 g/day), sulphur content of wool (3·02 *v.* 3·46%) and body weight gains (0·41 *v.* 0·66 kg/week) resulting from ruminal infusion of methionine compared with abomasal infusions indicate that much of the organic ^{35}S flowing to the omasum was not unmodified methionine. Reis (1967) has shown that daily abomasal infusions of 0·6 g methionine were as effective as 2·4 g doses of methionine in increasing wool growth and Langlands (1970) later found a similar effect with infusions of 0·36 and 2·16 g of methionine. It is therefore probable that less than one-quarter of the 1·38 g of potentially available methionine (i.e. 69% of 2 g infused methionine) could have been free methionine. Further work is in progress to identify the ^{35}S components of ruminal and omasal digesta after infusing [^{35}S]methionine. A considerable proportion of omasal digesta ^{35}S was apparently associated with particulate matter, either incorporated into or adsorbed to microbial cells, since straining the digesta reduced the ^{35}S activity of the strained fluid to 59–81% of the total activity. The percentage of ^{35}S extracted from the strained digesta with methionine solution was only 50–70% of the total ^{35}S activity in this material, therefore a large portion of the organic ^{35}S was apparently incorporated into the microbial cells. Nader and Walker (1970) estimated that 11% of methionine was directly incorporated by ruminal microorganisms *in vitro*, but it seems likely that that proportion would be increased by the presence of large numbers of protozoa which require pre-formed amino acids (Coleman 1967) and which may be stimulated by methionine (Patton *et al.* 1970). This factor may account for part of the difference between the estimate of Nader and Walker (1970) and that of Landis (1963) (75–83%) of the proportion of methionine directly incorporated into microbial protein. However, Nader and Walker's results also show that a large portion of the sulphide resulting from the degradation of methionine was incorporated into microbial protein. These authors proposed that methionine was decomposed to H_2S and that this sulphide was incorporated into protein, via the formation of cysteine (see Delavier-Klutchko and Flavin 1965; Kredich and Tomkins 1966). Zikakis and Salsbury (1969) suggested that CH_3SH, the sulphide identified from methionine decomposition, was incorporated into protein via *S*-methylcysteine formation (see also Tokuno, Strauss, and Tsuda 1962). This may occur, but the finding of [^{35}S]H_2S in the present

experiment, and in greater proportions than [^{35}S]CH_3SH, suggests that CH_3SH is also metabolized via H_2S and incorporated into protein.

Despite the fact that some of the ruminally infused [^{35}S]methionine was degraded and the ^{35}S absorbed, there was a higher output of total sulphur and of ^{35}S in the faeces (30·2% of dose) than from the abomasal infusion of [^{35}S]methionine (10·7% of dose). This was also apparent in the experiment of Downes *et al.* (1970*b*), where approximately 17% of the ^{35}S given as [^{35}S]methionine in the diet and approximately 9% of the abomasally infused ^{35}S was excreted in the faeces. Bray (1969) has shown that ^{35}S from intravenous infusions of [^{35}S]Na_2SO_4 was excreted in the faeces, therefore one explanation may be that since the elevation of the [^{35}S]SO_4^{2-} fraction in the blood is greater after the ingestion of [^{35}S]methionine than when the dose is given abomasally (Downes *et al.* 1970*b*), more [^{35}S]SO_4^{2-} might be recycled to the gut tract and subsequently excreted. However, sheep fed 1·1–1·5 g sulphur per day in the diet excreted in the faeces only 11·5% of the ^{35}S given intravenously as [^{35}S]Na_2SO_4 (Bird 1972*d*). Therefore an alternative explanation may be that part of the ruminally infused methionine was rendered indigestible, due to microbial modification or incorporation. About 93% of the ^{35}S in the omasal digesta and 99% of the faecal ^{35}S was in an organic form. Since the apparent digestibility of ^{35}S from ^{35}S-labelled ruminal microorganisms is approximately 71% (Bird 1972*b*) the loss of ^{35}S incorporated into microbial protein may account for portion of the faecal ^{35}S. In addition, a deaminated but not demethiolated product of methionine (e.g. α-keto-γ-methylmercaptobutyrate), if formed, might be less digestible than is methionine. A further possibility may be decarboxylation of methionine to yield 3-methylthiopropylamine (see Hagino and Nakayama 1968) or oxidation to yield methionine sulphoxide (Salsbury *et al.* 1971).

Since 99% of the faecal ^{35}S associated with the abomasal infusion of [^{35}S]-methionine was also in an organic form either some of the infused methionine was not absorbed or almost all of the recycled sulphate, arising from the transulphuration of methionine and subsequent oxidation of cysteine (see Mudd 1970), was transformed to organic sulphur in the gut tract or both events occurred. Since little organic ^{35}S is secreted in the biliary–pancreatic fluids of the sheep following infusions of [^{35}S]Na_2SO_4 (Bird 1972*c*) most of the faecal sulphur probably arises from the transformation of recycled sulphate by bacteria in the alimentary tract (see also Bird 1971).

There was four times as much ^{35}S incorporated into wool from abomasal infusions of [^{35}S]methionine (22·8%) than from ruminal infusions (5·6%), a result similar to that obtained by Downes *et al.* (1970*b*) (*c.* 20 and 4%, respectively). In the present experiment there was an increased incorporation of ^{35}S into wool during the second period with a concomitant reduction in the excretion of ^{35}S in the faeces and urine, irrespective of treatment (Table 2). The greater incorporation of ^{35}S into wool and the reduced faecal excretion of ^{35}S in the second period of ruminal infusion was associated with a smaller flow of infused ^{35}S to the omasum. However, there appeared to be a greater incorporation of ^{35}S into, or associated with, the microbial fraction of the omasal digesta in this period, which might explain the slightly greater incorporation of ^{35}S into wool. Sulphur and nitrogen balances, wool growth rate, sulphur content of wool, and body weight changes did not differ significantly with period of treatment, although they were generally smaller in the first period.

Ruminal infusions of methionine did not affect the apparent ruminal digestion of organic matter or the flow of protein (plant and microbial) to the omasum, and, since plant protein is probably largely degraded in the rumen (Hogan 1964), it is concluded that methionine did not stimulate the synthesis of microbial protein. ^{35}S incorporated by the microorganisms, either directly from methionine or from sulphides arising from its decomposition, could merely substitute for ingested organic or inorganic sulphur and therefore there would be no change in microbial protein synthesized. Roberts *et al.* (1955) found that the irreversible transformation of cysteine to homocysteine in *Escherichia coli* was a process which limited the rate of protein synthesis; added homocysteine or methionine increased the growth rate above that obtained from cystine or sulphate alone. Pittman and Bryant (1964) have, in fact, found that methionine was either stimulatory or essential for the growth of certain ruminal bacteria. However, where methionine is the sole source of sulphur the cysteine requirements of bacteria must be met from sulphides arising from the decomposition of methionines, owing to the irreversibility of the transulphuration reactions leading to methionine synthesis (Roberts *et al.* 1955; also Thompson 1967). Lampila (1967) found that, compared with cystine, methionine permitted little growth of mixed ruminal microbes *in vitro*. Gallup, Pope, and Whitehair (1952) and McClaren, Anderson, and Barth (1965) obtained a small increase in nitrogen balance when methionine was added to rations containing nitrogen and sulphur predominantly in the form of urea and inorganic sulphate. The rations contained adequate total sulphur but possibly insufficient methionine *per se* to meet the microbial requirements for maximal growth. The greater nitrogen balance obtained by Loosli and Harris (1945) and substantially greater weight gains and wool growth obtained by Garrigus *et al.* (1950) and Albert *et al.* (1956) when methionine replaced supplemented sulphate or elemental sulphur in the diet may perhaps be attributed to a similar effect on ruminal metabolism. However, in all cases a more attractive explanation for the increased growth rate and nitrogen retention associated with methionine feeding may be that sufficient methionine passed unmodified to the intestines and, since methionine is the limiting essential amino acid at the tissue level (Nimrick *et al.* 1970), this enabled more efficient utilization of the absorbed amino acid nitrogen, as indicated in the present experiment.

There have been several reports where methionine supplements have not given a response in growth rate, wool production, or nitrogen retention (e.g. Thompson *et al.* 1952, Whiting *et al.* 1954) and this has often been attributed to an adequate synthesis of microbial protein resulting from the sulphur of the basal diet. In other experiments alternative forms of supplemental sulphur have given a response similar to that obtained with methionine (e.g. Starks *et al.* 1954) and in both situations it is possible to argue that the supplemental methionine was more extensively degraded in the rumen than in other experiments where positive responses were obtained. The finding in the present experiment of significant period differences in the incorporation of ^{35}S into wool and excretion of ^{35}S, and a uniformly different (but not statistically significant) period response in parameters relating to production illustrate the variability which is inherent in this aspect of ruminant nutrition. Unfortunately there have been no critical studies made of factors affecting the metabolism of methionine in the rumen.

Both the production of wool and body weight gains were substantially increased by methionine infusions. The mean increase in wool growth rate above basal was 28 and 68% for the ruminal and abomasal infusions, respectively. There was also an associated increase of 4·5 and 19·7% above basal, respectively, in the sulphur content of the wool grown. The differential wool production response to methionine given by either route was comparable with that obtained by Graceva (1969), where 3 g methionine given abomasally per day or in the diet gave increases of 42 and 18% above basal, respectively. The observed increase in wool production, due to abomasal infusions of methionine in the present experiment, was consistent with that found by others (e.g. Reis 1967; Langlands 1970; Robards 1971) under pen or grazing conditions. Gains in liveweight due to abomasal infusions of methionine have been recorded by Reis (1967), while Robards (1971) found a greater retention of nitrogen in sheep receiving abomasal infusions compared with pre-infusion values. No comparisons were made between methionine infusion treatments and the control sheep with regard to nitrogen and sulphur balance in the present experiment, but abomasal infusions significantly improved these balances in conjunction with increased liveweight gains and wool production, when compared with ruminal infusions.

This experiment confirms the view that methionine fed in the diet is not capable of stimulating either wool growth or body weight gains to the extent that abomasal infusions of equivalent amounts of methionine may. However, there remains the possibility that by increasing the dietary intake of methionine sufficient could pass undegraded to the intestines to give a maximal response in either of these parameters. In this regard, Langlands (1970) has shown that as little as 0·36 g methionine per day when infused abomasally may give a wool growth response equivalent to that obtained from a similar infusion of 2·16 g methionine per day. The results obtained in the present experiment and previously (Bird 1972*a*) suggest that the rate of methionine degradation is sufficiently slow that this could be economically achieved. Alternatively, it may be practicable to manipulate the rate of flow of fluid from the rumen and thereby achieve a similar result.

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