SULPHUR METABOLISM AND EXCRETION STUDIES IN RUMINANTS

IX.* SULPHUR, NITROGEN, AND ENERGY UTILIZATION BY SHEEP FED A SULPHUR-DEFICIENT AND A SULPHATE-SUPPLEMENTED, ROUGHAGE-BASED DIET

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Abstract

Four Merino wethers were each fed a sulphur-deficient, roughage-based ration containing 1·05% nitrogen (79% as urea) and supplying 135 mg sulphur/day. Four other sheep were fed similar amounts of basal ration supplemented with Na_2SO_4. This ration supplied 494 mg sulphur/day. After 21-day periods the treatment groups were reversed.

Supplemental sulphate increased the daily flow of protein nitrogen to the omasum by 2·09 (±0·28) g/day (mean ± S.E.M.) ($P<0·001$) and changed the nitrogen balance from −2·38 (±0·09) g/day to 0·15 (±0·19) g/day ($P<0·001$). The sulphur balances were changed from −101 (±4·8) mg/day to 86 (±14·6) mg/day ($P<0·001$). The ratio of nitrogen to sulphur stored when sulphate was added to the basal ration was 13·5 (±0·58), therefore a nitrogen to sulphur ratio in the feed narrower than this is necessary for the optimal usage of dietary nitrogen by sheep.

The apparent digestibility of organic matter of the diet was increased from 43·8 (±2·59)% to 65·7 (±2·63)% ($P<0·01$) when sulphate was added to the basal ration. The intake of energy was thereby altered from a submaintenance level of 1065 (±65) kcal/day to an above-maintenance level of 1653 (±68) kcal/day ($P<0·001$). An estimated 10·1 (±6·33)% of the dietary organic matter digested was digested in the rumen on the basal treatment, compared with 74·8 (±3·85)% on the sulphur-supplemented ration ($P<0·001$).

The amount of sulphur apparently recycled to the rumen on the basal treatment was 192 (±30) mg/day; less was found on the sulphate treatment, due to inefficient utilization of the added sulphur.

The output of organic sulphur in faeces was increased ($P<0·001$) by the addition of sulphate to the diet. Urinary excretion of organic sulphur ($P<0·001$) and ester sulphur ($P<0·001$) was also increased, but the excretion of inorganic sulphate did not change, indicating a limiting supply of sulphate ions for sulphation reactions at these intakes of sulphur.

I. Introduction

The effect of supplemental sulphur in promoting weight gains in lambs fed synthetic diets containing urea as the major nitrogen source is well known (e.g. Thomas *et al.* 1951; Starks *et al.* 1953; Albert *et al.* 1956). The present experiment demonstrates quantitatively the effect of a deficiency of sulphur in the diet upon

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ruminal metabolism, particularly relating to the digestion of dietary organic matter and to microbial protein synthesis. Where roughage of low protein content only is available to ruminants, the operation of these related processes of ruminal fermentation are vital, if sufficient energy and protein are to be obtained to ensure survival and the production of meat, milk, and wool.

Whanger and Matrone (1970) have shown that, in sheep fed sulphur-deficient diets largely containing soluble carbohydrates, an altered ruminal fermentation resulted in the production and absorption of D-lactate from the rumen and the excretion of lactate in the urine. The extent of this urinary loss of energy following such biochemical changes subsequent to eating a sulphur-deficient ration composed mainly of oat hulls (83%) was examined in this experiment.

The present experiment also examines the effect of low intakes of sulphur (0·135–0·49 g sulphur/day) on the urinary excretion of inorganic *v.* ester sulphur and the faecal excretion of organic sulphur. Other experiments where sulphur intakes were greater (Bird 1971; Bird and Hume 1971) have shown that the urinary output of organic, ester, and inorganic sulphur and the faecal output of organic sulphur is very responsive to variation in sulphur intake. Where intakes exceeded 0·86 g sulphur/day the urinary inorganic sulphate output was linearly related to sulphur intake (Bird 1971); where intakes were 0·86 g sulphur/day or less (0·61 g sulphur/day) (Bird and Hume 1971) the outputs were both small (30 and 16 mg sulphur/day, respectively). In both instances at the lowest intake of sulphur the output of ester sulphur far exceeded that of inorganic sulphur, suggesting an obligatory demand for inorganic sulphate for sulphation reactions.

Finally, an assessment was made of the apparent recycling of sulphur to the rumen where recycling may be minimal due to low blood sulphate concentrations (Bray and Hemsley 1969), and of the ratio of nitrogen to sulphur stored when sulphate was added to the basal ration. In the latter instance this will indicate the minimum nitrogen : sulphur ratio in the feed required for optimal utilization of dietary nitrogen.

II. Materials and Methods

(*a*) *Experimental Design*

Eight mature Merino wethers weighing between 36 and 44 kg (mean live weights at the start and end of the experiment were 41·9 and 39·0 kg, respectively) were used. Each had an omasal and a ruminal cannula (see Hume, Moir, and Somers 1970). In the first period four sheep (G184, W27, O41, W21) were fed the basal ration and the remainder (B77, W29, B97, W24) the supplemented ration. After 21 days the treatments were reversed and the experiment was continued for a further 21 days. Each period consisted of 14 days adjustment to the ration, followed by a 7-day collection period. The sheep were confined to metabolism cages in a constantly lit room and fed at 2-hourly intervals throughout the experiment.

(*b*) *Diets*

The basal ration consisted of oat hulls (83%), starch (6%), sucrose (6%), minerals (3%), and urea (2%). The oat hulls contained 0·025% sulphur and 0·27% nitrogen. The complete ration contained, on a dry matter basis, 0·023% sulphur, 1·046% nitrogen (79·3% as urea), and 93·7% organic matter. The mineral mix used was that described by Hume and Bird (1970). The urea and sucrose were dissolved in water and mixed into the ration, which was then dried to 95% dry matter and stored in polythene bags prior to feeding.

Na_2SO_4 (0·25%) was added in solution with the urea and sucrose to the basal diet to give the sulphur-supplemented ration. The sulphur content of this ration was 0·082%.

Both rations were fed at the rate of 600 g dry matter/day. This amount was readily consumed on the sulphur-supplemented diet, but not as readily on the basal diet. Two sheep, G184 and B77, ate only 575 and 580 g dry matter/day, respectively, while a third sheep, W24, refused to eat at all after 2 weeks on the basal treatment and was removed from the experiment.

Deionized water was offered *ad libitum* and the daily intake measured. The water containers and the feed bins were plastic-coated to minimize ingestion of sulphur from sources other than the diet.

(c) *Collection of Samples*

Urine and faeces were collected and sampled daily for 7 days, as described by Hume, Moir, and Somers (1970).

Omasal and rumen digesta samples were obtained twice daily for 5 days during the 7-day collection period, as described by Bird and Moir (1972). A total of 100 g of digesta was removed daily in these samples. The 10 omasal samples were later combined for analysis of nitrogen, sulphur, organic matter, and lignin.

Feed residues, if any, were collected daily and the dry matter content determined after drying at 95°C for 24 hr.

Blood samples were obtained by jugular puncture at 1200 hr on the 19th and 21st day of each period, midway between 2-hourly feeding times. Potassium oxalate was used to prevent coagulation of the collected blood.

(d) *Chemical Analysis*

The total nitrogen content of rations, faeces, urine, and unstrained omasal digesta, the organic matter in rations and unstrained omasal digesta, and the total volatile fatty acids (VFA) in strained omasal digesta were determined as described by Hume, Moir, and Somers (1970).

Protein nitrogen in unstrained omasal digesta was determined by the method of Winter, Johnson, and Dehority (1964), except that, initially, equal volumes (10 ml) of the tungstate and sulphuric acid solutions were added to 10 g of sample.

Analysis of sulphur in rations, omasal digesta, rumen fluid, faeces, urine, and blood were as previously described (Bird and Fountain 1970; Bird 1971).

Total lactate in centrifuged omasal digesta was determined as described by Pennington and Sutherland (1956).

The lignin content of air-dried ration samples and of unstrained omasal digesta samples was determined by the method of Van Soest (1963), with the appropriate recommended alterations to the concentrations of sulphuric acid and cetyltrimethylammonium bromide, based on the dry matter content of the digesta samples. Dietary lignin was used as an indigestible marker (e.g. Hogan 1964; Thornton *et al.* 1970) and the rate of flow of digesta to the omasum was determined from the concentration of lignin in the omasal digesta in relation to the daily intake of lignin.

The caloric content of urine was determined as follows: 5 ml of sample was added to 1 g of cellulose in a crucible and dried at 45°C; a further 5 ml of sample was added and again dried; the crucible was then transferred into a Baird and Tatlock oxygen bomb calorimeter for combustion. The caloric value of the added cellulose was similarly determined and subtracted from the sample values.

(e) *Statistical Analysis*

The results were analysed by three-way analysis of variance, using the method described by Snedecor (1965) to allow for the absence of data from sheep W24 in one period.

III. Results

There were no significant differences due to treatment period in any of the measured parameters. Significant differences between sheep were found only in the

rate of flow of digesta to the omasum ($P<0\cdot05$), the concentration of protein sulphur ($P<0\cdot05$) and protein nitrogen ($P<0\cdot05$) in the omasal digesta, and the concentration of sulphide in the ruminal digesta ($P<0\cdot05$).

(*a*) *Flow of Digesta*

The daily flow of digesta to the omasum (Table 1) was not significantly affected by the sulphate treatment, but the percentage dry matter of the omasal digesta collected (see Table 2) was greater ($P<0\cdot01$) on the basal treatment.

TABLE 1

FLOW OF DIGESTA, NITROGEN, AND SULPHUR TO THE OMASUM AND NITROGEN AND SULPHUR BALANCE DATA

Treatment values are means ± standard error. Statistical differences between treatments are given thus: n.s., not significant; *** $P<0\cdot001$; ** $P<0\cdot01$; * $P<0\cdot05$

	Basal§	Basal + sulphate	Statistical difference
Digesta flow (g/day)	4306(±512)	5432(±552)	n.s.
Nitrogen (g/day)			
Intake	6·17(±0·048)	6·32(±0·003)	*
Omasal digesta total nitrogen	5·48(±0·314)	7·46(±0·435)	**
Omasal digesta protein nitrogen†	3·72(±0·115)	5·92(±0·230)	***
Omasal digesta protein	23·3(±0·72)	37·0(±1·44)	***
Faecal nitrogen	2·38(±0·050)	2·59(±0·128)	n.s.
Urinary nitrogen	6·17(±0·123)	3·58(±0·250)	***
Nitrogen balance	−2·380(±0·093)	0·146(±0·187)	***
Sulphur (mg/day)			
Intake	135(±1)	494(±0)	n.s.
Omasal digesta total sulphur	327(±29·5)	498(±32·6)	*
Omasal digesta protein sulphur‡	234(±14·7)	337(±20·6)	*
Omasal digesta soluble organic sulphur	60(±15·0)	15(±7·1)	n.s.
Omasal digesta reducible sulphur	47(±6·5)	111(±13·9)	**
Sulphur balance	−101(±4·8)	86(±14·6)	***
Treatment differences§			
Decrease in urinary nitrogen (g/day)	2·667(±0·345)		
Increment in nitrogen balance (g/day)	2·549(±0·241)		
Increment in sulphur balance (g/day)	0·189(±0·017)		
Ratio nitrogen : sulphur in stored nitrogen, sulphur	13·491(±0·583)		
Increment in tungstic acid nitrogen flow (g/day)	2·087(±0·281)		
Increment in tungstic acid nitrogen flow (as % increment in nitrogen balance)	86·9(±14·62)		

† Protein precipitated with tungstic acid. Protein = nitrogen × 6·25.

‡ Protein precipitated with trichloroacetic acid.

§ Excluding sheep W24.

(*b*) *Water Intake and Excretion*

The daily intake of water was not affected by treatment; the intakes were 964 (±60·8) and 919 (±61·1) ml/day on the basal and sulphate treatments, respec-

tively. The mean daily excretion of urine was 519 (±40·2) and 675 (±48·3) ml on the basal and sulphate treatments, respectively. The excretion of water in the faeces was greater ($P<0·05$) on the basal treatment (357±33·4 g/day) than on the sulphate treatment (210±20·3 g/day).

TABLE 2

APPARENT DIGESTIBILITY OF DRY MATTER AND ORGANIC MATTER OF THE DIET, CONCENTRATIONS OF VOLATILE FATTY ACIDS (VFA) AND LACTATE, AND DIGESTIBILITY AND RETENTION OF DIETARY ENERGY

Treatment values are means ± standard error. Statistical differences between treatments are indicated thus: n.s., not significant; *** $P<0·001$; ** $P<0·01$

	Basal§	Basal + sulphate	Statistical difference
Dry matter			
Intake (g/day)	594(±4·2)	600(±0)	n.s.
Apparent digestibility (%)†	43·1(±2·53)	63·7(±2·45)	**
Omasal digesta (%)	14·19(±1·57)	6·52(±0·48)	**
Organic matter (OM)			
Intake (g/day)	557(±3·8)	562(±0·2)	n.s.
Output (g/day)	305(±14·0)	191(±14·5)	**
Apparently digested (g/day)‡	238(±14·0)	365(±14·8)	**
Apparent digestibility (%)‡	43·8(±2·59)	65·7(±2·63)	**
Leaving rumen (g/day)	557(±13·3)	337(±23·6)	***
Bacterial OM leaving rumen (g/day)‖	23(±1·1)	50(±2·2)	
Dietary OM leaving rumen (g/day)	534(±13·1)	286(±20·9)	***
Dietary OM digested in rumen:			
Amount (g/day)	24(±15·0)	275(±21·1)	***
As % of total OM digested	10·1(±6·33)	74·8(±3·85)	***
As % of OM intake	4·1	49·1	
Omasal digesta VFA			
Total VFA (m-moles/l)	62·0(±10·22)	45·0(±5·69)	n.s.
Total VFA (m-moles/day)	239·6(±23·30)	235·7(±35·0)	n.s.
Omasal digesta lactate			
Total lactic acid (m-moles/l)	0·54(±0·17)	1·32(±0·42)	n.s.
Energy			
Digested energy (kcal/day)¶	1065(±65)	1653(±68)	***
Urine energy (kcal/day)	146(±18)	197(±16)	n.s.
Urine energy as (% of digested energy)	13·9(±0·85)	12·2(±1·36)	n.s.

† Allowing for omasal and ruminal digesta removed in sampling.

‡ Values adjusted to allow for omasal and ruminal digesta removed in sampling.

§ Excluding sheep W24.

‖ Estimated from tungstic acid nitrogen data [see Results, Section (*j*)].

¶ Estimated —see Moir (1961).

(*c*) *Flow of Nitrogen and Sulphur to the Omasum*

Adding sulphate to the basal sulphur-deficient ration increased the concentration of protein nitrogen ($P<0·05$) and reducible sulphur ($P<0·05$) in omasal digesta. Further, the Na_2SO_4 addition, as shown in Table 1, increased the daily flow of total nitrogen ($P<0·01$), protein nitrogen ($P<0·001$), total sulphur ($P<0·02$), protein sulphur ($P<0·02$), and reducible sulphur ($P<0·01$) to the omasum.

Supplemental sulphate increased the daily flow to the omasum of protein nitrogen by 2·2 g nitrogen (or 13·7 g of protein).

The proportion of the omasal digesta total sulphur found in trichloroacetic acid-precipitated protein was 73·5 (±4·80) and 71·9 (±3·82)% on the basal and sulphate treatments, respectively. The proportion of reducible sulphur (total sulphate sulphur) increased from 13·8 (±2·30)% on the basal treatment to 22·1 (±2·6)% on the sulphate treatment; a corresponding reduction ($P<0·05$) occurred in the proportion of soluble organic sulphur (18·2±3·73% *v.* 3·2±1·67%).

(*d*) *Rumen Fluid Sulphides*

The concentration of sulphides in strained rumen fluid was increased ($P<0·05$) from 0·07 (±0·03) to 0·37 (±0·09) μg sulphur/ml by the addition of sulphate to the diet.

(*e*) *Blood Reducible Sulphur*

Whole blood reducible sulphur concentrations were increased ($P<0·02$) from 11·50 (±0·78) to 15·94 (±0·79) μg/ml by the addition of sulphate to the diet.

(*f*) *Sulphur Recycling* (Table 1)

On the basal treatment the flow of sulphur to the omasum exceeded the intake of sulphur by 192 (±30·3) mg/day. However, on the sulphate-supplemented ration the apparent recycling of sulphur was only 4 (±32·6) mg/day.

(*g*) *Sulphur Excretion* (Table 3)

Supplemental sulphate increased the concentration and output in the faeces of total sulphur ($P<0·001$) and neutral sulphur ($P<0·001$) and the output in the urine

TABLE 3

EXCRETION OF SULPHUR IN URINE AND FAECES

Treatment values are means ± standard error. Statistical differences between treatments are indicated thus: n.s., not significant; *** $P<0·001$

	Basal†	Basal + sulphate	Statistical difference
Faeces sulphur (mg/day)			
Total sulphur	185(±2·1)	278(±7·6)	***
Neutral sulphur	177(±2·6)	269(±8·2)	***
Ester sulphate sulphur	7(±0·9)	8(±0·8)	n.s.
Inorganic sulphate sulphur	2(±1·1)	1(±0·6)	n.s.
Urine sulphur (mg/day)			
Total sulphur	50(±2·6)	130(±9·1)	***
Neutral sulphur	28(±1·3)	47(±2·3)	***
Ester sulphate sulphur	18(±1·7)	76(±6·4)	***
Inorganic sulphate sulphur	5(±1·1)	8(±2·4)	n.s.

† Excluding sheep W24.

of total sulphur ($P<0·001$), neutral sulphur ($P<0·001$), and ester sulphate ($P<0·001$). Inorganic sulphate excretion by either route was unaffected by treatment.

Organic sulphur compounds accounted for 95·4 (±0·99)% and 96·7 (±0·56)% of the faecal sulphur on the basal and sulphate treatments, respectively. Of the urinary sulphur excreted on the basal and sulphate treatments, respectively, neutral sulphur was 56·0 (±1·18) *v.* 37·1 (±2·72)%, ester sulphur 35·0 (±2·36) *v.* 57·9 (±1·39)%, and inorganic sulphur 9·0 (±2·36) *v.* 5·0 (±1·39)% of the total.

Sulphur balances (Table 1) were changed from negative to positive ($P<0·001$) by the addition of sulphate to the basal diet; the mean increment in sulphur balance was 189 mg/day.

(*h*) *Nitrogen Excretion* (Table 1)

Faecal nitrogen output was little affected by treatment but urinary nitrogen output was decreased ($P<0·001$) by 2·67 g/day on the sulphate treatment. The overall effect of this treatment was to convert a marked negative nitrogen balance on the basal ration to a slightly positive balance; the mean increment in nitrogen balance for the seven sheep that completed the experiment was 2·55 g/day.

The increased production of ruminal microbial protein accounted for approximately 87% of the observed increase in nitrogen balance.

(*i*) *Nitrogen : Sulphur Ratio in Stored Nitrogen and Sulphur* (Table 1)

The ratio of the increments in nitrogen balance and sulphur balance resulting from the addition of sulphate to the basal ration was 13·49 (±0·583).

(*j*) *Dry Matter and Organic Matter Digestibility* (Table 2)

The apparent digestibility of the dry matter and organic matter of the diet was substantially increased ($P<0·01$) by supplemental sulphate. This caused a significant increase ($P<0·001$) in the energy digested.

There was a marked effect of sulphate treatment on the amount of organic matter leaving the rumen daily ($P<0·001$). In order to estimate the approximate amount of dietary organic matter leaving the rumen on each treatment an estimate of the amount of organic matter present in the bacterial fraction was made. Non-dietary, presumably microbial, protein in omasal digesta was calculated by difference from total protein values and plant protein assumed to have escaped digestion within the rumen. In this regard it was assumed that similar proportions of dietary organic matter and protein were degraded in the rumen. Thus, *c.* 5 and 50% of the 8 g dietary protein may have been degraded in the rumen on the basal and sulphate treatments, respectively. Microbial organic matter was estimated on the basis that *c.* 10·5% of bacterial cells is nitrogen (see Hungate 1966). The estimate of dietary organic matter digested in the rumen on the basal treatment was 10·1% of the total organic matter digested and 74·8% on the sulphate treatment.

(*k*) *Energy Utilization for Microbial Growth*

The estimated amounts of dietary organic matter digested daily in the rumen were 24 and 275 g on the basal and sulphate treatments, respectively. Thus, the synthesis of microbial protein, due to the addition of sulphate to the basal diet, required 251 g organic matter. The increment in microbial protein synthesis was similarly estimated. If no dietary protein was digested on either treatment, 15·2 g

protein on the basal and 29·0 g on the sulphate treatment could have been synthesized daily. If all of the dietary protein was degraded, then these values could have been 23·3 and 37·0 g/day. The between-treatment differential, therefore, ranged from 13·7 to 21·7 g protein/day. If 5 and 50% of the dietary protein was digested on the basal and sulphate treatments, respectively, then 15·3 (±0·72) and 33·0 (±1·44) g of non-dietary protein flowed daily to the omesum on these treatments, so that approximately 17·7 g of microbial crude protein could have been synthesized from the 251 g organic matter apparently digested. The ratio of crude protein synthesized (g) per 100 g organic matter digested in the rumen was therefore 7·05.

(*l*) *VFA and Lactate in Omasal Digesta* (Table 2)

Neither the concentration of VFA or of total lactate in omasal digesta or the daily flow to the omasum of these compounds varied with treatment. Lactic acid concentrations were very small relative to VFA concentrations and were therefore not determined in samples obtained from the second period of treatments.

(*m*) *Retention of Energy* (Table 2)

Urinary excretion of energy, whether expressed on the basis of total daily output or as a percentage of the digested energy, did not differ between treatments.

IV. Discussion

A relatively small increase in the intake of sulphur (0·36 g/day) markedly improved the nutritive value to sheep of a sulphur-deficient, low protein roughage in which urea supplied 4·9 g nitrogen/day and oat hulls and starch together 1·28 g nitrogen/day. The increased digestibility of organic matter (22 units) was sufficient to elevate the energy intake from a submaintenance level of 1065 kcal/day up to 1653 kcal/day, which for penned sheep of similar body weight (40 kg) is above the maintenance requirement of 1500 kcal/day (or 340 g digestible organic matter) established by Langlands *et al.* (1963). There was an estimated 17·7 g/day increase in ruminal microbial protein synthesis on the sulphate-supplemented diet, with part of this gain being offset by the assumed degradation of about 4 g of dietary protein. The increased flow of protein to the omasum (13·7 g/day) was sufficient to bring the sheep from negative (−2·38 g nitrogen/day) to positive nitrogen balance (0·15 g nitrogen/day). These results are in accord with those of Bray and Hemsley (1969) and Playne (1969), where increases in the intake and digestibility of low protein roughage and in nitrogen retention accompanied the supplementation of such rations with Na_2SO_4. Earlier work (e.g. Thomas *et al.* 1951; Starks *et al.* 1953, 1954; Albert *et al.* 1956) has shown that growing lambs fed semipurified rations containing less than 0·1% sulphur and with urea the major source of nitrogen, made greater weight gains when Na_2SO_4 was added to the diets.

The consequence of the sulphur deficiency and wide nitrogen : sulphur ratio (45·7) in the basal ration is evident from the depressed ruminal microbial metabolism found when this ration was fed. Of the total dietary organic matter digested it was estimated that only 10% was digested in the rumen compared with 75% on the sulphate-supplemented ration (nitrogen : sulphur ratio of 12·8). The estimate on

the basal diet may be too low; only 24 g organic matter/day was apparently digested in the rumen but the efficiency of bacterial incorporation of fermented plant organic matter is less than 33% (Hogan and Weston 1971), therefore *c.* 72 g or more of organic matter would have been needed to support the synthesis of 15·3 g of microbial protein. Overestimating the flow of digesta, and hence protein, could account for this discrepancy of *c.* 50 g organic matter. Alternatively, much of the protein attributed to ruminal microbial cells may have been derived from the ruminal epithelium or possibly salivary proteins. Under conditions of severe sulphur deficiency portion of these proteins might escape degradation, due to the retarded rate of ruminal microbial metabolism. Bray and Hemsley (1969) have shown that the rate of cellulose digestion in the rumen was greatly decreased when the ration contained 0·06% sulphur compared with 0·14% sulphur, and it is probable that in the present experiment on the basal treatment where sulphur was even more limiting, that little more than the starch and sucrose components were digested in the rumen.

The increased synthesis of microbial protein due to supplementing the basal ration with sulphate was a relatively inefficient process, in terms of energy expenditure, since an estimated 7·05 g of crude protein was synthesized per 100 g organic matter digested, compared with values of 15–16 estimated by Hogan and Weston (1967), 17–23 (Hume 1970*b*; Hume and Bird 1970), and 14·4 (Walker and Nader 1970). However, the estimate made in the present experiment may be a little low since overestimating the amount of microbial protein synthesized on the basal treatment would decrease the differential between the two treatments. That is, due to the addition of sulphate to the basal diet more than the calculated 17·7 g/day of protein may have been synthesized. Further, overestimating the flow to the omasum of digesta and hence organic matter, would mean that less organic matter was digested in the rumen due to the addition of sulphate than calculated. The influence of these factors on the estimation of the efficiency of bacterial synthesis has been discussed by Hogan and Weston (1971).

Hume, Moir, and Somers (1970) found that energy was more efficiently used for growth and more protein was synthesized at higher intakes of nitrogen. Hume (1970*a*, 1970*b*) also found a similar effect on growth efficiency with the addition of casein, zein, and possibly branched-chain VFA to low protein diets. In the present experiment it is probable that a deficiency of sulphur was the major limitation to microbial growth, even on the sulphate-supplemented ration, but other limiting growth factors such as peptides or amino acids normally provided from dietary protein may have been implicated. On the basal diet there was a net gain of 192 mg sulphur leaving the rumen above that ingested, but only 4 mg on the sulphate-supplemented diet. Because the concentrations of ruminal sulphides and of reducible sulphur in blood were higher on the latter treatment, and since portion of the added sulphate passed to the omasum apparently undegraded, the supplemental sulphate was not efficiently incorporated into microbial sulphur. There was no apparent recycling of nitrogen on the basal treatment but at least 1·14 g nitrogen/day was recycled on the sulphate treatment.

The estimate of sulphur recycled on the basal diet is less than half that found by Bird and Hume (1970). In that experiment 0·44 g sulphur was apparently recycled when the sheep ate a diet supplying 0·61 g sulphur/day. The ration contained higher levels of soluble carbohydrates (25% starch, 12% sucrose) and nitrogen (2·5% urea

and 6·6% gelatin) than fed here and may have led to a greater incorporation of recycled sulphur into microbial protein. Lower blood sulphate concentrations under the present extreme conditions of sulphur deficiency could also result in less sulphur being recycled via salivary secretions, which are the main sources of recycled sulphur (Bray 1969). Bray and Hemsley (1969) have shown that total sulphur and reducible sulphur concentrations in parotid saliva were lower in sheep fed a sulphur-deficient diet and that less sulphur was recycled under those conditions. Concentrations of inorganic sulphate in blood have been shown to be severely depressed on sulphur-deficient diets (Weir and Rendig 1954; Bray and Hemsley 1969).

The concentration of total VFA and the flow of VFA from the rumen in the omasal digesta were similar in both treatments, despite a large difference (251 g) in the organic matter digested in the rumen. However, Moir (personal communication) found low concentrations of VFA in omasal fluid similar to those found here, irrespective of ruminal concentrations, therefore omasal VFA concentrations cannot be used to predict parameters of ruminal metabolism.

There did not appear to be significant production of lactate on the sulphur-deficient diet. The concentration of total lactate in the omasal digesta samples was less than 1 mM, compared with a concentration of up to 80 mM found in rumen fluid of sheep eating a sulphur-deficient diet composed largely of carbohydrate (Whanger and Matrone 1970). These authors suggest that the conversion of lactate to propionate via the acrylate pathway was inhibited and D-lactate accumulated in the rumen (Whanger and Matrone 1967). High concentrations of lactate were found in the urine, presumably due to the absorption and subsequent excretion of D-lactate which is apparently not metabolized by the tissues (Whanger and Matrone 1966). In the present experiment the loss of energy in the urine of sheep on the basal treatment was less than that on the sulphate-supplemented treatment; expressed as a percentage of the digested energy there was little difference, suggesting that excretion of lactate did not occur. Therefore, on a sulphur-deficient roughage diet the failure to digest the dietary organic matter is the important cause of the loss of energy potentially available to the sheep.

The proportion of the omasal digesta total sulphur found in trichloroacetic acid-precipitated protein was similar on both treatments (72–74%) and also similar to that found by Bird and Moir (1972) (73%) but greater than found by Bird and Hume (1971) (55–70%). The proportion of reducible sulphur found in the present experiment was also higher on either treatment than found by Bird and Hume (1971) but the proportion of soluble organic sulphur components was lower. In the present instance, assuming that the contribution of ester sulphates to the total sulphur in the omasal digesta were similar in both treatments, then *c.* 18% of the added sulphate passed unchanged to the omasum, compared with *c.* 3% found by Bird and Hume (1971) when 1·4 g sulphate sulphur was added daily to the basal diet which contained 0·6 g sulphur/day. In that case, however, another sheep was incapable of reducing sulphate to the same extent and 62% was not degraded. The failure of the ruminal micro-organisms to effectively reduce all of the added sulphate in the present experiment may be due to an absence of dissimilatory sulphur reducing organisms coupled with a limitation to assimilatory sulphate reduction (see Roy and Trudinger 1970), coincident with a restriction on microbial growth by other growth-limiting factors.

The nitrogen : sulphur ratio of tungstic acid-precipitated protein nitrogen to trichloroacetic acid-precipitated protein sulphur in the omasal digesta was 15·9 and 17·6 for the basal and sulphur-supplemented rations, respectively, compared with a mean of 18·3 calculated from the data of Bird and Hume (1971). These ratios are wider than found by Walker and Nader (1968) for microbial protein (*c.* 11·0) but the difference may be due, in part, to differing methods for precipitating protein. Hume (1969) has shown that at least 25% more nitrogen is precipitated by tungstic acid than by trichloroacetic acid, so that the nitrogen : sulphur ratio calculated from tungstic acid nitrogen and trichloroacetic acid sulphur could be wider than if trichloroacetic acid precipitates alone were used. The ratio of tungstic acid nitrogen to neutral sulphur may be closer to the true value; the ratios in the present experiment were 13·3 and 15·3 for basal and sulphate treatments, respectively, and 12·3 in the experiment of Bird and Hume (1971). These values are also lower than Walker and Nader's (1968) value for microbial protein but the difference may be due to the presence of plant protein and possibly also animal protein in the omasal digesta.

Organic sulphur compounds comprised 95–97% of the faecal sulphur compared with 87–94% found by Bird (1971) when up to 5 g of sulphate sulphur was infused daily into the rumen, or 92–95% found by Bird and Hume (1971). Faecal excretion of ester sulphate sulphur or inorganic sulphur was small and unaffected by change in sulphur intake. In the present experiment, and in others (Bird 1971, 1972*a*; Bird and Hume 1971) sulphur deficiency resulted in smaller outputs of faecal neutral sulphur, despite greater outputs of faecal dry matter which could possibly increase gut mucosal shedding or merely produce a greater loss of undigested plant protein sulphur. Increased bacterial growth associated with a greater digestibility of the diet and an increased faecal loss of bacterial sulphur due to the incomplete digestion of bacteria (see Bird 1972*b*) probably accounts for much of the greater excretion of faecal neutral sulphur found at high intakes of sulphur.

As previously reported (Bird 1971; Bird and Hume 1971), the urinary excretion of ester sulphate and of organic sulphur compounds increased with greater intakes of sulphur. However, in the present experiment inorganic sulphate excretion was not elevated when sulphate was added to the diet, showing that for an intake of sulphur less than 0·5 g/day the demand by the tissues for sulphate ions for sulphation reactions exceeds the supply.

The ratio of the nitrogen stored to sulphur stored by the sheep tissues, as deduced from the increment in nitrogen and sulphur balance following the addition of sulphate to the diet, was 13·5. The stored sulphur may not be entirely in protein since sulphate itself is required for a number of sulphation reactions in the body, including the formation of mucopolysaccharides of cartilage, bone, connective tissue, and mucous secretions (see Dziewiatkowski 1970; Roy and Trudinger 1970) and neither ration may have supplied sufficient sulphur to completely satisfy those requirements. The ratio of nitrogen to sulphur stored is wider than the value of 8·1 similarly calculated from Bray and Hemsley's (1969) data (see Bray 1965). One explanation for this difference may be a lesser incorporation of sulphur into wool in the present experiment.

The nitrogen : sulphur ratio in plants may vary according to species, chemical environment and stage of maturity. Begg and Freney's (1960) data for a number of

pasture species show a nitrogen : sulphur ratio between 10 and 23 over a range of plant sulphur contents. Stewart and Porter (1969), however, found that with maize and bean plants this ratio could be varied from 4 up to 55 by changing from a nitrogen-deficient, sulphur-adequate soil to a sulphur-deficient, nitrogen-adequate soil, although the plant protein nitrogen : sulphur ratio was constant at *c.* 15. From the data presented it is clear that a nitrogen : sulphur ratio wider than *c.* 13·5 in ruminant diets, whether in pasture plants or in compounded rations must result in uneconomical usage of the dietary nitrogen by ruminants.

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