

The effect of molybdenum on the conversion of sulphate to sulphide and microbial-protein-sulphur in the rumen of sheep

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1. [³⁵S]sulphate was used to measure the apparent turnover of sulphate, sulphide and microbial-protein-S in the rumen contents of four sheep that were intraruminally infused with 10 g sodium sulphate/d alone, or together with 126 mg sodium molybdate (50 mg molybdenum/d).

2. Infusion of molybdate increased the concentration of sulphate in rumen fluid from 2.2 to 7.2 μg S/ml and decreased the rate of reduction of sulphate to sulphide by 50%. Although the rate of sulphide production was slower, the concentration of sulphide in rumen contents was increased. A dual role for molybdate in the metabolism of sulphide in the rumen is suggested to explain these changes.

3. In animals that were not infused with molybdate, only one-third of the S (3.0 g/d) that passed through the sulphate pool in rumen fluid was converted to sulphide, decreasing to one-sixth when molybdate was infused.

4. The turnover of S amino acids in microbial protein was not significantly affected by molybdate. Only 52-57% of the S amino acid content of microbial protein was synthesized de novo by way of the sulphide pool.

Inorganic sulphate is significant in the nutrition of sheep both as a substrate for de novo synthesis of sulphur amino acids (Emery, Smith & Huffman, 1957) and as a modifying factor in the metabolism of copper and molybdenum (Dick, 1956; Underwood, 1971).

Micro-organisms in rumen digesta are capable of producing relatively large quantities of sulphide by the metabolic reduction of inorganic sulphate (Anderson, 1956). Some of this sulphide passes from the digesta across the rumen wall, a small amount flows down the gastrointestinal tract to the abomasum, and the remainder is used for de novo synthesis of S amino acids in microbial protein. Although some species of rumen micro-organism are able to incorporate preformed methionine or cysteine into cell protein, it is thought that this does not occur to a large extent in vivo because the concentration of free amino acids is very low in rumen fluid, and added S amino acids are rapidly degraded. Nevertheless, the extent of direct incorporation of the S amino acids of forage proteins into microbial protein has not been measured direct. The balance between de novo synthesis or direct incorporation of S amino acids by the microbial population in the rumen may vary depending on a number of factors, including the physical form of the forage, time after feeding, the solubility of the plant proteins, or the amount of inorganic S, nitrogen and readily digested carbohydrate simultaneously available.

Sodium molybdate is known to inhibit the rate of sulphate reduction by suspensions of washed rumen micro-organisms (Huisingh & Matrone, 1972). By decreasing the

supply of sulphide this compound may limit the extent of de novo synthesis of S amino acids in rumen contents. The inhibition of sulphate reduction is thought to be due to competition at the first stage of sulphate activation, catalysed by ATP:sulphate adenylyltransferase (*EC* 2.7.7.4) (Wilson & Bandurski, 1958; Robbins, 1962). The increase in sulphide concentration in the rumen fluid of sheep after feeding 50 mg Mo as $\text{Na}_2\text{MoO}_4/\text{d}$ (Mills, 1960; Bryden & Bray, 1972) appears anomalous, and has not been reconciled with the inhibitory action of molybdate in sulphate reduction; nor have the possible consequences of this action for microbial protein synthesis been studied.

Molybdate and sulphate must be supplied in the diet together before the Cu concentration in the liver of ruminant animals is decreased. Sulphate is not similarly effective when given with molybdate to non-ruminant animals such as the rat and rabbit. The possibility therefore exists that the rumen, with its microbial population carrying out interconversions of S compounds, may be an important site of interaction between Cu, Mo and S.

This paper presents the results of an experiment in which the effect of molybdate on the apparent turnover of relevant S pools in sheep rumen contents was measured using a radioactive tracer technique. An estimate of the extent of de novo synthesis of S amino acids by the rumen micro-organisms is given.

EXPERIMENTAL

Animals and diet

Four 2-year-old Merino wether sheep (33–36 kg), fitted with permanent rumen fistulas, were each given a daily ration of 800 g chopped wheaten hay as twenty-four equal-sized portions hourly (Gray, Weller, Pilgrim & Jones, 1967), to produce a relatively steady-state of metabolism within the rumen. The ration was adequate to maintain the body-weight of the animals, and supplied 10.16 g N, 0.975 g total S, 90.4 mg sulphate-S, 2.34 mg Cu and 0.14 mg Mo daily. Because the ration was low in cobalt (0.03–0.04 $\mu\text{g/g}$) it was necessary to protect the animals against vitamin B_{12} deficiency by injecting cyanocobalamin intramuscularly (250 $\mu\text{g/week}$). Subcutaneous injections of copper glycinate (75 mg/animal; Glaxo Laboratories (N.Z.) Ltd, Palmerston North, New Zealand) were also given. Rain-water was available for drinking at all times except during rumen fluid volume determinations.

Treatments

Two treatments were superimposed on the dietary regimen mentioned previously: (1) intraruminal infusion of 10 g sodium sulphate/d, (2) intraruminal infusion of 10 g Na_2SO_4 plus 126 mg $\text{Na}_2\text{MoO}_4/\text{d}$. These treatments supplied 2.2 g sulphate-S and 50 mg Mo daily. The treatments were randomly allocated to the animals, and each animal eventually received each treatment.

There was an equilibration period of 18 d before any turnover measurements were made, to allow the rumen microbial populations to adjust to the continuous infusions and to reach a steady rate of metabolism. The infusion volume was 150 ml during the

first 14 d of the equilibration period, but during the last 4 d, and for the subsequent 10 d sampling period, most of the daily water intake was continuously infused intraruminally (2500 ml) to minimize variations that may have resulted from irregular fluid intakes during turnover measurements.

On the 1st day of the sampling period, and for each of the next 6 d thereafter, 60 μCi [^{35}S]sulphate ($\text{H}_2^{35}\text{SO}_4$, specific radioactivity 28.8 mCi/mmol; The Radiochemical Centre, Amersham, Bucks., UK) was included in the continuous intraruminal infusion. From the 8th day of the sampling period, the radioactive tracer was omitted from the infusion and the decrease in specific radioactivity of relevant S compounds in rumen digesta was followed.

Samples of rumen fluid and digesta were taken at 8 h intervals during the sampling period. More frequent samples of rumen fluid were taken during the 2 h after stopping the radioactive infusion. Rumen fluid was sampled from a polyethylene tube that passed through the closed rumen cannula into the mid-ventral sac of the rumen. The fluid passed through fine-meshed nylon voile and was then drawn through the tube with a glass syringe. Representative samples of rumen digesta (50 g) were obtained with a stainless-steel sampling device similar to that described by Smith & Marston (1970).

The rumen fluid volume and fluid flow-rate for each sheep was determined on the day before and the day after each sampling period, using the single injection method with chromium ethylenediaminetetraacetate (Downes & McDonald, 1964; Binnerts, van't Klooster & Frens, 1968).

Methods of analysis

Inorganic sulphate. The concentration of sulphate-S in solutions that were infused into the rumen was determined titrimetrically (Bird & Fountain, 1970), after reduction of the sulphate to sulphide (Johnson & Nishita, 1952).

The inorganic sulphate in rumen fluid was isolated by passing 4 ml through a column of Amberlite IRA-400 anion-exchange resin (Rohme & Haas Co., USA) and eluting it with 6 ml of a solution containing 35 g sodium chloride/l (Bingley & Dick, 1967). One drop of 6 M-hydrochloric acid was added to the eluate and the sulphate was precipitated by adding 2 ml of a solution containing 100 g barium chloride/l. The precipitate was quantitatively recovered with a Pregl stick (porosity 4), and the sulphate-S in it was determined by the method of Bird & Fountain (1970). The recovery of 100 μg sulphate-S added to rumen fluid averaged 94 % using this procedure.

The amount of radioactivity in the sulphide produced from the reduction of sulphate in the Johnson & Nishita (1952) apparatus was measured by the following method.

Acid-volatile sulphide. All storage vials and apparatus used for sulphide analyses were cleaned with detergent, soaked in 3 M-HCl for 20 min, and thoroughly rinsed with glass-distilled water.

The concentration of sulphide in rumen fluid was determined for duplicate 10 ml samples. The soluble sulphide was trapped at the time of sampling by mixing the fluid with 0.2 ml of a solution containing 50 g cadmium acetate/l in a preweighed glass test-tube. This was found to be essential for quantitative recovery of acid-volatile sulphide. If cadmium acetate was not added, the amount of sulphide that

could be released by acidification of 10 ml rumen fluid containing 94 μg sulphide-S decreased rapidly to 89 μg S after 1 min and to 56.5 μg S after 1 h, even though the fluid was held in an air-tight container at 0°. Solutions containing cadmium acetate could be stored at room temperature for several hours without loss of sulphide.

The tube and contents were weighed and were connected to an apparatus similar to that described by Bray (1965) for the displacement of sulphide. The N_2 flow was stopped after 20 min and the sulphide content of a 5 ml portion of the trapping solution was measured titrimetrically (Bird & Fountain, 1970). Another 5 ml portion of this solution was prepared for the measurement of radioactivity by first oxidizing the sulphide to sulphate with 1 ml hydrogen peroxide (100 vol.) for 1 h, adding 10 ml of 0.6 M-HCl with carrier H_2SO_4 (0.5 mmol) and then precipitating the sulphate with 10 ml of a solution containing 100 g BaCl_2/l . The precipitate was recovered on a 25 mm glass filter disc (Whatman GF/C; W. & R. Balston Ltd, Kent, UK) and was washed thoroughly with water and ethanol. It was then dried at 105° and was suspended together with 400 mg fine silica (Cab-O-Sil; Packard Instrument Co., Downers Grove, Illinois, USA) in 10 ml of a solution containing 5 g 2,5-diphenyl-oxazole/l toluene. The radioactivity of the suspension was measured using a liquid scintillation counter (Model 3375; Packard Instrument Co.).

This method measured the concentration and specific radioactivity of hydrogen sulphide, as well as acid-volatile metal sulphides, including soluble thiomolybdates.

Microbial-protein-S. The amount of microbial-protein-S in rumen digesta was calculated by dividing the ^{35}S content of acid-washed digesta samples by the specific radioactivity of S in acid-precipitated, plant-free isolates of rumen micro-organisms that had been taken from the sheep on the same day.

For the determination of the ^{35}S content of digesta, 4 ml 6 M-HCl was added to 20 g digesta and boiled with stirring for 2 min. The plant solids and acid-precipitated micro-organisms were quantitatively recovered on a 90 mm filter-paper (Whatman no. 42; W. & R. Balston Ltd) plus powdered cellulose. The filtrate was refiltered if cloudy. The 'filter cake' was washed with 20 ml aqueous ethanol (400 ml/l), three 20 ml portions of 0.05 M- Na_2SO_4 and 0.05 M- Na_2S in aqueous ethanol, and three final rinses with aqueous ethanol. The air-dry 'filter cake' was digested with 20 ml concentrated nitric acid and 0.5 mmol carrier H_2SO_4 for 2 h in a 500 ml Kjeldahl flask, 20 ml perchloric acid (700 ml/l) was added, and the temperature gradually increased until HClO_4 fumes appeared. The contents were simmered for 1 h, cooled, diluted and filtered. The filtrate was boiled, 10 ml of a solution containing 100 g BaCl_2/l was added and the barium sulphate precipitate was recovered and prepared for determination of radioactivity content as previously described.

The specific radioactivity of S in acid-precipitated rumen micro-organisms isolated substantially free of plant material was determined as follows. 150 ml samples of rumen fluid were centrifuged at 2° and 400 g for 1 min (Highspeed 18 centrifuge; Measuring and Scientific Equipment Ltd, London SW1, UK) to sediment large plant particles. The supernatant fraction was centrifuged at 22000 g for 20 min and the resulting 'pellet' of micro-organisms was resuspended in 100 ml water and recentrifuged. The cells were transferred to a flask containing 5 ml 6 M-HCl and were boiled

for 1 min, cooled, and centrifuged. The precipitate was washed twice with 10 ml aqueous ethanol, four times with a mixture of 0.05 M-Na₂SO₄ and 0.05 M-Na₂S in aqueous ethanol, and was finally rinsed six times with 10 ml aqueous ethanol to remove all soluble S compounds. The washed precipitate was then oxidized with 10 ml concentrated HNO₃ and 10 ml HClO₄ (700 g/l). BaSO₄ was precipitated as described previously, but without the addition of carrier H₂SO₄. The specific radioactivity of S was determined as described previously for acid-volatile sulphide after the reduction of the isolated sulphate to sulphide (Johnson & Nishita, 1952).

Cr. The concentration of Cr in samples of rumen fluid taken for the determination of rumen fluid volume and the rate of fluid flow was determined by atomic absorption spectroscopy (William, David & Iismaa, 1962) using a Varian-Techtron spectrophotometer (Model AA4; Varian-Techtron, Victoria, Australia).

RESULTS

The concentrations and specific radioactivities of S compounds in rumen contents

The results of previous experiments had indicated that the specific radioactivities of sulphate-S, sulphide-S, and microbial-protein-S in rumen contents from sheep fed hourly were relatively constant within 3 d of the start of a continuous intraruminal infusion of [³⁵S]sulphate (Walker & Nader, unpublished results). In the present experiment the specific radioactivities of these compounds after 5, 6 and 7 d of radioactive infusion were taken to be representative of the steady-states of microbial metabolism that were maintained in the presence and absence of added molybdate.

Inorganic sulphate in rumen fluid. The concentration and specific radioactivity of inorganic sulphate in the rumen fluid of the sheep is given in Table 1. Although comparatively large amounts of sulphate were infused daily, the sulphate concentration was so low that 16 ml fluid had to be used to give sufficient sulphate-S for accurate analysis. There were pronounced differences in the concentration of sulphate in the rumen fluid of animals given the same treatment, but the effect of molybdate in increasing sulphate concentrations was consistent. In one animal, the concentration of sulphate in rumen fluid increased to very high values (170 µg S/ml) when molybdate was infused. These sulphate concentrations were not included in the mean value given in Table 1.

The specific radioactivity of inorganic sulphate in the rumen fluid of all animals was significantly lower than that of the infused sulphate (Table 1, $P < 0.001$). There was insufficient sulphate in the food ingested by the sheep to account for this difference (Table 2), indicating that recycling of sulphate to the rumen contents by way of the saliva, or across the rumen wall, must have been significant (Table 2).

Acid-volatile sulphide in rumen fluid. Molybdate increased both the concentration and the total amount of sulphide in the rumen fluid (Tables 1 and 3).

The specific radioactivity of sulphide in rumen fluid was approximately 30 counts/min per µg S in the presence or absence of molybdate, and was not significantly different from that of rumen fluid sulphate (Table 1).

Table 1. *The effect of intraruminal infusion of sodium molybdate on the concentrations and specific radioactivities of sulphur in sulphate, sulphide, and microbial-protein in rumen contents of sheep*

(Mean values with their standard errors for four animals. Approximately 10 g sodium sulphate was infused into the rumen of each sheep daily, and where indicated 126 mg $\text{Na}_2\text{MoO}_4/\text{d}$ was also given)

Treatment	Concentration				Specific radioactivity (counts/min per $\mu\text{g S}$)			
	Rumen fluid		Digesta microbial-protein-S ($\mu\text{g S/g}$)		Rumen fluid		Digesta microbial-protein-S	
	Sulphate-S ($\mu\text{g S/ml}$)	Sulphide-S ($\mu\text{g S/ml}$)	Sulphide-S ($\mu\text{g S/g}$)	Digesta microbial-protein-S ($\mu\text{g S/g}$)	Sulphate-S	Sulphide-S	Sulphate-S	Digesta microbial-protein-S
No molybdate infused	2.2	5.6	102.8	102.8	41.9	29.2	29.2	16.3
Molybdate infused intraruminally	7.2*	6.9	100.8	100.8	42.8	32.1	32.1	17.1
SEM	1.5	0.3	3.2	3.2	0.8	1.7	1.7	0.5

* Mean value for three animals; in one animal values were very high (170 $\mu\text{g S/ml}$) and were not therefore included in the mean.

Table 2. *The effect of intraruminal infusion of sodium molybdate on the pool sizes, turnover times, and daily amounts of sulphate-sulphur entering and leaving the rumen contents of sheep*

(Mean values with their standard errors for four animals. Approximately 10 g sodium sulphate was infused into the rumen of each sheep daily, and where indicated 126 mg $\text{Na}_2\text{MoO}_4/\text{d}$ was also given)

Treatment	Infused sulphate (g S/d)	Ingested in food (g S/d)	Apparently recycled to rumen (g S/d)	Pool size (mg S)	Apparent turnover time (min)	Apparent turnover (g S/d)	Sulphate remainder* (g S/d)	Outflow in rumen fluid (g S/d)
No molybdate infused	2.18	0.09	0.88	12.6	5.8	3.14	1.94	0.03
Molybdate infused intraruminally	2.16	0.07	0.64	38.6	19.0	2.87	2.20	0.12
SEM	0.07	0.01	0.12	8.6	3.7	0.10	0.10	0.03

* Sulphate turnover - (sulphide turnover + sulphate turnover in rumen fluid).

Table 3. *The effect of intraruminal infusion of sodium molybdate on the pool sizes, turnover times, and daily amounts of sulphide-sulphur and microbial-protein-S metabolized in the rumen of sheep*

(Mean values with their standard errors for four animals. Approximately 10 g sodium sulphate was infused into the rumen of each sheep daily, and where indicated 126 mg $\text{Na}_2\text{MoO}_4/\text{d}$ was also given)

Treatment	Rumen fluid sulphide-S					Microbial-protein-S				
	Pool size (mg S)	Apparent turnover time (min)	Apparent turnover (g S/d)	Outflow in fluid (g S/d)	Apparently absorbed (g S/d)	Pool size (mg S)	Apparent turnover time (h)	Apparent turnover (g S/d)	Protein-S from sulphide (g S/d)	Protein-S not from sulphide (g S/d)
No molybdate infused	30.5	37.8	1.17	0.08	0.67	642	18.7	0.81	0.42	0.39
Molybdate infused intraruminally	37.6	106.9	0.55	0.11	0.93	565	19.1	0.71	0.41	0.30
SEM	2.2	14.5	0.04	0.01	0.06	33	1.1	0.06	0.03	0.04

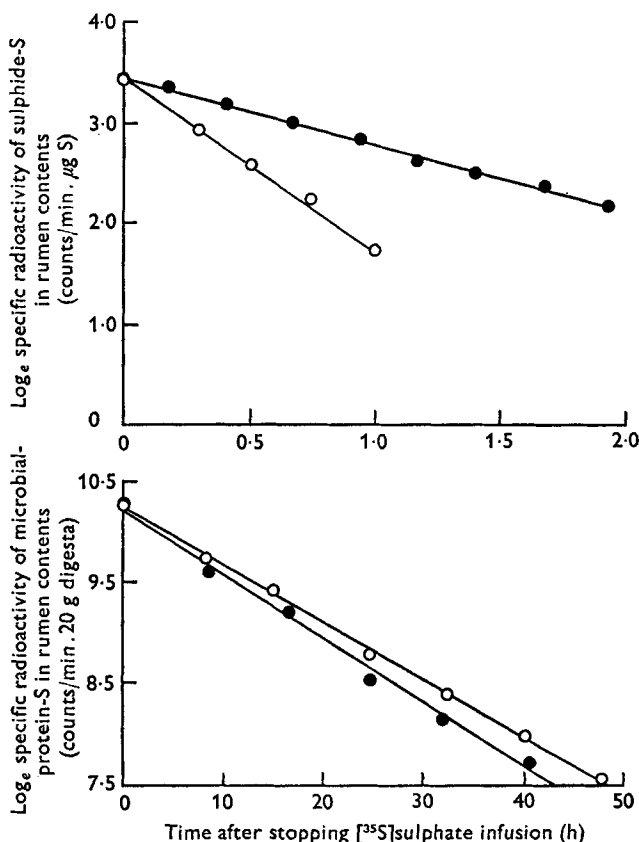


Fig. 1. The decrease in specific radioactivity of sulphide-sulphur and microbial-protein-S in the rumen contents of a sheep after stopping a continuous intraruminal infusion of $[^{35}\text{S}]$ -sulphate. The sheep was given chopped wheaten hay at hourly intervals and was intraruminally infused with 10 g sodium sulphate/d (○) or 10 g Na_2SO_4 plus 126 mg sodium molybdate/d (●) during and after the infusion of $[^{35}\text{S}]$ sulphate. For details of experimental procedure, see p. 13.

Microbial-protein-S. The amount of microbial-protein-S in rumen digesta differed between sheep, and ranged from 74 to 138 $\mu\text{g S/g}$ (mean 101.8 $\mu\text{g S/g}$). Molybdate had no significant effect on the quantity of microbial-protein-S/g rumen digesta, nor on the specific radioactivity of that S (Table 1).

The specific radioactivity of microbial-protein-S was approximately half that of sulphide-S ($P < 0.001$), regardless of molybdate treatment (Table 1), indicating that a substantial amount of microbial-protein-S was obtained from sources other than sulphide.

The apparent turnover of S compounds in rumen contents

Sulphate turnover. The daily turnover of sulphate in rumen fluid was predominantly determined by the relatively large amounts infused (2.2 g S/d). A minimum daily turnover of sulphate in rumen fluid was calculated by multiplying this amount of S by the specific radioactivity ratio, sulphate-S in the infusion:sulphate-S in rumen fluid. This calculation assumes that all sulphate-S that entered the rumen, other than

Table 4. *The effect of intraruminal infusion of sodium molybdate on fluid volume, turnover time, and flow-rate in the rumen of sheep*

(Mean values with their standard errors for two separate determinations for each of four sheep. Approximately 10 g sodium sulphate was infused into the rumen of each sheep daily, and where indicated 126 mg Na₂MoO₄/d was also given)

Treatment	Volume (ml)	Turnover time (h)	Flow-rate (l/d)
No molybdate infused	5536	9.6	14.6
Molybdate infused intraruminally	5042	8.8	14.4
SEM	244	0.5	1.2

the infused sulphate, was not radioactive. Since some radioactive sulphate may have passed into the rumen by way of the saliva or across the rumen wall during the experiment, the values for daily sulphate-S turnover given in Table 2 must be regarded as minimum estimates.

Of the 3.0 g S/d that passed through the sulphate pool, only about one-third was converted to sulphide in the absence of added molybdate, and this value decreased to one-sixth in the presence of molybdate (Tables 2 and 3). Very little of the sulphate which was not converted to sulphide flowed down the gastrointestinal tract with rumen fluid (Table 2). The remainder passed from the rumen by another pathway (Table 2).

Sulphide-S and microbial-protein-S turnover. After omitting the [³⁵S]sulphate from intraruminal infusions on the 8th day of the respective sampling periods, the decrease in the specific radioactivity of S in sulphide and microbial protein was followed (Fig. 1). The decrease could be adequately described by a single exponential curve in each instance. The apparent turnover time was calculated as the reciprocal of the first-order rate-constant, and the apparent daily turnover (g S/d) was derived from the following equation:

$$\text{Apparent turnover (g S/d)} = \frac{\text{pool size (g S/rumen)} \times 24}{\text{apparent turnover time (h)}}$$

Molybdate decreased the apparent turnover of sulphide from 1.17 to 0.55 g S/d (Table 3, $P < 0.01$). This effect was the result of an increased turnover time for sulphide (from 37.8 to 106.9 min) not a decreased pool size (Table 3). Molybdate did not significantly affect the amount of sulphide-S that was incorporated into microbial protein (Table 3), nor did it influence the total turnover (from 0.81 to 0.71 g S/d) and turnover time of the microbial-protein-S (Table 3).

The flow of fluid and S compounds from the rumen

The rumen fluid volumes and fluid flow-rates for each sheep and treatment are shown in Table 4. The flow of sulphide and sulphate from the rumen (Tables 2 and 3) was calculated by multiplying the fluid flow-rates by the respective concentrations in rumen fluid. For microbial protein, the flow down the gastrointestinal tract equalled the daily turnover because microbial protein is not absorbed from the rumen.

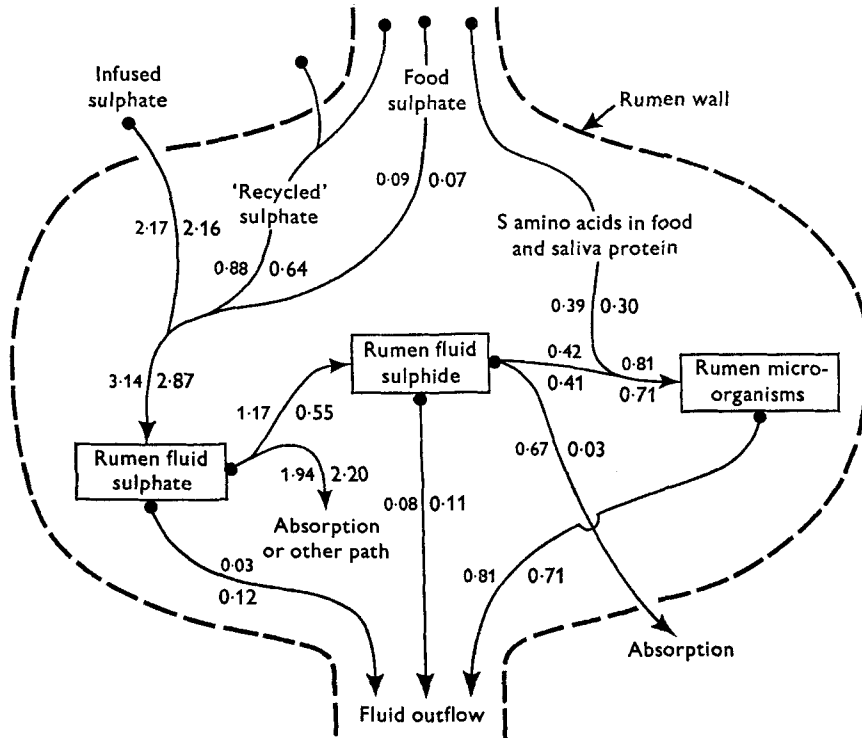


Fig. 2. Schematic diagram of the daily turnover (g sulphur/d) of sulphate, sulphide, and microbial-protein-S in the rumen of sheep intraruminally infused with 10 g sodium sulphate (values in small type)/d or with 10 g Na_2SO_4 plus 126 mg sodium molybdate/d (values in large type)/d.

Summary of S metabolism in the rumen

A schematic representation of the apparent turnover of sulphate, sulphide, and microbial-protein-S in the rumen, in the presence and absence of added molybdate is shown in Fig. 2, which is constructed from the values given in Tables 2 and 3.

DISCUSSION

Other workers have attributed the increase in sulphide concentrations found in the rumen fluid of animals given Na_2MoO_4 (50 mg Mo/d) to a stimulation of sulphide production in rumen micro-organisms (Mills, 1960; Hartmans & Bosman, 1970; Bryden & Bray, 1972). Implicit in this conclusion is the assumption that the concentration of sulphide in rumen fluid is directly related to the rate of sulphide production. Our results indicate that this relationship is not always valid. Sulphide concentrations were increased in rumen fluid *in vivo* although there was a 50% decrease in the rate of sulphide production when molybdate was infused (Table 3). The slower rate of production did not lead to a lower concentration of sulphide in this instance because it was compensated by a second action of molybdate to inhibit the rate of apparent absorption of sulphide from the rumen (Table 3 and Fig. 2).

The inhibition of sulphide production caused by molybdate *in vivo* (Table 3) is in

accord with *in vitro* results (Huisingsh & Matrone, 1972), and can confidently be ascribed to inhibition of the first stage in sulphate reduction catalysed by ATP: sulphate adenylyltransferase (Wilson & Bandurski, 1958; Robbins, 1962).

The apparent absorption of sulphide from the rumen was decreased by 20 mmol/d when only 0.52 mmol molybdate was infused daily (Table 3), suggesting that this action of molybdate was catalytic, and was not a quantitative reaction with sulphide to form a non-absorbed substance.

The reaction between soluble molybdates and H_2S in dilute aqueous solution leads to the formation of a series of thiomolybdate salts, of the type $R_2^1MoO_{4-n}S_n$, where n is 1-4 (Tridot & Bernard, 1962; Aymonino, Ranade & Muller, 1969). The incubation of a suspension of washed rumen micro-organisms in a solution containing Na_2MoO_4 and sulphate results in the production of di-, tri-, and tetrathiomolybdates (Gawthorne, unpublished results). It is therefore likely that thiomolybdates form within the rumen and it is possible that the slow dissociation of these compounds (Aymonino, Ranade, Diemann & Muller, 1969) may change the kinetics of absorption of soluble sulphide. On the other hand, molybdate or a compound formed from molybdate may inhibit the transport system for sulphide in the rumen wall. Mills, Monty, Ichihara & Pearson (1958) found that the rate of conversion of sulphide to thiosulphate in rat liver homogenates was decreased by feeding molybdate. Our results could be explained if a similar inhibition occurs in sheep to an extent that limits the rate of transport of sulphide across the rumen wall, or the clearance of sulphide from blood.

Since the apparent absorption of sulphide from the rumen was almost entirely inhibited by infusing 50 mg Mo/d, it can be predicted that larger quantities of molybdate would not cause proportionate decreases in the rate of absorption. Thus, further decreases in the rate of sulphide production caused by these amounts of molybdate would no longer be compensated by corresponding decreases in the rate of absorption, and sulphide concentrations would decrease. This decrease has been found *in vivo* when sheep are given between 300 and 1800 mg Mo/d (Bryden & Bray, 1972).

Bray (1969) found that the loss of sulphide from a buffer solution placed in the emptied rumen of sheep was exponential, with a half-time of 10.7-21.5 min. Moir (1970) calculated half-times of 20 and 35 min from the results of Anderson (1956) and Spais, Lazaridis & Agiannidis (1968) where Na_2S had been injected into actively-metabolizing rumen contents. In our experiment, the physiological half-time of sulphide in rumen fluid ($0.693 \times$ apparent turnover time; Table 3) depended on the daily intake of molybdate, which increased it from 26.2-74.1 min. This previously unrecognized effect of Mo may have contributed to the differences in sulphide half-time found by other workers.

Of the 3.0 g S/d that passed through the sulphate pool in rumen fluid, only one-third was converted to sulphide in the absence of added molybdate, and one-sixth in its presence (Tables 2 and 3). A small amount of the remaining sulphate flowed with the rumen fluid down the gastrointestinal tract (Table 2), but most of the sulphate left the rumen by another pathway. A possible pathway could be absorption across the rumen wall, but Bray (1969) has found that sulphate is not absorbed from buffer

solutions placed in the rumen. If sulphate was not absorbed from the rumen of animals in the present experiment, it either escaped analysis, or was converted to a S compound (other than sulphide) that was absorbed. Some sulphate may have escaped analysis if it was converted to sparingly-soluble sulphates, for example calcium sulphate, and remained attached to large plant particles. Because strained rumen fluid was used for sulphate analyses, the portion of the sulphate pool that moved with the solid digesta would not have been measured. However, the sulphate that was not reduced to sulphide or flowed with the rumen fluid cannot all be accounted for in this way even if the entire Ca content of the food (2 g Ca/kg food) was converted to CaSO_4 .

Only that portion of the food-protein-S incorporated into microbial protein is shown in Fig. 2. The fate of the remainder cannot be stated with certainty, but as the specific radioactivity of the rumen fluid sulphide pool was not significantly lower than that of the rumen fluid sulphate pool (Table 1) it can be concluded that very little food-protein-S was degraded to sulphide. Most of it may have passed from the rumen with the outflowing digesta.

The specific radioactivity ratio, microbial-protein-S:sulphide-S (Table 1) indicates that only 52–57% of the S in microbial protein originated from the sulphide pool (Table 3). In animals that were not infused with molybdate this occurred although there was an abundant supply of sulphide (Table 3 and Fig. 2). We interpret these results to suggest that approximately half the S amino acid content of microbial protein in rumen digesta was synthesized *de novo* from sulphide, and the remainder resulted from the direct incorporation of non-radioactive amino acids from digested plant and salivary proteins. Nader & Walker (1970) used rumen digesta from sheep given a mixture of lucerne and wheaten hay in an *in vitro* incubation, and found that up to 44% of the methionine and 6% of the cysteine in the protein of rumen micro-organisms could have arisen by direct incorporation of food amino acids. Since there is twice as much methionine as cysteine in rumen micro-organisms (Bird, 1972), this represents a mean direct incorporation of 31.3% for the S amino acids, a value that compares favourably with our results of 43–48%. Thus, the extent of direct incorporation of S amino acids by rumen micro-organisms appears to be greater than generally believed (see Moir, 1970).

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