SULPHUR METABOLISM AND EXCRETION STUDIES IN RUMINANTS

X.* SULPHIDE TOXICITY IN SHEEP

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Abstract

The effect of ruminal infusions of sulphide on feed intake, water intake, rumen motility and rumination, and of single ruminal or ileal infusions of sulphide on other physiological functions was determined.

Single ruminal or ileal infusions of sulphide (0·94 g sulphur) in solution (Na_2S–H_2S) resulted in temporary respiratory distress and collapse. Rumen motility was temporally abolished when ruminal infusions of sulphide (0·94 g sulphur) were given; smaller doses resulted in a moderate transitory depression of motility.

Continuous ruminal infusions of sulphide (2·93 g sulphur/day) solution (sodium sulphide in water) resulted in a significant decrease in dry matter intake but water intake was not greatly affected. Ruminatory activity was decreased but not in relation to the reduced feed intake. Rumen motility was also decreased, but at least partly as a result of the decreased intake.

Inappetence resulting from the excessive intake of sulphate or other sulphur-containing compounds by ruminants may be mediated via the formation of sulphide in the rumen. Intake by sheep of not more than 4 g sulphur/day, or the addition to ruminant diets of 0·2% sulphate sulphur or *S*-amino acid sulphur, should satisfy microbial and tissue sulphur requirements without adversely affecting feed intake.

I. Introduction

The significance of the intake of sulphur as a determinant of the utilization of nitrogen for ruminal microbial protein synthesis, and of the digestion of dietary organic matter, is well established (e.g. Bray and Hemsley 1969; Hume and Bird 1970; Bird 1972). Where supplements of sulphur are given in an effort to increase the supply of protein and energy to the ruminant tissues, the possibility of deleterious effects of excessive intakes of sulphur must be considered.

Elemental sulphur (Christensen *et al.* 1947) and sodium sulphate (e.g. Albert *et al.* 1956; McBarron and McInnes 1968; L'Estrange, Clarke, and McAleese 1969) have been found to decrease feed intake when given in sufficient quantity. These compounds have been used to regulate the intake of feed supplements, but toxicity symptoms have been reported only with the use of elemental sulphur (e.g. Coghlin 1944; White 1964).

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Dougherty, Mullenax, and Allison (1965) and Bray (1969) have shown that infusions of H_2S gas into the rumen caused sheep to collapse or otherwise display signs of nervous excitability. In other mammals sulphide may be inhaled, ingested, or absorbed through the skin in sufficient quantities to cause toxicity. The toxic effect is exerted primarily through paralysis of the nervous system (see review by Evans 1967). The evolution of H_2S gas from sewers and marshes, due to the activity of dissimilatory sulphur-reducing bacteria (see Roy and Trudinger 1970) has occasionally caused human fatalities. Inhalation of air containing 20 p.p.m. H_2S is tolerated but 1000 p.p.m. is rapidly fatal (Evans 1967). Sulphide toxicity has, however, apparently not been associated with the feeding to ruminants of supplemental sulphate (e.g. Boyazoglu, Jordan, and Meade 1967) or with high levels of sulphates in drinking waters (Peirce 1960).

During an investigation into the fate of sulphate infused ruminally or duodenally (Bird and Moir 1971) sheep receiving a continuous ruminal infusion of 5–6 g/day of sulphate sulphur eventually refused to eat or drink. Details of these occurrences are given in the present paper. Since duodenal infusions of sulphate did not affect intake, and in view of the presence of dissimilatory sulphate-reducing bacteria in the rumen (Peck 1962) and their ability to rapidly adapt to sulphate infusions (e.g. Lewis 1954; Bird and Hume 1971; Bird and Moir 1971), the adverse effects of sulphate infusion noted by Bird and Moir (1971) were attributed to sulphide toxicity.

In the experiments described in the present paper the consequences and probable mechanisms of sulphide action in the ruminant have been further examined. Intakes of sulphur sufficient to satisfy the requirements of the ruminant system without adversely affecting feed intake are suggested.

II. Methods

(*a*) *Experiment 1*

Four sheep were given continuous duodenal or ruminal infusions of from 0 to 6 g sulphur (as sodium sulphate) daily, as described by Bird and Moir (1971). The basal diet contained 80·4% oaten chaff, 12·5% starch, 3·7% casein, 1·4% urea, and 2·0% minerals and it supplied from 0·7 to 1·1 g sulphur/day.

The effect of route of infusion and amount of sulphate sulphur infused on dry matter intake and water intake was recorded. Concentrations of sulphide in rumen liquor were also measured (see Bird and Moir 1971).

(*b*) *Experiment 2*

Single infusions of sulphide were given, at varying time intervals, to two sheep (A and B) and the effect on respiration, rumen movement, and general behaviour observed. Hydrogen sulphide was obtained after acidification with 6N HCl of an aqueous solution of sodium sulphide contained in a syringe. The acid was introduced into the sulphide solution via a three-way tap and the gas plus liquid contents of the syringe immediately injected into the rumen via a ruminal cannula. The amounts of sulphide infused (as H_2S–Na_2S solution) were 0·94, 0·47, or 0·24 g.

Rumen motility was recorded in sheep B by means of a kymograph and a weighted balloon partly filled with water and suspended in the ventral sac of the rumen from the rumen cannula.

(*c*) *Experiment 3*

Continuous infusions of sodium sulphide in aqueous solution were given ruminally to two sheep (07 and 08) over a period of 8 days. Each day 2·93 g S^{2-} was infused in 800 ml of solution.

The sulphide content of the infused solution was determined by the method of Bird and Fountain (1970), 12 hr after mixing and commencing the daily infusion. Possible losses of S^{2-} due to oxidation by the air could then be assessed in determining the actual amount of sulphide infused.

The intakes of feed and water were recorded daily during the period of infusion and were compared with intakes recorded over 5-day control periods prior to and following the infusion period. The diet given in experiments 2–5 consisted of oaten hulls (97%) and a sulphur-free mineral mix (3%) and contained 0·03% sulphur.

Ruminal movements were recorded continuously as tracings on paper fed from spools. Each day the tracing was divided into segments representing an arbitrary classification of five differing amplitudes of contraction. Thus, maximal amplitude of contraction was found during rumination and greatly diminished amplitudes during eating. The results were recorded as the percentage of total time occupied by each degree of motility. In this way a semiquantitative comparison of ruminal motility before, during, and after the infusion was obtained. Since the effect of feed intake *per se* rather than the direct effect of hydrogen sulphide on the nervous system may determine the ruminal motility in this experiment, the sheep were later given amounts of feed from 1000 to 200 g/day over intervals each of 5 days and the rumen motility was recorded during the last 2 days of each period. A comparison could then be made with the experiment in which sulphide infusions produced a decline in intake.

Rumination and other jaw movements were recorded by the metering device described by Peirce and Moir (1964). The apparatus was fitted to the sheep several days prior to the commencement of the experiment.

Samples of rumen fluid were obtained via the ruminal cannula by the method described by Bird (1972) and the sulphide concentration determined as described by Bird and Fountain (1970).

(*d*) *Experiment 4*

Sulphide (3·2 g S^{2-}) in aqueous solution was infused into the rumen of two sheep (07 and 08) over a 4-hr period on each of two consecutive days. The intake of feed was recorded on these days and compared with that for a period of 4 days prior to and 4 days after the infusion period.

(*e*) *Experiment 5*

Hydrogen sulphide was infused into the ileum of one sheep (012) in single doses. The sheep was fitted with a re-entrant cannula in the distal ileum (Thornton *et al.* 1970) and the infusion was made into the proximal cannula which was subsequently blocked for the duration of the test. The effect of the infusions on respiration and other behaviour were noted.

(*f*) *Experiment 6*

Sulphide (3 g S^{2-}/day) in aqueous solution was continuously infused into the distal ileum of sheep 012, over a period of 4 days, and the effect of the infusion on dry matter intake and water intake was compared with that for a control period before, and a recovery period after, the infusion.

III. Results

(*a*) *Experiment 1*

In the first period a sheep receiving a ruminal infusion of 6 g sulphate sulphur per day refused to eat or drink after 9 days of the infusion. Appetite and water intake were restored soon after the infusion was stopped. Similar effects were observed in the second period with another sheep receiving the same amount of sulphate, and in a following period when a third animal succumbed when only 5 g of sulphate sulphur per day was infused. These responses have been summarized in Table 1. The affected animals appeared drowsy and did not react to handling or general disturbance in the manner of the other sheep. Rumen stasis was suggested

by the apparent impaction and unusually unpleasant odour of the digesta. Elevated concentrations of sulphide in the rumen (Table 1) corresponded with the onset of the symptoms. Ruminal infusions of 4 g sulphate sulphur per day, or less, did not adversely affect the sheep, nor did duodenal infusions of up to 6 g sulphate sulphur per day.

TABLE 1

EFFECT OF CONTINUOUS RUMINAL INFUSION OF SODIUM SULPHATE SOLUTIONS ON THE INTAKE OF FEED AND WATER, AND INDUCTION OF TOXICITY SYMPTOMS

Parameter	Sheep 2	Sheep 1	Sheep 4	Sheep 3
Sulphate sulphur infused (g/day)	6	6	5	4
Initial dry matter intake (g/day)	800	800	800	800
Prior rumen sulphide level (μg/ml)*	0·2	0·4	0·6	0·2
Toxicity induction time (days)	9	3	4	0
Maximum rumen sulphide level (μg/ml)*	18	6	11	11
Minimum dry matter intake (g/day)	0	0	253	800
Minimum voluntary water intake (g/day)	0	0	—	—
Toxicity remission time (days)†	1	1–2	1–2	—
Reestablished infusion level (g sulphur/day)	—	5	4	—

* Samples taken 4 hr after feed presentation.

† After stopping the infusion of sodium sulphate solution.

(*b*) *Experiment 2*

There was no obvious response by sheep A within 45 min after infusing a single dose of H_2S–Na_2S solution (0·94 g S^{2-}) into the rumen. The dose was repeated and after 1 min muscular twitching was observed and the sheep collapsed suddenly, staring vacantly. Five minutes later the sheep stood up and displayed no further sign of the treatment.

A third infusion (0·94 g S^{2-}) was given 45 min after the second infusion and within 2 min respiratory distress was evident. Respiration was at first rapid and shallow then became laboured. The tongue lolled out and violent movements of the diaphragm or abdomen were seen. The sheep collapsed and remained comatose for 1 hr before reviving. No free sulphides were detected in a sample of jugular blood obtained immediately upon collapse of the animal. Five hours later the sheep stood, but unsteadily. The concentration of sulphide in the rumen fluid, 20 μg/ml, was still high at the time. A day later the animal commenced eating and was apparently completely recovered. However, it was soon observed that the sulphide infusions had resulted in blindness, which was not alleviated even after 4 weeks.

With sheep B, as with sheep A, no response to 0·94 g S^{2-} was observed 45 min after the initial infusion. A second infusion produced only a cessation in eating, but a third infusion 45 min later induced, within 3 min, violent flank movements and muscular trembling. These symptoms were transitory and no others were evident. Three hours later the concentration of sulphide in the rumen fluid was 5·6 μg/ml. The sheep resumed eating and consumed 1 kg of its ration during the next 5 hr.

On the second day, 6 min after the ruminal infusion of 0·94 g S^{2-}, ruminal movements were completely abolished [Fig. 1(*a*)]. In this time hydrogen sulphide was

detected on the breath and the sheep was agitated and continually stumbled. The respiratory rate and heart rate were moderately elevated initially then returned to

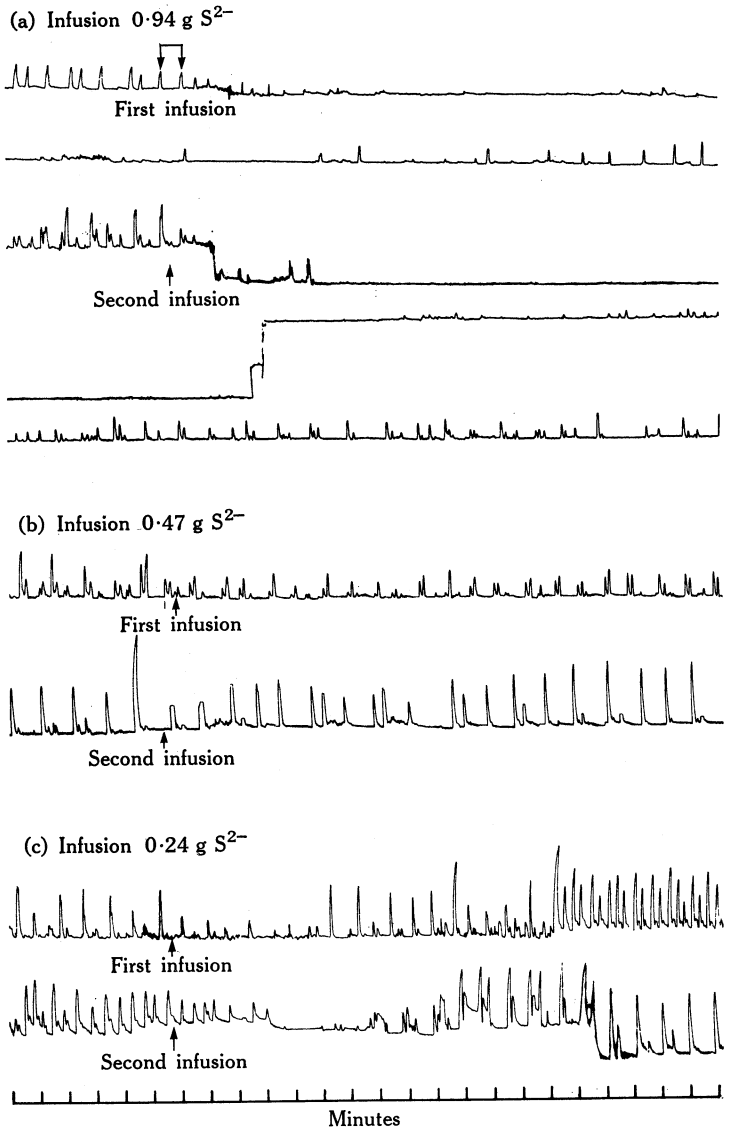

Fig. 1.—Effect of single ruminal infusions of sulphide from acidified sodium sulphide solution on rumen motility. (*a*) Infusions of 0·94 g S^{2-} given 150 min apart. Each trace is continuous. (*b*) Infusions of 0·47 g S^{2-} given 75 min apart, the first 6 hr after the second infusion of 0·94 g S^{2-}. (*c*) Infusion of 0·24 g S^{2-} given 34 min apart, on the following day. Trace speed was 1 cm/min.

normal. Ruminal motility was partially regained 20 min after the infusion and fully recovered after a further 25 min. The animal was seen to eat and later to ruminate.

A second infusion of 0·94 g S^{2-} made 2½ hr after the first infusion caused a collapse within 2 min. After a further 1½ min respiration became laboured for 5 min, and then became shallow and difficult to detect. The heart rate was slow but irregular. The sheep rose from the prone position 37 min after the infusion was given. The occasions when the sheep collapsed then regained its feet may be seen from changes in the base line in the second trace of Figure 1(*a*).

Six hours after the second infusion a single infusion of 0·47 g S^{2-} was given and although hydrogen sulphide was detected on the breath and for several minutes there was a moderate decrease in ruminal motility [Fig. 1(*b*)], accompanied by spasmodic intervals of heavy breathing, no other distress symptoms were noted. Repetition of the dose, after a further 1¼ hr, gave a similar result [Fig. 1(*b*)]. On the following day, infusions of 0·24 g S^{2-} also produced a moderate effect on ruminal motility [Fig. 1(*c*)].

TABLE 2

EFFECT OF CONTINUOUS INTRA RUMINAL INFUSION OF SODIUM SULPHIDE SOLUTION ON THE INTAKE OF FEED AND WATER, RUMINATION, AND RUMEN MOTILITY

Values given represent means ± standard error for each period. Significant differences between successive periods means are indicated by similar superscripts: ** $P < 0·01$

Parameter	Sheep 07			Sheep 08		
	Days 1–5	Days 6–13	Days 14–18	Days 1–5	Days 6–13	Days 14–18
Sulphide infused (g/day)	0	2·93	0	0	2·93	0
Dry matter intake (g/day)	1062**	840**	943	1165**	533**	990
	±36	±28	±41	±18	±162	±167
Water intake (g/day)†	1614	1774	1882	1664	1380	1768
	±171	±34	±202	±206	±242	±296
Number of chews/day	54,735	48,608	48,220	73,923**	33,320**	61,632
	±2,850	±2,268	±2,623	±2,494	±7,599	±10,056
Number of chews per gram	53·6	58·1	51·3	64·8	73·5	67·2
	±0·44	±2·45	±2·78	±2·58	±12·77	±9·30
Rumen fluid sulphide (μg/ml)‡	2·3**	27·2**	1·9**	0·3**	11·3**	1·6**
	±1·91	±5·69	±1·70	±0·30	±2·08	±0·20
Rumen fluid pH‡	6·14	6·35	6·41	6·30	6·69	6·95
	±0·02	±0·05	±0·04		±0·10	±0·40
Rumen motility scale§						
1	0	6	0	0	27	21
2	0	60	7	0	29	51
3	15	22	43	0	15	16
4	65	12	7	38	10	7
5	20	0	3	62	19	5

† Including water infused into the rumen over days 6–13.

§ Arbitrary scale 1–5 represents minimal contractility through to maximal contractility (see text). Values given are estimated percentage of total time occupied by each activity.

‡ Sampled 4 hr after feed presentation.

(*c*) *Experiment 3*

There was a significant decrease ($P < 0·01$) in the intake of dry matter due to the continuous ruminal infusion of sulphide solution (2·93 g S^{2-} /day). The mean values ± standard error are recorded in Table 2. With sheep 07, the dry matter

intake did not fall below 740 g/day when sulphide was infused, but after the third day of infusion the intake of sheep 08 varied from 0 to 464 g/day. The intake of feed during the recovery period was also greater than during the infusion period. The intake of water was not significantly affected by infusions of sulphide. In general, ruminatory activity was great, irrespective of treatment. The greatest number of jaw movements recorded in a day was 57,330 for sheep 07 and 93,400 for sheep 08. The decline in rumination during the infusion period with sheep 08 ($P < 0{\cdot}01$) could be attributed to a decline in intake, since between periods there were no differences in the number of chews per gram of dry matter eaten.

Decreased rumen motility was associated with the infusion of sulphide into the rumen. The normal motility was regained in sheep 07 during the recovery period but not with sheep 08. Decreased ruminal movements were, however, also caused by a decreased feed intake, as shown in Table 3. Contractions of greater amplitude were

TABLE 3

EFFECT OF FEED INTAKE ON RUMINAL MOTILITY

Rumen motility scale*	Sheep 07: dry matter intake (g/day)			Sheep 08: dry matter intake (g/day)		
	1000	600	200	1000	600	200
1	0	22	19	0	8	4
2	40	20	55	0	21	59
3	55	36	26	8	43	37
4	5	14	0	41	23	0
5	0	8	0	51	5	0

* See Table 2 for units used.

recorded at higher intakes. Comparing the results in Table 2 with those in Table 3 it appears that a decreased intake *per se* was not entirely responsible for the decreased ruminal motility in sheep 07, but may have been in sheep 08.

(*d*) *Experiment 4*

The intake of dry matter by sheep 07 was decreased from a mean of 922 g/day for the 4 days prior to the infusion to 280 and 160 g/day, respectively, for the first and second days of the infusion of 3·2 g S^{2-} into the rumen over a 4-hourly period. The mean feed intake during the recovery period was 1067 g/day. The dry matter intake for sheep 08 was 1141 g/day prior to the infusion, 112 and 0 g/day during the infusions, and 1300 g/day after the infusions.

(*e*) *Experiment 5*

A single infusion of H_2S–Na_2S (0·94 g S^{2-}) into the ileum of sheep 012 had no effect within 10 min of dosing, thereupon a further 0·47 g S^{2-} was infused. The sheep immediately began to sway and stagger, but did not fall; marked increases in the movements of the diaphragm were noted; the animal became increasingly

agitated and there was an increased respiratory rate. No hydrogen sulphide was detected on the breath. After 15 min the sheep lay down, with its hind legs outstretched, and appeared to suffer some abdominal discomfort. Evidence of considerable mucosal shedding was found the next day when removing a blockage from the ileal cannula, but the animal had fully regained appetite within 2 days.

(*f*) *Experiment 6*

The continuous infusion of sulphide into the ileum at the rate of 3 g/day over 4 days produced a significant decrease in the intake of dry matter ($P < 0{\cdot}001$) and of water ($P < 0{\cdot}01$) compared with the values from the preliminary period (Table 4). Little water and no food was consumed during the first day of the recovery period but full appetite was regained after 4 days.

TABLE 4

EFFECT OF CONTINUOUS ILEAL INFUSIONS OF SODIUM SULPHIDE SOLUTION ON THE INTAKE OF FEED AND WATER BY SHEEP 012

Values are means ± standard error. Significant differences between successive treatments are indicated by similar superscripts: *** $P < 0{\cdot}001$: ** $P < 0{\cdot}01$

Parameter	Days 1–8	Days 9–12	Days 13–19
Sulphide infused (g/day)	0	3·0	0
Dry matter intake (g/day)	1781 (±38)***	203 (±239)***	1228 (±281)
Water intake (g/day)†	3873 (±370)**	1260 (±304)**	3377 (±704)

† Including water infused into the ileum over days 9–12.

IV. DISCUSSION

The changes in respiration and heart rate observed in sheep when hydrogen sulphide was infused ruminally are consistent with those found in non-ruminant species by Evans (1967) and others. The results from experiment 1 also show that ruminal infusions of sulphide were able to completely inhibit ruminal movements, during which time the animal exhibited marked excitement and activity of the peripheral musculature suggestive of sympathetic stimulation. The effect of sulphide on the nervous system was not immediately reversible, in that with both sheep there appeared to be an accumulative effect of hydrogen sulphide infusions over time. Initially 0·94 g S^{2-} failed to affect respiration but after subsequent infusions respiratory distress resulted. Ruminal movements were eventually inhibited by the infusion of as little as 0·24 g S^{2-}. The resultant blindness in one sheep further indicates that exposure to sulphide may irreversibly affect the nervous system.

When sulphide was infused slowly over several days (exp. 2), however, it was not clear whether the observed decline in ruminal motility was caused directly by sulphide or indirectly through a decline in feed intake. The decreased intake associated with ruminal infusions of sulphide (expts. 2 and 3) may have been caused by a retarded rate of rumen emptying associated with poor ruminal contractions, by a direct effect of sulphide ion on the hypothalamus, or even a local effect of hydrogen sulphide on the nervous tissue within the rumen walls. There was some increase in ruminal pH

associated with the infusion of sodium sulphide in aqueous solution during experiments 2 and 3, but high ruminal pH (as induced by HCO_3^-) does not necessarily interfere with ruminal motility or feed intake by sheep (Grosskopf and Briel 1966). It is possible that high concentrations of sulphide within the rumen may depress the fermentative activity of the microorganisms, decreasing the rate of digestion of roughage and therefore the rate of intake. Hubbert, Cheng, and Burroughs (1958) reported that cellulose digestion by ruminal microorganisms *in vitro* was depressed slightly by 1000 μg sulphate sulphur per millilitre of medium. Kennedy, Mitchell, and Little (1971), however, found no depression of starch digestion by ruminal microorganisms *in vitro* when sulphur up to 11,000 μg/ml was added (as sodium sulphate) and no diminution of growth *in vitro* by ruminal microbes was observed in our own laboratory when sulphide levels up to 1500 μg/ml were used. The absorption of sulphide from the rumen is a rapid process (Bray 1969) and high concentrations are not maintained (Lewis 1954; Anderson 1956; Spais *et al.* 1969; Bird 1972); therefore it is unlikely that sulphide *in vivo* would substantially affect the activity of the ruminal organisms. Another adverse effect on appetite mediated through the excessive ingestion of sulphate, in conjunction with moderate or excessive intakes of molybdenum, is the induction of a copper deficiency (Dick 1954; Marcilese *et al.* 1969). As with the possible effect of sulphide on microbial activity, this effect would not account for the immediate depression of intake observed when sulphide is infused.

In the ruminant the toxic effect of sulphide on the nervous system is apparently mediated via eructation of hydrogen sulphide, with other gases, from the rumen and absorption through the lungs. Most of the eructated ruminal gases enter the lungs and infusion of hydrogen sulphide into the rumen of sheep with blocked trachea produced no symptoms, whereas sheep with open trachea collapsed after several eructations (Dougherty, Mullenax, and Allison 1965). Sulphide absorbed from the rumen may be detoxified by oxygenated haemoglobin in the blood; *in vivo* the reduction of oxyhaemoglobin is reversible (Evans 1967). Plasma itself does not oxidize HS^-. In cats, at least, the rate of sulphide oxidation is slow, presumably due to the time taken for HS^- to penetrate the red cells (Evans 1967). From Evan's data, whole blood from a cat could oxidize hydrogen sulphide at the rate of between 20 and 40 μg S^{2-}/ml/min. Assuming that this rate is applicable to sheep, that 3 g S^{2-} is daily absorbed directly into the portal vein (2·08 mg/min) and that 20% of a cardiac output of 4 litres/min flows through the liver, then *c.* 24 mg S^{2-}/min might be detoxified, or roughly 10 times the rate of sulphide absorption. The liver itself, through the action of a sulphide oxidase system, is also clearly capable of detoxifying sulphide (see Anderson 1956), hence it is unlikely that much free sulphide would reach the brain after being absorbed from the rumen into the portal system. The direct and shorter route to the heart and brain afforded by the inspiration of hydrogen sulphide and transfer into the pulmonary vein effectively by-passes the liver and enables hydrogen sulphide to exert its toxic effect on the respiratory–circulatory systems. Evans (1967) concluded that spinal vasomotor centres were affected in the same manner by hydrogen sulphide as were medullary centres. The finding in the present experiment that infusions of sulphide into the ileum produced responses similar to that seen with ruminal infusions may indicate a similar effect. Altern-

atively, that experiment may have indicated that the capacity of the liver and blood to detoxify absorbed sulphide was inadequate and that hydrogen sulphide did reach the chemoreceptors in the circulatory system.

In the experiment described by Bird and Moir (1971) where sulphate was infused into the rumen at a constant rate the concentration of sulphate in the duodenal digesta was substantially elevated within a day or so but these levels declined with the increasing conversion of sulphate to sulphide within the rumen. Lewis (1954) first showed that the ability of the ruminal microbes to reduce sulphate depended upon a period of adaptation. The data from Bird and Hume (1971) shows that after 9 days of supplementing the ration with sulphate (1 g sulphur/day) 3 of 4 sheep degraded almost all of the added sulphur to sulphide, some of which was incorporated into microbial protein. Little, if any, sulphate is absorbed directly from the rumen (Bray 1969). Thus, while differences between sheep may exist, in general after adaptation one may equate the rate of infusion of sulphate sulphur with the rate of sulphide production. In the experiment of Bird and Moir (1971) the maximum rate of sulphide production could have been up to 6 g S^{2-}/day, or double the rate of sulphide infused in the current experiments. Similar effects on feed intake were found in both cases.

One would expect differences to arise when comparing the consequences of infusing sulphate or giving it in the diet. A continuous infusion would favour the build-up and maintenance of the dissimilatory sulphate-reducing bacteria in the rumen. A decreased intake of feed would, presumably, not substantially affect these organisms, since sulphate is used as the terminal electron acceptor in anaerobic metabolism. The continued production of hydrogen sulphide, in conjunction with an energy-limited sulphide utilization potential and perhaps decreased rate of ruminal emptying, would perpetuate and aggravate the toxic effect of the sulphate infusion. Where sulphate is ingested, the cycle is broken by the refusal of feed and the symptoms may be unrecognised. Excess intake of sulphate or cyst(e)ine, both of which are rapidly converted to hydrogen sulphide (Bird and Hume 1971; Bird 1972) could, therefore, exert an effect, perhaps marginal, on intake. Alhassan and Satter (1968) found that the daily infusion of from 60 to 202 g of sodium sulphite progressively decreased the feed intake of cows to zero. Since sulphite is an intermediate in the reduction of sulphate to sulphide (Henderickx 1961) it is possible that the production of excessive amounts of hydrogen sulphide within the rumen was responsible for the adverse effect of sulphite on intake. Sulphates have long been used to prevent excess intake of feed supplements and have been found to cause reduced weight gains when included in the rations of growing lambs (Albert *et al.* 1956). In that experiment additions of sodium sulphate up to 1·76% of the diet improved gains, but further increases reduced feed intake and growth rates. The inclusion of sodium sulphate or sulphuric acid in the diet (sulphur = 1% of dry matter) of mature sheep has also been shown to markedly reduce feed intake (L'Estrange, Clarke, and McAleese 1969).

As with the feeding of urea (e.g. McBarron and McInnes 1968) it is suggested that the consumption of excessive amounts of sulphate may lead to toxicity, although the outcome may result only in an impaired intake. This effect would be manifested only after adaptation by the ruminal microorganisms to the ingested sulphate and

may be more apparent where conditions within the rumen (e.g. energy or nitrogen limitation) do not favour the utilization of the released H_2S for microbial growth. It is suggested that the daily intake by sheep of sulphur, in any form, should not exceed 4 g, which is more than required for optimal ruminal protein synthesis (Hume and Bird 1970) but less than is likely to reduce feed intake. Alternatively, a concentration of added sulphate sulphur, cystine sulphur, or methionine sulphur in the diet of 0·2% of the dry matter should permit efficient usage of dietary energy and nitrogen for growth and production by sheep (e.g. Starks *et al.* 1954; Whiting *et al.* 1954; Albert *et al.* 1956; Rendig and Weir 1957; Hume and Bird 1970) and by cattle (Jones, Haag, and Weswig 1952; Jacobson *et al.* 1967) without reducing feed intake.

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