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Transfer of Sulphur to the Digestive Tract of Sheep

P. M. Kennedy,[A] *J. P. Hogan*,[B] *J. R. Lindsay*[B] *and R. M. Hogan*[B]

[A] Division of Animal Production, CSIRO, Davies Laboratory, Townsville, Qld 4810.
Present address: Department of Animal Science,
University of Alberta, Edmonton, Alberta, Canada T6G 2E3.
[B] Division of Animal Production, CSIRO, Ian Clunies Ross Animal Research Laboratory,
P.O. Box 239, Blacktown, N.S.W. 2148.

Abstract

The transfer of sulphate from plasma to digestive tract and from digestive tract to plasma in cross-bred sheep was estimated by the use of isotope dilution techniques with $Na_2{}^{35}SO_4$. The passage of ^{35}S along the digestive tract was simultaneously measured by reference to two inert radioactive markers infused intraruminally.

In the first experiment, three sheep given a roughage-based diet containing 174 ± 7 mg S/day received an intravenous infusion of $Na_2{}^{35}SO_4$ for 7 days before collections were made of plasma and of digesta from the rumen, abomasum and terminal ileum. Similar collections were made in the second experiment in which four sheep received intraruminal infusions of $Na_2{}^{35}SO_4$. From estimates of infusion rate of ^{35}S, specific radioactivity of ^{35}S in plasma and digesta and rate of flow of sulphur in the digestive tract the following calculations were made:

The transfer of sulphate from the plasma to the rumen was calculated as 29 mg S/day. Of this only 12 mg S/day passed as organic sulphur in digesta from the stomach. As the net gain of sulphur in the stomach in this experiment was 153 mg/day, sulphate transferred from the plasma contributed only a small amount of sulphur derived from endogenous sources in the stomach.

In contrast, the substantial passage of ^{35}S into the intestinal lumen during intravenous infusion of $^{35}SO_4$ suggested that 38 and 41 mg S/day of the 236 and 145 mg organic S/day flowing from the small and large intestine respectively was derived from plasma sulphate, corresponding to about 26% of the dose.

Introduction

The rate of synthesis of microbial protein in the rumen of sheep fed low sulphur diets may be increased by supplementation with inorganic sulphate (Hume and Bird 1970; Bird 1972*b*), with consequent improvement in the retention of nitrogen and energy in the body. Endogenous sulphate recycled from the blood to the rumen is undoubtedly also used in this manner. In fact, sulphate may be the only form of recycled sulphur to give a net increase in protein passing from the rumen, as recycled organic sulphur which is largely present in protein cannot support the synthesis of additional protein (Bray and Hemsley 1969).

The available estimates suggest that the transfer of sulphate to the ovine rumen is small (Bray 1969*a*, 1969*c*; Kennedy *et al.* 1975) in contrast to the substantial net influx of sulphur, as assessed by measurement of sulphur flow from the omasum (Hume and Bird 1970; Bird 1972*b*). However, simultaneous estimates of the influx of organic sulphur and sulphate are not available.

Although only a small amount of ^{35}S returned to the ovine rumen after intravenous injection with [^{35}S]sulphate, the appearance of approximately 31% of the dose in the faeces (Kennedy *et al.* 1975) suggested that a major route of excretion of ^{35}S from the

blood sulphate pool was via the post-ruminal tract. Sulphate recycling could thus be analogous to the recycling and degradation of blood urea which, in the sheep, appears to occur mainly in the post-ruminal tract (Nolan and Leng 1972). The passage of both urea and sulphate into the post-ruminal tract may seriously reduce the amounts available for recycling to the rumen and incorporation into microbial organic matter.

The present experiments were conducted to estimate the input of sulphate and organic sulphur into, and the net flow of sulphur from, the stomach of sheep fed a low sulphur diet. In addition, data on the net flow of sulphur from the small and large intestines were obtained to provide information on the post-ruminal secretion of endogenous sulphur.

Materials and Methods

Sheep and Diet

Seven crossbred wethers [Dorset ♂ × (Border Leicester × Merino)♀] weighing about 35 kg were used. All were fitted with permanent cannulae in the rumen and abomasum, and three were also fitted with a cannula in the ileum, about 15 cm from the ileocaecal junction. The sheep were maintained in single pens indoors and were dosed with vitamins A and D_3, and with thiabendazole to control helminths.

The diet was similar to the basal diet of Bird (1972*b*) and consisted of oat hulls (83%), starch (6%), sucrose (6%), minerals (3%), and urea (2%). The mineral mixture was that described by Hume and Bird (1970). The diet contained 11·8 g nitrogen, 0·25 g sulphur and 913 g organic matter per kilogram dry matter. Sodium sulphate was added in solution to the feed of all sheep to provide 50 mg additional sulphur daily; the ration was offered in equal 80 g portions each 3 h by means of interval feeders. Food residues were collected daily and bulked before subsequent analysis.

Experimental Design

Experiment 1 was designed to measure the passage from the abomasum, ileum and in faeces of ^{35}S infused as $Na_2{}^{35}SO_4$ into the jugular vein, in order to estimate the net entry of sulphate from plasma into the stomach (reticulorumen, omasum, and abomasum), small intestine, and large intestine. Estimates of transfer of plasma sulphate into the rumen were also made. Three sheep were offered the experimental diet for 7 days prior to continuous infusion of $Na_2{}^{35}SO_4$ (carrier free) at a constant rate of 211 μCi/day into the jugular vein. Two days after the start of the intravenous infusion a continuous ruminal infusion of two radioactive markers, radioactive chromium complexed with EDTA (^{51}Cr–EDTA) (Downes and McDonald 1964) and ^{103}Ru-labelled tris-(1,10 phenanthroline)-ruthenium (II) chloride (^{103}Ru–P) (Tan *et al.* 1971) was commenced. After 7 days of intravenous infusion, collections were commenced of plasma and of digesta samples from the rumen, abomasum and terminal ileum; these continued twice daily from the 14th to the 16th day.

Experiment 2 was designed to provide information on the irreversible loss of sulphate from the rumen pool, in order to estimate transfer of sulphate from the blood to the rumen. Four sheep with rumen and abomasal cannulae were offered the diet for 10 days prior to the start of intraruminal infusion of $Na_2{}^{35}SO_4$ (45 μCi/day) together with the two radioactive digesta markers. Samples of ruminal and abomasal digesta were collected three times daily from the 4th to the 6th day of the continuous infusions.

Sample Collection and Analyses

Samples of abomasal and ileal digesta were analysed for ^{51}Cr, ^{103}Ru, organic matter and nitrogen (Hogan 1973). Rumen liquor was collected through a gauze-covered tube four times daily during the sampling period (days 14–16) and treated with H_2O_2 as described by Kennedy *et al.* (1975) to oxidize sulphide to sulphate. Total sulphur, sulphate and ^{35}S were analysed by the methods of Bird and Fountain (1970). Organic sulphur was calculated as the difference between total sulphur and sulphate. Bacterial samples were obtained from each sheep on day 16 (expt 1) and separated from other particulate material by differential centrifugation (Meyer *et al.* 1967). Bacterial samples were freeze-dried and analysed for sulphur and ^{35}S. Jugular blood was sampled six times per sheep during the sampling period, with potassium oxalate used as anticoagulant, centrifuged and the

plasma analysed for total sulphate. Sulphate bound to plasma protein was not separated from inorganic sulphate before analysis, since preliminary analysis using trichloroacetic acid to precipitate plasma protein showed that the specific radioactivity (SR) of the two fractions was similar.

Measurements of Digesta Flow, Irreversible Loss of Plasma and Rumen Sulphate, and Transfer of Plasma Sulphate to the Digestive Tract

The movement of digesta through the abomasum and terminal ileum was estimated by reference to ^{51}Cr–EDTA and ^{103}Ru–P as described by Hogan (1973).

The rate of irreversible loss (IL) of sulphate from the plasma and rumen was calculated as

$$\frac{\text{infusion rate of } {}^{35}SO_4 \text{ (mCi/day)}}{\text{SR of sulphate (mCi/g S)}}.$$

The value so derived for the rumen 'sulphate' pool does not truly represent a flow of sulphur since several pools (sulphide, inorganic sulphate, ester sulphate) were included in the analysis. However, this would not affect estimates of transfer of sulphate from the plasma to the rumen.

The proportion of sulphur in a secondary pool derived from sulphate in a primary pool (either in plasma or rumen) was calculated as the ratio of the SR of sulphur in the secondary pool to that of sulphate sulphur in the primary pool.

Transfer of sulphate from the plasma to the rumen was calculated as

$$\frac{a \times \text{IL rumen sulphate}}{1-(a \times b)},$$

where a and b are the proportions of rumen sulphate derived from plasma sulphate, and of plasma sulphate derived from rumen sulphate respectively (cf. Nolan *et al.* 1976).

The flow of ^{35}S in organic sulphur in digesta (expt 1) was expressed as the indicated flow of plasma sulphate sulphur (mg S/day) into the organic sulphur pool, calculated as the product of the flow of organic sulphur and the proportion of organic sulphur derived from plasma sulphate.

Results

Movement of Digesta

In experiment 1, the flow of digesta was 6·62 (±0·61) kg/day from the abomasum, 3·77 (±0·38) kg/day from the ileum, and faecal output was 499 (±18) g/day.

Digestion of Organic Matter and Nitrogen

Wide variations between animals were observed both in total food intake and in the degree of selection of oat hulls compared with other dietary components. This is reflected in the variability in the passage of organic matter from the stomach and intestines (Table 1). About 44% of dietary organic matter was apparently digested in the whole tract, 40% of the digestion occurring in the stomach.

The digestibility of nitrogen was less variable. The nitrogen that left the stomach in forms other than ammonia was 16–17% less than nitrogen intake; net losses in the intestines were about 47% of the amounts that left the stomach, most of the loss occurring in the small intestine. The amounts of ammonia in digesta were 0·4 g N/day leaving the abomasum, 0·3 g leaving the ileum and 0·2 g in the faeces.

Movement of Sulphur through the Digestive Tract

The endogenous transfer of sulphur to the stomach resulted in a daily net gain of 153 mg S in experiment 1 (Table 1), equivalent to about 85% of sulphur ingested in the diet. Total sulphur passing to the small intestine from the stomach comprised

bacterial sulphur (189±63 mg S/day, equivalent to 58% of total leaving stomach), small amounts of sulphate sulphur (1–2%) and an appreciable amount of non-bacterial organic sulphur which was equivalent to 81% of intake.

Table 1. Amounts of organic matter, nitrogen and sulphur (means ± s.e.m.) passing through the stomach and intestine of three sheep offered a semipurified diet

	Organic matter	Nitrogen[A]	Total sulphur	Sulphate sulphur
Intake (g/day)	470±19	6·07±0·25	0·174±0·007	0·050
Leaving stomach (g/day)	379±37	5·06±0·41	0·327±0·060	0·006±0·001
Leaving ileum (g/day)	321±36	3·08±0·28	0·246±0·018	0·010±0·002
Faecal output (g/day)	262±41	2·69±0·27	0·151±0·009	0·006±0·002
Apparent digestibility				
In whole tract				
(g/100 g intake)	44·3±6·7	55·7±3·6	13·6±5·5	
In stomach				
(g/100 g intake)	19·5±4·6	16·4±8·1	−87·3±33·5	
(g/100 g digested in tract)	43·6±4·5	30·1±16·7	−685±115	
In small intestine (g/100 g leaving stomach)	14·7±13·3	39·1±3·3	22·9±15·5	
In small and large intestines (g/100 g leaving stomach)	31·0±4·8	46·5±9·7	52·6±10·5	

[A] Nitrogen in forms other than ammonia.

The apparent digestion of sulphur in the intestines amounted to 176 mg S/day, representing about 50 g S per 100 g S entering the small intestine. Net losses of approximately equal amounts of sulphur occurred from the small and the large intestines.

Table 2. Specific radioactivity of plasma sulphate sulphur, rumen sulphate sulphur and organic sulphur in digesta of sheep during a continuous intravenous infusion of $Na_2{}^{35}SO_4$

Calculations of the proportions of organic sulphur in digesta derived from plasma sulphate are given

Form of sulphur	SR (mCi/g S)	SR in pool/ SR in plasma sulphate-S[A]	Sulphur flow through pool (mg S/day)	Indicated flow of plasma sulphate-S through pool (mg S/day)
Sulphate				
Plasma	1·127			
Rumen	0·090	0·080		
Organic				
Abomasal	0·042	0·038	321	12·2
Ileal	0·181	0·161	236	38·0
Faecal	0·319	0·283	145	41·0

[A] Ratio of SR in secondary pool to that in plasma sulphate sulphur; these values represent the proportions of the sulphur in these pools derived from plasma sulphate sulphur.

Movement of ^{35}S and Sulphate into the Digestive Tract

The intravenous infusion of 211 μCi $^{35}SO_4$ per day in experiment 1 maintained SR in plasma and rumen pools at approximately 1·13 and 0·09 mCi/g S respectively (Table 2). Corresponding values associated with the intraruminal infusion of

45·3 μCi $^{35}SO_4$/day in experiment 2 were 0·53 and 0·13 mCi/g S. The concentrations of plasma sulphate in the two experiments were 8·3±0·1 and 8·7±1·3 mg S/litre respectively. Calculations from the data of experiment 1 indicated that the irreversible loss of plasma sulphate was 185 mg S/day and that 8·0% of rumen sulphate was derived from plasma sulphate. As the irreversible loss of rumen sulphate in experiment 2 was calculated as 359±33 mg S/day, the transfer of sulphate from plasma to rumen in experiment 1 was probably 28·9 mg S/day, corresponding to 32·6 μCi ^{35}S. Data from experiment 2 suggest that 7·7±1·2% of plasma sulphate was derived from rumen sulphate.

Calculations based on SR of abomasal sulphur and rates of digesta flow from the stomach indicate that in experiment 1 about 46% of the 32·6 μCi/day of ^{35}S entering the rumen passed in digesta from the stomach. Similarly in experiment 2 approximately 43% of the ^{35}S infused as sulphate into the rumen passed to the duodenum. In the two experiments only 10 and 3% respectively of the ^{35}S in digesta passed from the stomach in the form of sulphate. Expressed as a proportion of the dose infused intravenously, approximately 7·7% of the ^{35}S passed in digesta from the stomach. By contrast, the amounts of ^{35}S that passed from the ileum and in the faeces were both equivalent to approximately 26% of the dose, with most (88·2 and 94·2% respectively) of the ^{35}S present as organic sulphur.

Sulphur derived from plasma sulphate represented 12·2, 38·0 and 41·0 mg S/day of organic sulphur flowing from the stomach, small intestine and large intestine respectively (Table 2). These amounts represented 3·8, 16·1 and 28·2% respectively of the flow of organic sulphur.

Discussion

Despite the variability between sheep in feed intake and digestion, two important points appear to have been established in the present experiments. The first is that, of the sulphur recycled to the rumen from the plasma, only a small amount is sulphate. The second is that appreciable quantities of ^{35}S, present in the plasma as sulphate, cross the intestinal wall and are found in the intestines in non-sulphate forms.

In the experiment in which it was measured, plasma sulphate could have directly contributed only 12 mg of the observed net gain of 146 mg organic sulphur in the stomach; because little sulphur appears to reach the abomasum from endogenous sources (Hume 1974), much of the remainder was probably derived from salivary proteins or sloughed cells. The amount of sulphur involved could be provided by 10 litre/day mixed saliva containing 15 mg organic S/litre and such rates of salivary secretion and concentrations of organic sulphur in saliva are readily attained in sheep (Tribe and Peel 1963; Bray 1969*a*).

The calculated transfer of ^{35}S into the rumen, 32·6 μCi/day, represented 15·5% of ^{35}S infused as sulphate into the blood. This may be compared with the proportion (0·3–1·4%) of an intravenous dose of [^{35}S]sulphate that crossed the rumen wall, and a similar proportion (0·1–1·5%) that was collected in mixed saliva over a period of 4 h in the experiments of Bray (1969*c*) in which the rumen contents of sheep were replaced with buffer solutions. In the present experiments if all ^{35}S entered the stomach as sulphate the corresponding quantity of recycled sulphate (29 mg S/day) was intermediate between that for sheep fed a mature roughage (4 mg S/day) and that for sheep fed a medium-quality legume (98 mg S/day) (Kennedy *et al.* 1975). Even

this may be an overestimate because with the long-term infusions of [^{35}S]sulphate employed in the present experiments it is possible that some ^{35}S incorporated into the organic sulphur components of the blood and tissues could have been included in the ^{35}S recycled to the stomach.

Other estimates (P. M. Kennedy and L. P. Milligan, unpublished data) show that sulphate transfer to the rumen reached about 150 mg S/day in sheep receiving, in addition to a diet of brome grass pellets, sodium sulphate infused into the rumen or abomasum. Higher values (640–880 mg S/day) were calculated by Gawthorne and Nader (1976), but these estimates were derived by difference and could be subject to appreciable errors.

In both experiments, the amount of organic ^{35}S leaving the stomach was about 40% of ^{35}S entering the rumen as sulphate. The efficiency of incorporation of recycled sulphate into microbial protein was thus low, even when the primary limitation to microbial growth on this same diet appeared to be sulphur deficiency (Bird 1972*b*), and probably reflected the rapidity of absorption of sulphide from the rumen. For example, Bray's (1969*b*) data indicate that the mean time spent by sulphide molecules in the rumen may be as low as 14–31 min. Probably for this reason Bird (1972*b*) recovered at the duodenum only 48% of 360 mg sulphate sulphur added as a supplement to a diet providing 135 mg S, whilst Leibholz (1972) found in duodenal digesta only 4·4–10·5% of ^{35}S infused as sulphate into the rumen.

The observation that more organically bound ^{35}S left the distal end of the small intestine than passed from the stomach and that even more organically bound ^{35}S was excreted in the faeces indicates that appreciable quantities of ^{35}S originally present in the plasma as sulphate were transported across the wall of both the small and large intestines. The net gain of ^{35}S in the intestines was equivalent to approximately 18% of the daily infusion of [^{35}S]sulphate. The total influx of ^{35}S into the intestines probably represented a larger proportion of the dose, since net absorption of sulphur from the intestine represented 50% of the amount flowing from the stomach. Sulphur influx into the intestines may be attributed to the secretion of bile and pancreatic fluids into the small intestine (Bird 1972*a*) and to secretion by goblet cells of sulphated glycoproteins and mucopolysaccharides (Hecker 1973). The secretion of organic sulphur into the small intestine was noted by Clarke *et al.* (1966) and by Bird and Thornton (1972), who suggested that 30% of flow of sulphur to the ileum was mucoprotein in their experiments. The gain of organic ^{35}S in the large intestine was probably due to synthesis of microbial protein from urea and sulphate secreted into the large intestine (Bird and Thornton 1972).

From the data available, it appears that sulphate constitutes only a small part of the sulphur returned from blood to rumen with sheep fed a diet low in sulphur, and that saliva is probably the major source of recycled sulphur. This situation is similar to that prevailing with salivary urea and protein (Nolan and Leng 1972; J. P. Hogan and P. J. Connell, unpublished data) as relative sources of recycled nitrogen. Salivary proteins thus play a vital role, by acting as a source of sulphide, ammonia and amino acids, in maintaining bacterial growth and activity with sheep fed low sulphur, low protein diets. The effectiveness of salivary proteins in this role will be modified not only by the relative rates at which the metabolites are released, but also, as Bray and Till (1975) have indicated, by the relative rates of absorption of sulphide and ammonia from the rumen. Studies in both areas are clearly important to any attempt to improve the efficiency of utilization of recycled nutrients by the sheep.

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