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Sulphate Recycling and Metabolism in Sheep and Cattle

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Abstract

Merino wethers and Brahman × Shorthorn steers, offered lucerne or spear grass hay, were used to study the movements of sulphate through pools in plasma and ruminal liquor. The irreversible loss of sulphate from ruminal liquor was 60 and 76% of sulphur ingested for both species fed lucerne and spear grass respectively. The irreversible loss of sulphate from the plasma averaged 67 and 56% of sulphur ingested for animals fed lucerne and spear grass respectively.

Daily recycling of sulphate to the rumen of sheep was 98 mg sulphur on the lucerne diet and 3·9 mg sulphur on the spear grass diet. Sulphate recycling in cattle feed lucerne was 533 mg sulphur; in cattle fed spear grass the value was 234 mg sulphur.

Over 6 days following an intravenous injection of [^{35}S]sulphate into sheep and cattle fed lucerne, 5–10% of the dose was excreted in the faeces and *c.* 10% was retained. Corresponding values for animals fed spear grass were 23–31% in faeces and 40–51% of the dose retained. After intraruminal injections of [^{35}S]sulphate, animals fed lucerne excreted 15–18% of the dose in the faeces and retained 25–30% of the dose over 6 days. Values for animals fed spear grass were 22–26% in faeces and 62–70% retained.

It was concluded that sulphate recycling to the rumen is a limiting factor in microbial synthesis for sheep fed low-quality roughage, and that secretion of endogenous sulphur into the postruminal tract of ruminants is of importance in the metabolism of sulphate.

Introduction

The transfer of urea from blood to the rumen is a major source of nitrogen for ruminal bacteria of an animal fed a low-protein diet (Houpt 1970). The extent of incorporation of recycled nitrogen into microbial protein determines the amount of this nitrogen that becomes available to the ruminant as amino acid nitrogen. The extent of this incorporation may in turn be limited by the quantity of recycled sulphate (Bray and Hemsley 1969). Estimates of the recycling of endogenous sulphur vary considerably. Bray (1969*b*) found in sheep that a small proportion only of an intravenous dose of [^{35}S]sulphate was transferred across the rumen wall. The contribution of sulphate in parotid saliva was also small relative to the output of urea and total sulphur (Bray 1969*a*; Bray and Hemsley 1969), although mixed saliva contained a higher concentration of total sulphur (Bray 1969*a*) and sulphate (Goss 1969). However, estimates from digesta flowing from the rumen indicated that a large proportion of sulphur ingested was recycled to the rumen (Bird and Hume 1971; Bird 1972*b*).

When animals were fed a low-quality roughage diet without urea supplements, the addition of sulphate to the diet caused an increase in food intake and digestibility for sheep (Playne 1969; Kennedy and Siebert 1972) but not for cattle (Kennedy 1974). These different responses between the species have been attributed to more efficient

recycling of sulphur to the bovine rumen compared with the ovine rumen (Kennedy and Siebert 1972).

In the experiments reported here, quantitative estimates of the recycling of sulphate to the rumen were made by radiotracer techniques. Comparisons were made between sheep and cattle fed either a good- or a poor-quality roughage.

Materials and Methods

Diets

The diets used were lucerne (*Medicago sativa*) hay, and spear grass (*Heteropogon contortus*) hay, ground to pass through a 28-mm screen. The lucerne and spear grass contained respectively 2·4–3·1 and 0·42–0·84 g nitrogen, and 0·16–0·34 and 0·065–0·07 g sulphur per 100 g of dry matter.

Animals and their Management

Six adult Merino wethers (30–35 kg body weight, aged 2–3 years) and six Brahman × Shorthorn (Droughtmaster) crossbred steers (180–220 kg body weight, aged 2–2½ years) were used in these experiments. Animals were dosed with thiabendazole at intervals of 4 weeks to control helminths.

The following stepwise procedure was adopted throughout the experiment, firstly for sheep, then for cattle.

Lucerne diet

1. Six sheep and six cattle were pre-fed for 6 weeks with 800 g and 5·5 kg food per day respectively.
2. Three animals were placed in metabolism cages and fed the daily ration at 2-h (sheep) or 3-h intervals (cattle), using an automatic feeding device.
3. After 3 days in metabolism cages, faeces and urine were collected for a further 7 days for determination of nitrogen and sulphur balance.
4. Radiotracer ($Na_2{}^{35}SO_4$) was injected or infused intravenously or intraruminally, and excreta were collected for 6 days.
5. The first group of three animals were returned to pens and fed lucerne (800 g/day for sheep, 5·5 kg/day for cattle), and the second group were placed in metabolism cages, and steps 2–4 were repeated.
6. After the second group of animals had been removed, two animals from the first group were returned to metabolism cages, fed automatically for 3 days, then injected as in step 4.

Spear grass diet

7. Sheep and cattle were fed *ad libitum* for 3 weeks.
8. As for steps 2–4.
9. The first group of three animals were returned to pens and fed lucerne *ad libitum*, the second group were placed in metabolism cages, and steps 2–4 were repeated.
10. As for step 6, except animals had been fed lucerne for 2 weeks, then spear grass *ad libitum* for 3 weeks.

The metabolism cages were continuously illuminated during the period of the measurements. A total of four single injections (two intravenous, two intraruminal) and four continuous infusions (two intravenous, two intraruminal) were carried out on both groups of animals.

Experimental Procedures

Catheters were inserted into each jugular vein of each animal for the administration of ^{35}S and for collection of blood samples. The radiotracer ($Na_2{}^{35}SO_4$) was injected as a carrier-free solution in isotonic saline (5–10 ml) and washed in with saline (5 ml) or given as a continuous infusion using a peristaltic pump (25 ml/h for 24–30 h for sheep; 40 ml/h for 48 h for cattle). For administration into the rumen, a volume of 50 ml in saline solution (including washings) was injected into the ventral sac in about 10 sites, or given as a continuous infusion into the anterior part of the ventral sac at the same rate as for intravenous infusions. Dosage was 1 mCi of ^{35}S for cattle and 300 μCi for sheep.

The volume of liquid in the rumen was estimated by studying the disappearance of polyethylene glycol 4000 (PEG) injected into the rumen just prior to injection of isotope.

Sampling Procedures

Feed, feed residues, urine and faeces were sampled as previously described (Siebert and Kennedy 1972) except that $CuCl_2$ and HCl were added to the urine buckets as preservatives. For collection of urine a funnel-shaped collection apparatus was strapped to each sheep.

Following the injection of the radiotracer, blood and ruminal samples were taken frequently over the first 8 h, especially from the primary labelled pool. From 8 to 48 h after injection or following infusion, samples were taken every 4–6 h. Plasma was separated by centrifugation using potassium oxalate as an anticoagulant, and was stored at $-5°C$. After thawing, 3 ml of plasma were mixed with 9 ml of 6·6% trichloracetic acid, and 3 ml of supernatant was taken immediately for sulphate analysis. Samples of ruminal fluid (100 ml) were withdrawn into a syringe using a perforated plastic tube covered by a fine nylon gauze. Each sample was mixed, and 5 ml were immediately treated with 1 ml of hydrogen peroxide (100 vol.) in a tube containing *c.* 25 mg of $HgCl_2$ as a preservative. The mixture was allowed to stand for 30 min before addition of 2 ml of 20% trichloracetic acid. After centrifugation samples of supernatant were stored at $-5°C$. A further 5 ml of rumen fluid were immediately treated with 5 ml of 4N H_2SO_4 prior to ammonia analysis. The excess of sample was immediately returned to the rumen.

Analytical Methods

Nitrogen in feed, feed residues, faeces and urine was determined after Kjeldahl digestion, using an autoanalyser (Technicon Equipment Corp., indophenol blue reaction). Ammonia in ruminal liquor was similarly determined after dilution with 4N H_2SO_4. PEG in ruminal liquor was determined by the method of Smith (1959) and the volume of rumen fluid determined by extrapolation to zero time. The concentration and radioactivity of total sulphur, inorganic sulphate and ester sulphate were determined by the methods of Bird and Fountain (1970). The [^{35}S]sulphate activity in plasma was considered to be that from inorganic sulphate, since ester sulphate is principally bound to plasma proteins, and was therefore precipitated by trichloracetic acid. By contrast, [^{35}S]sulphate measured in ruminal liquor included sulphide (after peroxide oxidation), ester sulphate and inorganic sulphate. Total radioactivity in urine, plasma and rumen liquor was also determined by diluting plasma or rumen liquor (1 ml) or urine (0·1 ml) to 6·5 ml with water and adding 10 ml of scintillation mixture [the toluene–Triton-X (7 : 6) mixture of Patterson and Greene (1965)]. For faeces, sulphur was oxidized to sulphate by digestion with a mixture of $HClO_4$–HNO_3. The sulphate was then precipitated, and assayed for radioactivity by the method of Nader and Walker (1970) using a liquid scintillation spectrometer (Packard Instrument Corp.). Efficiency of counting was calculated using internal and external standards, and corrections were made accordingly.

Calculations

Irreversible loss, i.e. the rate at which sulphate leaves the sulphate pool and does not return to it during the experimental period, and pool size were calculated by standard procedures (Baker and Rostami 1969; Leng 1970) using the method of curve peeling (Perl 1960).

After a single injection of ^{35}S into the rumen of animals fed either diet, a rapid decline in specific activity occurred before mixing was complete. Consequently, it was difficult to obtain accurate estimates for the first component, and so the line was constrained to pass through the zero-time intercept as calculated using the measured (PEG) volume and the injected dose. For animals fed spear grass it appeared that a considerable proportion of the dose had been incorporated into (or absorbed onto) the microorganisms before mixing was complete, thereby obscuring the first component and yielding unrealistically high values for irreversible loss. Therefore, estimates of irreversible loss of sulphate from the rumen for animals fed spear grass were obtained from continuous infusions only.

The rate of transfer of plasma sulphate to ruminal sulphate (i.e. recycling) was calculated as the product of the irreversible loss of sulphate from ruminal liquor and the proportion of ruminal sulphate derived from plasma sulphate (calculated from the plateau values of specific radioactivity of sulphate after continuous intravenous infusion, or by the relative areas under the curves describing the decline in radioactivity of sulphate with time in plasma and ruminal fluid—see Nolan and Leng 1972).

Results

Intake and Excretion of Nitrogen and Sulphur

The intake and excretion of nitrogen and sulphur for sheep and cattle fed a diet of lucerne or spear grass is shown in Table 1. For both sheep and cattle fed spear grass, the excretion of nitrogen, total sulphur, inorganic sulphate and ester sulphate in urine, and of total sulphate in faeces, was small compared with animals fed the lucerne diet. Both nitrogen and sulphur balances were negative for animals fed spear grass. These balances did not include nitrogen and sulphur synthesized into wool or hair.

Table 1. Intake and excretion of nitrogen and sulphur for six sheep and six cattle fed lucerne or spear grass

Values given are means ± S.E.M.

Measurement	Sheep		Cattle	
	Lucerne	Spear grass	Lucerne	Spear grass
Nitrogen intake (g/day)	19·9±2·3	3·43±0·06	117·9±0	15·4±2·0
Faecal nitrogen (g/day)	5·04±0·49	2·29±0·23	31·3±2·6	14·8±3·6
Urinary nitrogen (g/day)	13·9±2·6	1·28±0·47	86·4±2·9	5·17±3·00
Nitrogen balance (g/day)	1·01±0·33	−0·14±0·10	0·02±1·61	−4·57±2·91
Sulphur intake (g/day)	2·32±0·14	0·27±0·01	9·80±0	1·79±0·27
Faecal sulphur (g/day)	0·70±0·10	0·24±0·01	3·60±0·37	2·03±0·55
Urinary sulphur (g/day)	1·05±0·21	0·04±0·01	4·68±1·00	0·35±0·16
Sulphur balance (g/day)	0·57±0·25	−0·01±0·01	1·52±0·72	−0·59±0·43
Faecal total sulphate sulphur (g/day)	0·12±0·05	0·04±0·04	0·37±0·06	0·07±0·06
Urinary total sulphate sulphur (g/day)	0·66±0·31	0·02±0·01	2·85±1·13	0·19±0·08
Urinary ester sulphate sulphur (g/day)	0·36±0·04	0·01±0·004	1·18±0·93	0·12±0·06

Table 2. Measurements of concentrations of ammonia and sulphate, rumen fluid volume and irreversible loss of sulphate from the rumen pool of six sheep and six cattle fed a diet of lucerne or spear grass

Diet	Animal No.	Tech-nique[A]	Animal weight (kg)	Ruminal measurements: Mean NH_3 concn (mg N/100 ml)	Mean sulph-ate+sulphide concn (μg S/ml)	Fluid vol. (l)	Sulphate pool size (mg)	Irreversible sulphate loss: (g S/day)	(% S in-gested)
				Sheep					
Spear grass	4	CI	26·5	3·3	2·5	5·2	13·0	0·16	77
	5	CI	29·5	2·0	2·2	4·6	10·1	0·18	71
Lucerne	4	SI	29·8	20·9	9·5	3·5	33·3	1·43	58
	6	SI	33·2	15·4	10·5	3·2	33·6	1·53	62
	1	CI	35·5	17·5	12·9	3·6	46·4	1·67	72
	2	CI	28·5	15·6	7·5	4·1	30·8	1·52	64
				Cattle					
Spear grass	5	CI	187	0·75	4·0	31·5	126	1·68	85
	6	CI	205	0·61	4·4	47·3	208	1·75	73
Lucerne	2	SI	210	18·2	10·5	18·7	196	4·88	60
	6	SI	220	20·5	5·9	25·2	149	5·48	56
	1	CI	220	20·3	7·8	22·1	172	5·33	54
	4	CI	225	21·1	11·5	28·6	329	4·46	55

[A] CI = continuous infusion; SI = single injection.

Single Injections and Constant Infusions into the Rumen

After an initial mixing phase the decline in specific radioactivity of sulphate in ruminal fluid following injection of the dose exhibited three exponential components. Examples of the curves obtained after single injection or continuous infusion of radiotracer for steers fed lucerne hay are given in Fig. 1. Estimates of the irreversible loss of sulphate are given in Table 2. The irreversible loss of sulphate for both sheep and

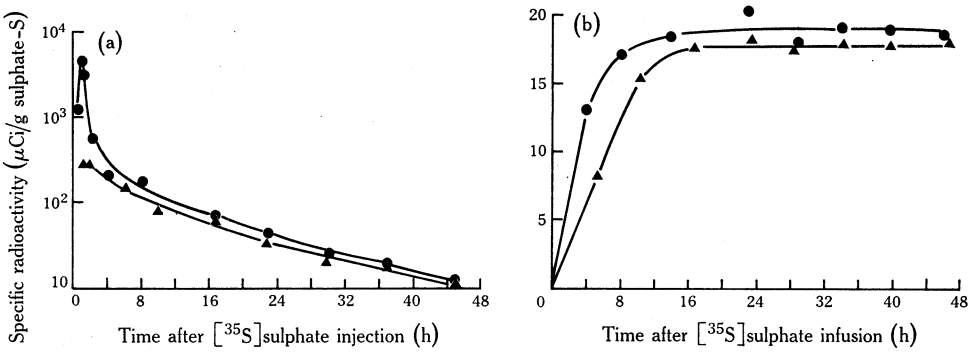

Fig. 1. Time course of change in specific radioactivity of sulphate sulphur in plasma (▲) and ruminal liquor (●) after a single injection (*a*) or continuous infusion (*b*) of $Na_2{}^{35}SO_4$ into the rumen of a steer fed lucerne.

cattle was approximately 60% of sulphur ingested when fed lucerne and approximately 77% of sulphur ingested when fed spear grass. On the spear grass diet, ruminal liquor from sheep contained less sulphate but more ammonia than did ruminal liquor from cattle; concentrations of ammonia and sulphate were low compared with animals fed the lucerne diet.

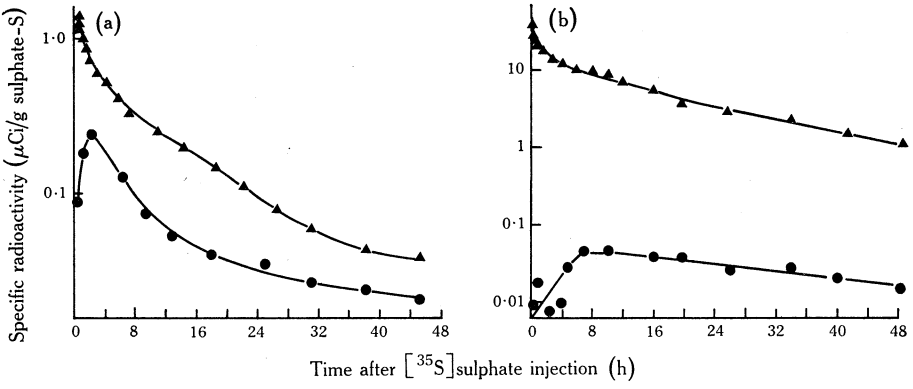

Fig. 2. Decline in specific radioactivity of sulphate sulphur in plasma (▲) and ruminal liquor (●) after an intravenous injection of 1 mCi of $Na_2{}^{35}SO_4$ into a steer (*a*) and a sheep (*b*) fed spear grass.

Single Injections and Constant Infusions of ^{35}S into the Blood

The decline in specific radioactivity of sulphate after an intravenous injection is shown for a steer and a sheep fed spear grass (Figs 2*a* and 2*b*). The curves were described adequately by three exponential components. The transfer of [^{35}S]sulphate from the plasma to ruminal liquor was markedly greater for steers than for sheep on

the spear grass diet; 11·1–15·6% of ruminal sulphate was derived from the plasma for cattle, compared with 1·1–4·1% for sheep (Table 3). For the lucerne diet, 8·5–13·6% of sulphate in ruminal liquor of cattle was derived from plasma, compared with 5·5–7·2% for sheep (Table 3).

Table 3. Measurements of concentration of plasma sulphate, body sulphate pool, body sulphate space, irreversible loss of sulphate from the body pool and the proportion of ruminal sulphate derived from plasma sulphate for six sheep and six cattle fed a diet of lucerne or spear grass

Diet	Animal No.	Tech-nique[A]	Animal weight (kg)	Plasma sulphate concn (μg S/ml)	Body sulphate pool (mg S)	Body sulphate space (% body wt)	Irreversible loss of sulphate: (g/day)	(% S in-gested)	Ruminal sulphate from plasma (%)
					Sheep				
Spear grass	3	SI	26·0	7	53	29	0·155	52	1·1
	5	SI	28·8	10	66	23	0·203	63	2·2
	1	CI	32·3	6			0·130	53	1·8
	2	CI	27·5	11			0·174	65	4·1
Lucerne	3	SI	28·1	49	360	26	1·26	53	7·2
	4	SI	29·3	51	448	30	1·54	69	6·1
	5	CI	31·0	50			1·52	68	5·5
	6	CI	31·5	52			1·49	67	6·7
					Cattle				
Spear grass	3	SI	197	15	798	27	0·94	46	13·9
	4	SI	214	15	931	29	0·89	49	15·6
	1	CI	201	11			1·05	59	11·1
	2	CI	202	12			0·87	59	14·0
Lucerne	3	SI	197	45	2660	30	6·38	79	9·2
	5	SI	194	42	2200	27	5·85	60	8·5
	1	CI	217	46			7·53	70	13·6
	6	CI	227	41			7·23	67	11·0

[A] CI = continuous infusion; SI = single injection.

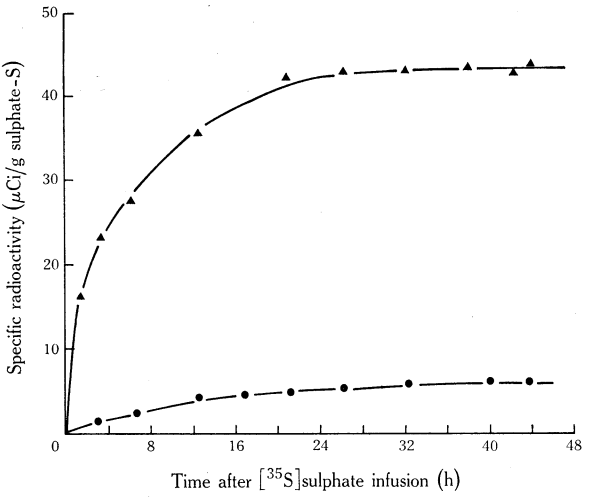

Fig. 3. Increase in specific radioactivity of sulphate sulphur in plasma (▲) and ruminal liquor (●) during a continuous intravenous infusion of $Na_2{}^{35}SO_4$ into a steer fed lucerne.

After intravenous infusion of [^{35}S]sulphate, the specific radioactivity of sulphate in plasma and ruminal liquor rapidly attained a plateau value (Fig. 3). The irreversible

loss of sulphate from the plasma pool averaged 56 and 67% of sulphur ingested for all animals fed spear grass and lucerne respectively (Table 3).

The decline in radioactivity of the sulphate and total sulphur fractions in plasma, and the appearance of [^{35}S]sulphate in ruminal liquor after an intravenous injection of [^{35}S]sulphate, is shown in Fig. 4 for a steer fed lucerne. The proportion of radioactivity in organic sulphur in plasma and ruminal liquor rapidly increased with time after injection of ^{35}S for all animals on both diets.

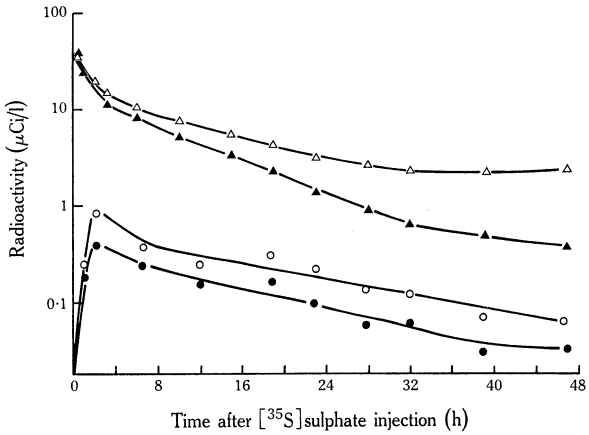

Fig. 4. Decline in radioactivity of total sulphur (△) and sulphate (▲) in plasma, and of total sulphur (○) and sulphate sulphur (●) in ruminal liquor, after an intravenous injection of $Na_2{}^{35}SO_4$ into a steer fed lucerne.

Recycling of Sulphate

The rate of transfer of sulphate from the plasma pool to the rumen, calculated from mean values from Tables 2 and 3 as previously described, was 3·9 and 98 mg sulphur per day for sheep fed spear grass and lucerne respectively. For cattle fed spear grass and lucerne this rate of transfer was 234 and 533 mg sulphur per day respectively.

Excretion of ^{35}S in Urine and Faeces

The cumulative excretion of ^{35}S after intravenous or intraruminal injections is shown in Fig. 5. On the lucerne diet 15–18% of the dose appeared in faeces and approximately 55% in urine within 6 days after an intraruminal injection of [^{35}S]-sulphate (Fig. 5*a*). When the dose was given intravenously only 5–10% of the dose appeared in the faeces, with 80–85% in the urine for all animals (Fig. 5*b*). On the spear grass diet cattle and sheep excreted respectively 22 and 26% of the dose in faeces and 8 and 12% of the dose in the urine after an intraruminal injection (Fig. 5*c*). After an intravenous injection cattle excreted 23% of the dose in faeces and 26% in urine, while sheep excreted 31% in faeces and 29% in urine (Fig. 5*d*). The retention of ^{35}S after 6 days for animals fed lucerne was approximately 10% of the dose after intravenous injection and 25–30% after intraruminal injection. For animals fed spear grass 40–50% of the dose was retained after intravenous injection, while 62–70% was retained after intraruminal injection.

The decline in specific radioactivity of urinary sulphate was described by the same curve as the decline in plasma sulphate (Fig. 6). The specific radioactivity of faecal sulphate was higher than that of urinary sulphate on the same day. The curves describing the decline in specific radioactivity of urinary sulphate and faecal sulphate

required two exponential components to describe them. The proportion of radioactivity in cattle faeces due to the sulphate fraction was high on the first day after intravenous injection (30–60% of total activity), but thereafter declined.

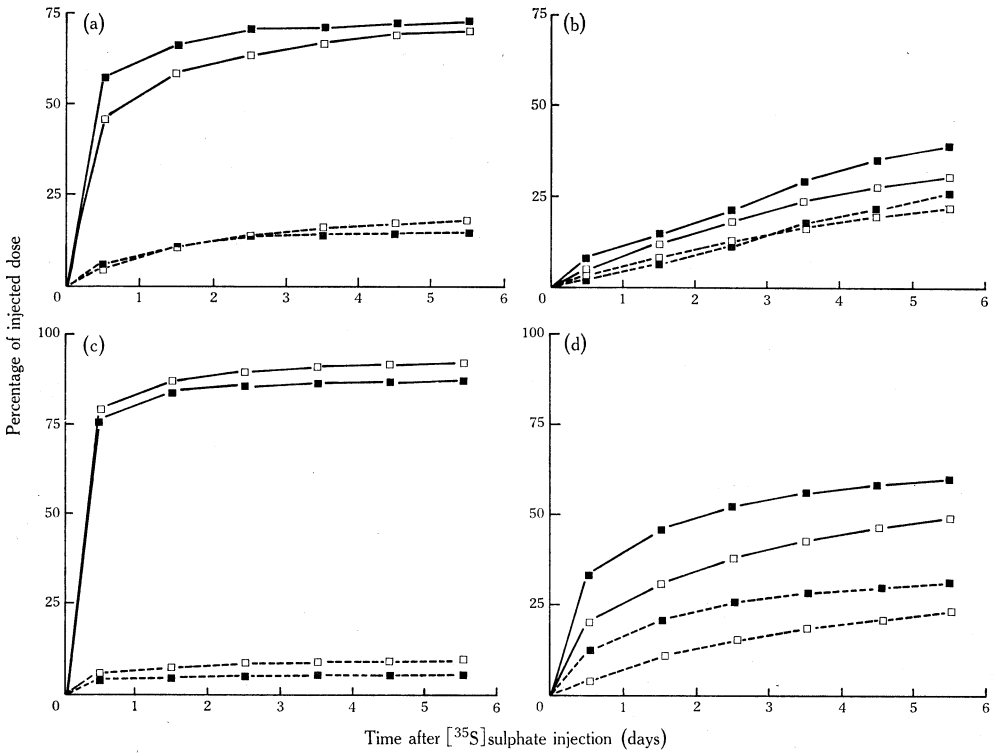

Fig. 5. Excretion of radioactive sulphur by sheep (■) and cattle (□) (means for two animals) after an injection of $Na_2{}^{35}SO_4$. – – – Faecal excretion. —— Total (urinary+faecal) excretion. (*a*) Intraruminal dose, lucerne diet. (*b*) Intravenous dose, lucerne diet. (*c*) Intraruminal dose, spear grass diet. (*d*) Intravenous dose, spear grass diet.

The proportion of faecal organic sulphur derived from plasma was calculated for two steers from the relative areas under the plasma sulphate and the faecal organic sulphur curves (Fig. 6). The proportion was 10·9% for the steer fed the lucerne diet, compared with 16·4% for the steer fed spear grass.

Discussion

The estimated daily recycling of sulphate to the rumen of sheep was 98 mg sulphur on the lucerne diet and 3·9 mg sulphur on the spear grass diet. For cattle the estimates were 533 mg sulphur for the lucerne diet and 234 mg sulphur for the spear grass diet. Expressed on the basis of body weight, daily recycling of sulphate was 3·2 and 2·5 mg sulphur/kg for sheep and cattle respectively when fed lucerne, and 0·14 and 1·2 mg sulphur/kg respectively when fed spear grass. Thus sulphate recycling to the rumen of sheep fed spear grass is considerably less than for cattle, confirming the suggestion of Kennedy and Siebert (1972). This finding, supported by the work of Bray (1969*b*) and

Bray and Hemsley (1969) on sheep, has important nutritional implications, since with low sulphate recycling only 20% of recycled urea nitrogen may be used for protein synthesis (Bray and Hemsley 1969). Bray and Hemsley (1969) also found that the concentration of sulphate in parotid saliva was low relative to the concentration of inorganic sulphate in blood. Their experiments and those reported here suggest that

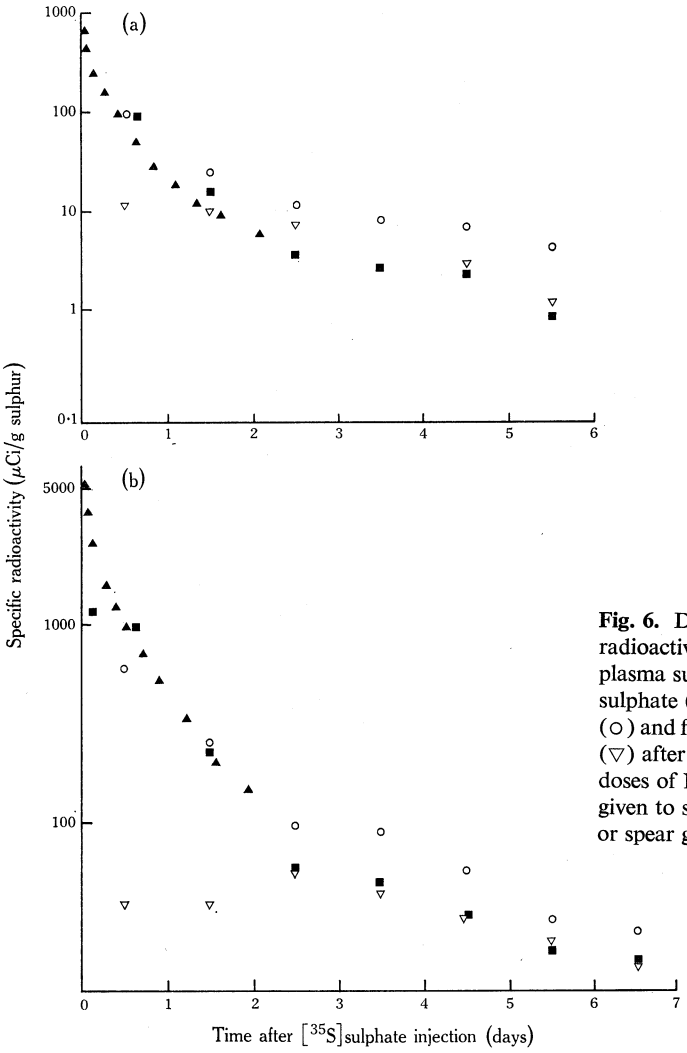

Fig. 6. Decline in specific radioactivity of sulphur in plasma sulphate (▲), urinary sulphate (■), faecal sulphate (○) and faecal organic sulphur (▽) after equal intravenous doses of $Na_2{}^{35}SO_4$ had been given to steers fed lucerne (*a*) or spear grass (*b*).

(1) the concentration of salivary sulphate is small relative to blood sulphate, and (2) there is a relationship between the concentrations of sulphate in blood and in saliva, and the amount of sulphate recycled to the rumen. The degree of sulphate recycling appears to be more closely related to the concentration of that part of the sulphate in plasma that is not bound to plasma proteins than to the total concentration of sulphate in plasma (Kennedy, unpublished data).

Other estimates of recycling of salivary sulphate to the bovine rumen are in agreement with the values presented in this paper. When mixed saliva was collected from

oesophageal fistulae of cattle fed spear grass, it was estimated that saliva contributed about 0·5–1·6 g sulphate sulphur per day (Kennedy, unpublished data). Goss (1969) estimated that sulphur recycling in the resting saliva secretion of cattle was 1·0–2·3 g sulphur per day, of which approximately 62% was sulphate sulphur. The N : S ratio of mixed saliva lay between 1·6 : 1 and 7 : 1 in both studies.

Recycling of sulphur to the rumen of sheep has been estimated as 0·44 and 0·19 g/day for sheep ingesting 0·61 and 0·14 g sulphur/day respectively (Bird and Hume 1971; Bird 1972*b*). The contribution of sulphate was not measured, but was probably small. Thus in sheep a large proportion of recycled sulphur may be amino acid sulphur in salivary proteins, and therefore would not increase net synthesis of protein in the rumen.

The transfer of sulphate from plasma to ruminal liquor in sheep fed lucerne which was measured in this study may be compared with the transfer of urea from blood to the rumen which was estimated by Nolan and Leng (1972), using similar sheep, techniques and diet. Taking the estimates of nitrogen and sulphur recycling of 1·2 g urea nitrogen/day (Nolan and Leng 1972) and 0·1 g sulphate sulphur/day (this study), the ratio of N : S recycled would be 12 : 1. Since the N : S ratio required for optimal utilization of nitrogen by the rumen microorganisms is about 10 : 1 (Moir *et al.* 1967–8), sheep on the lucerne diet recycle sulphate at a rate near optimal for utilization of recycled urea. Other estimates made of recycling of urea to the rumen are greater than that of Nolan and Leng (1972). For example, Weston and Hogan (1967) estimated that 4–5 g of urea nitrogen was recycled to the rumen when sheep had a concentration of blood urea nitrogen similar to the sheep studied by Nolan and Leng (1972). If this estimate is accepted, the ratio of N : S recycled would be as high as 50 : 1.

The assumptions underlying the mathematical treatment of the data are that (1) the system is in a steady state, with turnover rates and pool sizes remaining constant, and (2) the injected label is mixed instantaneously within the sampled pool. Animals fed the lucerne diet had been maintaining weight for 3–4 weeks prior to injection of ^{35}S, and the daily ration was given at frequent intervals in an attempt to achieve a steady state. When fed spear grass animals lost weight and were in negative nitrogen and sulphur balance, and thus were not in a steady state. However, the decrease in size of the sampled pools would be small during the sampling period. To standardize comparisons, measurements on animals fed spear grass were started 3 weeks after the animals were removed from the lucerne diet. Prior to analysis the sulphide in ruminal liquor was oxidized to sulphate, and the specific activity of ester + inorganic sulphate was subsequently estimated in a protein-free solution. There appeared to be rapid interchange of sulphur atoms between these three ruminal pools; therefore combining the three pools was considered justifiable, and would not affect calculations of recycled sulphate.

The sulphate space of all animals averaged 27·5 litres/100 kg body weight. This estimate is in good agreement with a previous estimate for sheep of 27 litres/100 kg (Bray 1969*b*). In the present experiment mixing of the injected [^{35}S]sulphate with the extracellular space was complete by 30 and 50 min respectively for sheep and cattle fed lucerne, but took approximately 50% longer for animals fed spear grass.

In this study the irreversible loss of sulphate from the body pool was approximately 1·45 g sulphur/day for sheep and 6·75 g sulphur/day for cattle fed lucerne. Subtraction of the values of sulphate excretion (Table 1) yields 0·67 g sulphur/day for sheep and 3·53 g sulphur/day for cattle which left the body pool of sulphate but which

was not excreted as sulphate. Even allowing for maximal incorporation of recycled sulphate into microbial protein in the rumen, the daily loss of sulphate not accounted for was 0·57 and 3·0 g sulphur for sheep and cattle respectively. This difference represents sulphate that was (1) secreted into the intestinal tract as organic sulphur, or as sulphate which was subsequently synthesized into microbial protein and (2) incorporated as sulphate into body tissues, e.g. connective tissue. Similar calculations for animals fed spear grass may be unreliable, due to the absence of steady-state conditions. Nolan and Leng (1972) estimated that 5·1 g urea nitrogen/day was apparently degraded in the postruminal tract. Thus the ratio of urea nitrogen : sulphate sulphur apparently degraded in the postruminal tract for sheep fed lucerne may be approximately 9 : 1.

When [^{35}S]sulphate was given as an intravenous injection to sheep and cattle fed a diet adequate in sulphur, the proportion of the dose excreted in the faeces was 11–19% (Hansard and Mohammed 1968, 1969; Bray 1969*a*; Bird and Thornton 1972). Bray (1969*a*) suggested that faecal ^{35}S was primarily derived from secretion of [^{35}S]sulphate into the postruminal tract in the form of esters or inorganic sulphate, but Bird and Thornton (1972) estimated that 90% of faecal ^{35}S was organic. In the present experiment the excretion of [^{35}S]sulphate in sheep faeces was not measured, but would probably contribute a higher proportion of the total ^{35}S in faeces than in the experiments of Bird and Thornton (1972), since the concentration of faecal sulphate relative to total sulphur was approximately double. However, the excretion of ^{35}S in faeces of sheep fed spear grass after injection of [^{35}S]sulphate was greater than could be attributed to microbial residues and postruminal sulphate secretion, implicating secretion of organic ^{35}S, or synthesis of organic ^{35}S from sulphate secreted in the postruminal tract. The specific activity of faecal sulphate measured for two steers (Fig. 6) indicated that faecal sulphate arose directly from the plasma pool, probably after secretion into the hind gut (Bird and Thornton 1972).

The apparent digestibility of sulphur was 11 and −13% respectively for sheep and cattle fed spear grass (calculated from Table 1), indicating that significant amounts of endogenous sulphur were secreted into the gut contents. A small amount of sulphur from bile and pancreatic fluids may be excreted in the faeces (Bird 1972*a*), but the major endogenous excretion of sulphur may arise from mucoprotein (Pasternak *et al.* 1958; Clarke *et al.* 1966).

The small quantities of sulphate recycled to the rumen of sheep fed a low-quality diet is presumably related to low concentrations of inorganic sulphate in blood. A sheep that is losing weight would be releasing sulphur-containing amino acids into the blood from the breakdown of tissue protein. Because a portion of these sulphur-containing amino acids is diverted into wool synthesis, a lesser amount would be degraded to sulphate in body tissues. The small sulphate pool available for satisfying tissue requirements for sulphate and for recycling to the rumen would be insufficient to utilize recycled nitrogen, leading to high values for blood urea nitrogen (Kennedy and Siebert 1972). In contrast, cattle hair requires relatively small amounts of cystine (Springell 1966), allowing greater recycling of sulphate to the rumen.

References

Baker, N., and Rostami, H. (1969). *J. Lipid Res.* **10**, 83.
Bird, P. R. (1972*a*). *Aust. J. Biol. Sci.* **25**, 817.
Bird, P. R. (1972*b*). *Aust. J. Biol. Sci.* **25**, 1073.

Bird, P. R., and Fountain, R. D. (1970). *Analyst (Lond.)* **95**, 98.
Bird, P. R., and Hume, I. D. (1971). *Aust. J. Agric. Res.* **22**, 443.
Bird, P. R., and Thornton, R. F. (1972). *Aust. J. Biol. Sci.* **25**, 1299.
Bray, A. C. (1969*a*). *Aust. J. Agric. Res.* **20**, 725.
Bray, A. C. (1969*b*). *Aust. J. Agric. Res.* **20**, 749.
Bray, A. C., and Hemsley, J. A. (1969). *Aust. J. Agric. Res.* **20**, 759.
Clarke, E. M. W., Ellinger, G. M., and Phillipson, A. T. (1966). *Proc. R. Soc. Lond. B* **166**, 63.
Goss, K. (1969). B.Sc.(Hons) Thesis, University of Western Australia.
Hansard, S. L., and Mohammed, A. S. (1968). *J. Nutr.* **96**, 247.
Hansard, S. L., and Mohammed, A. S. (1969). *J. Anim. Sci.* **28**, 283.
Houpt, T. R. (1970). In 'Physiology of Digestion and Metabolism in the Ruminant'. (Ed. A. T. Phillipson.) p. 119. (Oriel Press: Newcastle-upon-Tyne.)
Kennedy, P. M. (1974). *Aust. J. Agric. Res.* **25**, 1015.
Kennedy, P. M., and Siebert, B. D. (1972). *Aust. J. Agric. Res.* **23**, 45.
Leng, R. A. (1970). *Adv. Vet. Sci.* **14**, 209.
Moir, R. J., Somers, M., and Bray, A. C. (1967–8). *J. Sulphur Inst.* **3**, 15.
Nader, C. J., and Walker, D. J. (1970). *Appl. Microbiol.* **20**, 677.
Nolan, J. V., and Leng, R. A. (1972). *Br. J. Nutr.* **27**, 177.
Patterson, M. S., and Greene, R. C. (1965). *Anal. Chem.* **37**, 854.
Pasternak, C. A., Kent, P. W., and Davies, R. E. (1958). *Biochem. J.* **68**, 512.
Perl, W. (1960). *Int. J. Appl. Radiat. Isot.* **8**, 211.
Playne, M. J. (1969). *Aust. J. Exp. Agric. Anim. Husb.* **9**, 393.
Siebert, B. D., and Kennedy, P. M. (1972). *Aust. J. Agric. Res.* **23**, 35.
Smith, R. H. (1959). *J. Agric. Sci.* **52**, 72.
Springell, P. H. (1966). *Proc. Aust. Soc. Anim. Prod.* **6**, 399.
Weston, R. H., and Hogan, J. P. (1967). *Aust. J. Biol. Sci.* **20**, 967.

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