Quantitative aspects of the transformations of sulphur in sheep

BY P. M. KENNEDY AND L. P. MILLIGAN
Department of Animal Science, University of Alberta, Edmonton, Alberta T6G 2E3, Canada

(*Received* 26 *August* 1976 – *Accepted* 27 *May* 1977)

1. [^{35}S]sulphate was used to obtain quantitative estimates of the transfer of sulphur between the blood, rumen and postruminal tract of four sheep given brome grass (*Bromus inermis*) pellets or lucerne (*Medicago sativa*) pellets at the rate of 33 or 66 g/h. Sodium sulphate (0–4 g S/d) was infused into the rumen or abomasum of sheep given brome grass during four periods of 19 d and was not infused into the sheep during a subsequent period in which lucerne was given. The flow of sulphide, sulphate, microbial S and non-microbial organic S from the abomasum was estimated using ^{103}Ru and ^{51}Cr.

2. The concentration of inorganic sulphate in serum was increased to maximum values of 35–46 mg S/l by infusion of sulphate into the rumen or abomasum. The rate of irreversible loss of serum sulphate and rumen sulphide was positively related to the amount of sulphate infused.

3. Reabsorption of sulphate by the kidney reached a maximum of 0·69–1·1 mmol sulphate/l glomerular filtrate.

4. The transfer of sulphate from blood to the rumen was related to the concentration of inorganic sulphate in serum, attaining maximum values of 133 (± 13) mg S/d for sheep given brome grass plus sulphate, and 127–159 mg S/d for sheep given lucerne.

5. Bacteria derived 0·52–0·67 of organic S from rumen sulphide in sheep given brome grass, and approximately 0·45 of bacterial organic S was derived from sulphide for sheep given lucerne. Protozoa derived approximately 0·90 of organic S from bacteria.

6. It was estimated that endogenous organic S contributed 300–340 mg S/d to the rumen, and that 0·24–0·45 of S digested in the rumen was derived from endogenous sources.

Sulphide, like ammonia, is produced in the rumen by bacterial metabolism (Postgate, 1965) from dietary and endogenous protein and inorganic sources (Moir, 1970). Rumen bacteria can utilize sulphide in the synthesis of bacterial protein, therefore ^{35}S has been used to label bacterial protein via the sulphide pool to provide a marker for estimation of the flow of microbial protein from the rumen (Beever, Harrison, Thomson, Cammell & Osbourn, 1974). However, there is little information available on the rates of sulphide production in the rumen, or the extent of incorportion into microbial protein of sulphide compared to organic S derived from dietary and endogenous protein.

Bray & Hemsley (1969) have suggested that the extent of transfer of sulphate to the rumen from the blood may limit the incorporation of recycled urea-nitrogen into microbial protein. In sheep given a roughage diet of low protein content, the transfer of sulphate to the rumen may be as low as 4 mg S/d (Kennedy, Williams & Siebert, 1975). However, the input of organic S into the rumen from endogenous sources may be greater than from sulphate (Kennedy, Hogan, Lindsay & Hogan, 1976), but quantitative estimates are not available.

The studies described in this paper were designed to estimate in vivo the rates of production of sulphide in the rumen, to assess the importance of sulphide as a precursor of microbial S, and to determine the rate of entry of endogenous S into the rumen. Rates of transfer of S between pools in the blood and the rumen have been calculated.

EXPERIMENTAL

Animals and feeding regimen

Four Suffolk wethers, approximately 2 years of age, were fitted with permanent simple cannulas in the dorsal sac of the rumen and in the abomasum close to the pylorus. The animals were individually housed indoors in metabolism cages at 18–20° with continuous illumination. At intervals of 4 weeks sheep were dosed with an anthelmintic and injected with retinol, cholecalciferol and α-tocopherol.

The diet used in experimental periods 1–4 was brome grass (*Bromus inermis*) pellets containing (g/kg dry matter (DM)) 19·6 N, 1·44 S, and 901 organic matter (OM), while that in experimental period 5 was lucerne (*Medicago sativa*) pellets, containing (g/kg DM) 26·3 N, 2·14 S and 870 OM. The ration was offered at intervals of 1 h from an automatic feeder. Two sheep (sheep nos. 1 and 3) received 33·0 ($\pm$0·8) g DM/h. Sheep nos. 2 and 4 received 66·0 ($\pm$1·6) g/h. Each sheep was offered cobalt-iodized salt (20 g/d) and water was available *ad lib.*

Experimental procedures

The experiment comprised five sequential periods, each of 19 d; 11 d of additional dietary adaptation were allowed between periods 4 and 5. Two sheep, nos. 1 and 2, received continuous infusions (520 ml/d) of sodium sulphate in water solution into the abomasum at the rates of approximately 0, 0·5, 2 and 4 g S/d during periods 1 to 4 respectively. Sheep nos. 3 and 4 received sulphate infusions into the rumen at the same rates as sheep nos. 1 and 2 during each period. During period 5 the sheep were given lucerne pellets at 1 h intervals to achieve the same DM intake for each animal as in periods 1–4 and there was not infusion of unlabelled, supplementary Na_2SO_4.

After an adaptation period (days 1–10), catheters were inserted into both jugular veins of each sheep for the administration of isotopes and collection of blood samples. During days 11 and 12, carrier-free $Na_2{}^{35}SO_4$ was infused (approximately 55 ml/d, 5·5 μCi/ml) in sterile physiological saline (9 g sodium chloride/l). After infusion of $Na_2{}^{35}SO_4$ for 40 h, ^{51}Cr complexed with EDTA (^{51}Cr-EDTA; 250 μCi, 50 μCi/ml) was injected into the jugular vein and washed in with physiological saline (10 ml) in order to estimate glomerular filtration rate (GFR). Samples of blood (10 ml) and rumen fluid (20 ml) were taken at 1 h intervals for 8 h.

On day 12, after termination of the intravenous infusion, a priming dose (520 ml) of ^{51}Cr-EDTA (120 μCi), ^{103}Ru-labelled Tris-(1,10, phenanthroline)-ruthenium (II) chloride (^{103}Ru-P; 20 μCi) and $Na_2{}^{35}SO_4$ (80 μCi) was injected into the rumen, followed by a continuous infusion (520 ml/d containing 120 μCi ^{51}Cr-EDTA, 20 μCi ^{103}Ru-P, and 80 μCi [^{35}S]sulphate) for 6·5 d in order to measure flow of digesta (Tan, Weston & Hogan, 1971) and of microbial S (Kennedy, Christopherson & Milligan, 1976) from the abomasum. During the final 3 d of infusion (days 16–18) twelve samples (150 ml) of abomasal digesta were taken as described previously (Kennedy *et al.* 1976). In addition, four samples (40 ml) of rumen fluid were taken from each sheep at intervals of 4 h on days 16–18 for sulphide analysis, and isolation of bacteria and protozoa.

Faeces and urine were collected for 6 d (days 10–16) as described by Kennedy *et al.* (1976).

Analytical methods

To determine DM content, samples were heated at 95° to constant weight, and were subsequently ignited at 550° for 6 h to determine OM. Sulphide, total S, ester-sulphate, inorganic sulphate and ^{35}S were estimated by the methods of Bird & Fountain (1970). Serum was

mixed (1:1) with trichloroacetic acid solution (100 g/l) before analysis of the supernatant fluid for inorganic sulphate. Total N was determined by the Kjeldahl method.

Samples of bacteria and protozoa were separated from rumen fluid by differential centrifugation (Blackburn & Hobson, 1960), after filtration through cheesecloth, and were freeze-dried before analysis.

Abomasal digesta was separated into two fractions (Kennedy *et al.* 1976) and ^{51}Cr and ^{103}Ru were measured using a gamma counter (Biogamma; Beckman Intsruments Inc., Fullerton, California, USA). ^{35}S was estimated using a liquid-scintillation counter (Nuclear Chicago, Mark I; Searle Analytic Inc., Des Plaines, Illinois, USA).

Mathematical procedures

GFR was estimated during 8 h after injection of ^{51}Cr-EDTA (Stacy & Thorburn, 1966) assuming first-order kinetics.

The flow of digesta from the abomasum and of fluid from the rumen was calculated by reference to ^{51}Cr-EDTA and ^{103}Ru-P as described by Faichney (1975).

The flow from the rumen of organic S in microbes was calculated from the measured flow of organic ^{35}S in the abomasum and the specific radioactivity of the microbial fraction isolated from abomasal digesta. For purposes of calculation of S flows in the model it was assumed that bacteria accounted for 0·66 of microbial S flow as Walker & Nader (1975) have shown this to be the bacterial portion of microbial N flow.

The rate of irreversible loss of serum inorganic sulphate was estimated at plateau using standard procedures (Shipley & Clark, 1972). Irreversible loss of sulphide from rumen fluid was estimated after allowing for [^{35}S]sulphate flowing from the rumen, as follows:

irreversible loss of sulphide (g/d) =

$$\frac{[^{35}S]\text{sulphate infused } (\mu Ci/d) - [^{35}S]\text{sulphate leaving rumen } (\mu Ci/d) \text{ in rumen fluid}}{\text{asymptotic specific radioactivity of sulphide } (\mu Ci/g\ S)}.$$

The proportion (a) of rumen sulphide derived from serum sulphate was calculated at plateau during intravenous infusion as:

$$\frac{\text{specific radioactivity of sulphide}}{\text{specific radioactivity of sulphate}}.$$

The proportion (b) of serum sulphate derived from rumen sulphide, the proportion of bacterial organic S derived from rumen sulphide, and the proportion of protozoal organic S derived from bacteria during intraruminal infusions were similarly calculated.

The rate of transfer of S from rumen sulphide into the serum inorganic sulphate pool was calculated as:

$$\frac{b \times \text{irreversible loss of sulphate-S}}{1-(a \times b)}.$$

Similarly, transfer of inorganic sulphate-S from serum to rumen sulphide was calculated as:

$$\frac{a \times \text{irreversible loss of sulphide-S}}{1-(a \times b)}.$$

These equations, derived from equations presented by Shipley & Clark (1972) and Nolan, Norton & Leng (1976), yield better estimates of total movement between pools with extensive interchange than those employed previously (Nolan & Leng, 1972; Kennedy *et al.* 1975).

The utilization of serum inorganic sulphate in the body and gut was calculated as the difference between irreversible loss of sulphate and urinary excretion of inorganic sulphate.

Table 1. *Body-weight (kg), intake, flow from the stomach and faecal excretion of dry matter (DM), organic matter (OM) and nitrogen (g/d) for sheep given brome grass (Bromus inermis) or lucerne (Medicago sativa) pellets*

(Measurements were made during 6 d digestibility trials, followed by 3 d during which estimates were made of flow from the abomasum. Values for sheep given brome grass are means with their standard errors for four periods, see p. 66)

	Brome grass				Lucerne			
Sheep no. ...	1 and 3		2 and 4		1 and 3		2 and 4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body-wt (kg)	46	4·5	62	7·1	45	0·5	65	0·7
DM intake	784	11	1568	23	819	0	1637	0
OM intake	706	10	1412	21	712	0	1426	0
OM leaving abomasum	433	26	912	34	360	4	687	18
OM apparently digested in stomach	274	26	500	34	353	4	740	18
Faecal OM output	266	29	553	47	325	6	681	0
N intake	15·9	0·4	31·8	0·8	21·6	0	43·1	0
Non-ammonia-N leaving abomasum	17·2	0·9	37·6	0·6	17·5	1·5	34·9	5·6
Ammonia-N leaving abomasum	1·8	1·0	2·7	2·2	2·9	0·2	5·8	0·8
Faecal N output	5·2	0·4	10·7	0·7	6·2	0·2	13·5	0·6

Table 2. *Estimates of sulphur intake in food, and the irreversible loss, utilization in tissues and gut (mg S/d) and concentration (mg S/l) of inorganic serum sulphate in sheep given lucerne (Medicago sativa) pellets**

	Sheep no.			
	1	2	3	4
S intake	1755	3510	1755	3510
Serum inorganic sulphate concentration	40·5	32·5	45·0	44·4
Irreversible loss of inorganic sulphate from serum	1504	1762	1599	2166
Excretion of urinary inorganic sulphate	451	661	427	956
Utilization of inorganic serum sulphate in tissues and gut	1053	1101	1172	1210
Utilization of inorganic serum sulphate in gut	595	429	724	714

* For experimental details and calculations, see p. 67.

Further subtraction of urinary ester-sulphate excretion yielded estimates for utilization of sulphate in the gut.

Absorption of sulphide from the rumen was calculated as:

$$\frac{\text{irreversible loss of sulphide}}{1-(a\times b)} - \text{sulphide flowing from rumen and incorporated into microbes.}$$

RESULTS

Digestion of organic matter and N

Measurements of intakes of DM, OM and N, and of apparent digestion of OM and N in the stomach (reticulo-rumen, omasum and abomasum) and intestines are given in Table 1. The site or amount of infused sulphate did not significantly change any of these indices for sheep given brome grass. When sheep were given lucerne pellets, at least 0·9 of the apparent digestion of OM occurred in the stomach, in contrast to sheep given brome grass pellets, in which this value was approximately 0·6. There was a daily net gain in the stomach from

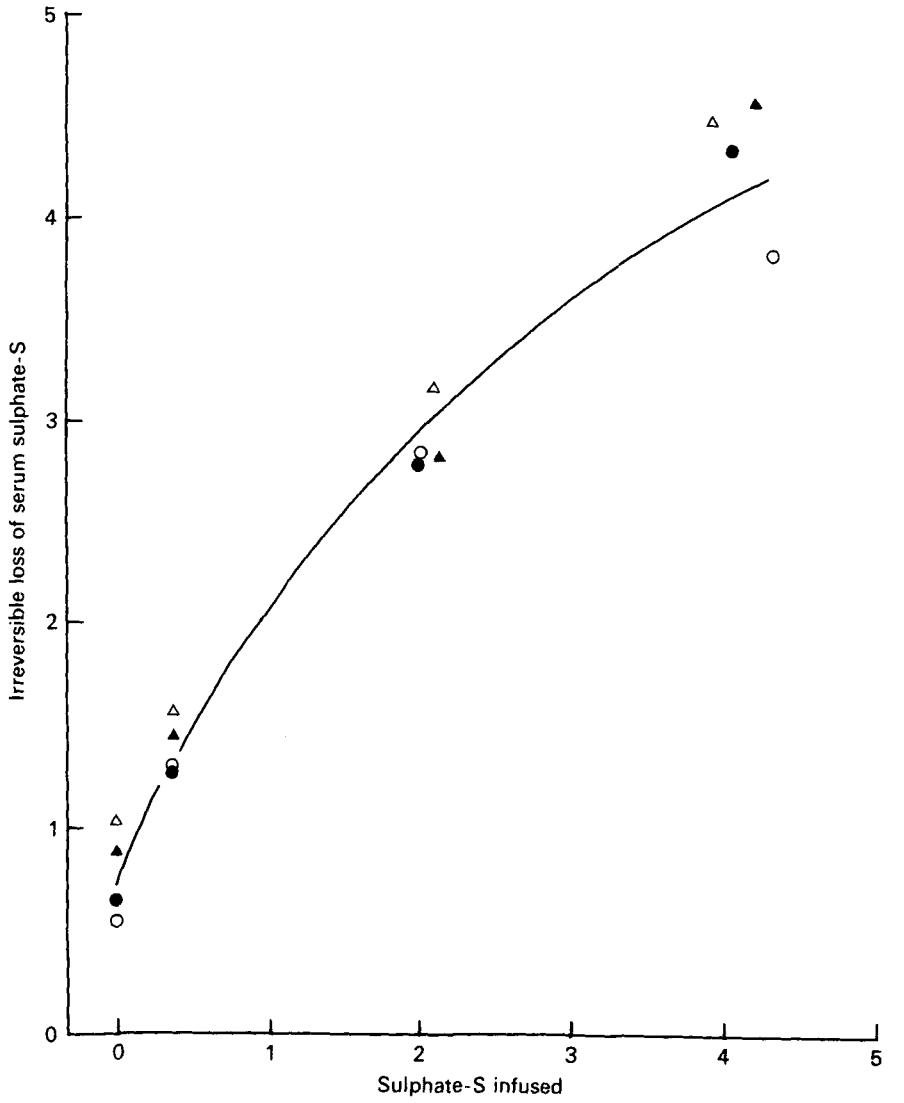

Fig. 1. Relationship between irreversible loss of inorganic sulphate from serum (g sulphur/d) and amount of sulphate infused into the rumen or abomasum (g S/d) in sheep given brome grass (*Bromus inermis*); (○), (△), sheep nos. 1, 2 (intra-abomasal infusion); (●), (▲), sheep nos. 3, 4 (intraruminal infusion). For details of procedures, see p. 66.

endogenous sources of 1–6 g non-ammonia-N in sheep given brome grass, compared to a daily net loss of 4–8 g non-ammonia-N in sheep given lucerne.

Effect of sulphate infusion on concentration and irreversible loss of sulphate in serum, and on utilization and excretion of sulphate

Infusions of sulphate increased the concentration of serum inorganic sulphate in sheep given brome grass to maximum values of approximately 35 mg S/l for sheep nos. 1–3, and 46 mg S/l for sheep no. 4. Serum sulphate levels in sheep given lucerne were 33–45 mg S/l (Table 2).

Sheep given brome grass without sulphate infusion excreted 21–68 mg S as urinary

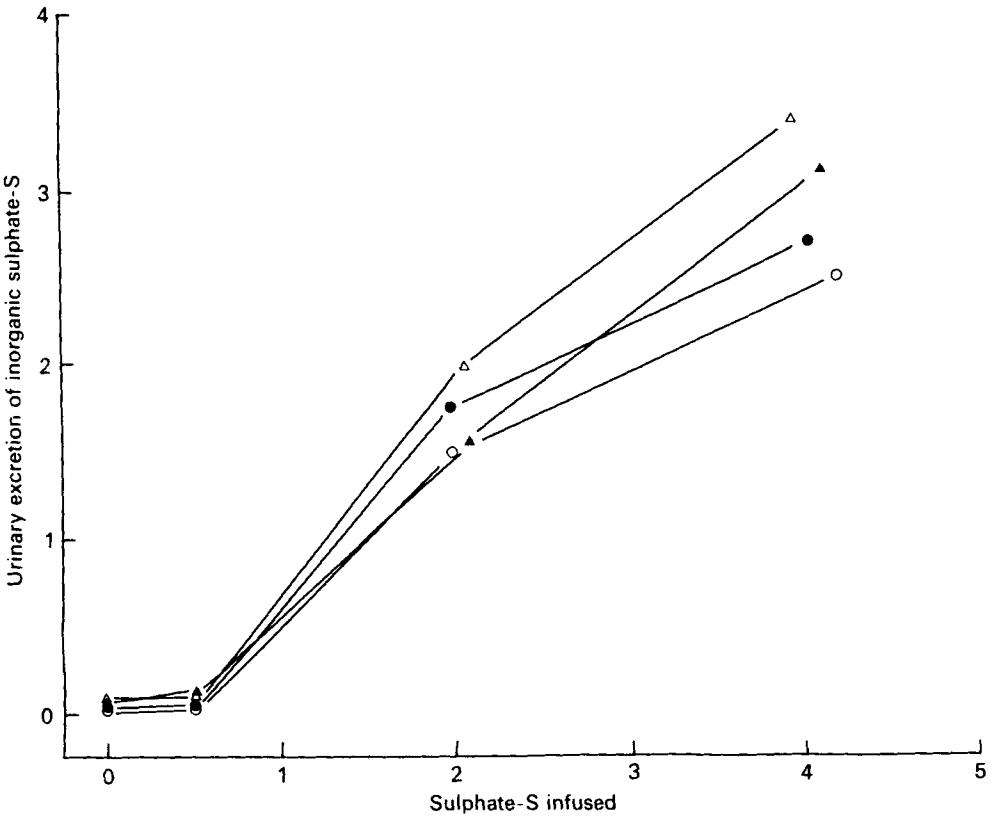

Fig. 2. Effects of sulphate infusion (g sulphur/d) on excretion of urinary inorganic sulphate (g S/d) in sheep given brome grass (*Bromus inermis*); (○), (△), sheep nos, 1, 2 (intra-abomasal infusion); (●), (▲), sheep nos. 3, 4 (intraruminal infusion). For details of procedures, see p. 66.

inorganic sulphate/d; this value increased to 427–956 mg S/d for sheep given lucerne. The irreversible loss of serum sulphate and excretion of urinary inorganic sulphate increased with the amount of sulphate infused into either the rumen or abomasum (Figs 1, 2). Values for the proportion of serum sulphate derived from rumen sulphide are given in Fig. 3.

The utilization of inorganic sulphate in the body tissues and gut of sheep given brome grass was increased from basal values of 560–960 to 1030–1560 mg S/d by infusion of sulphate. The values for sheep given lucerne were 1053–1210 mg S/d (Table 2). Utilization of sulphate in body tissues was 250–500, 470–600, 450–670 mg S/d in sheep given brome grass, brome grass plus sulphate, and lucerne respectively, and thus accounted for approximately 0·5 of total utilization. When incorporation of sulphate recycled to the rumen into microbial protein was calculated as the product of (proportion of ruminal sulphide derived from serum sulphate) × (proportion of microbial S derived from ruminal sulphide) × (quantity of microbial S leaving rumen) it appeared that (mean ± SEM) 0·90 ± 0·05 of gut utilization of sulphate occurred in the intestines.

Sulphate reabsorption in the kidney

Reabsorption of sulphate in the kidney tubules increased linearly to a maximum value (T_m) which differed markedly between sheep (Fig. 4). When T_m was expressed as a function of GFR to allow for body-weight differences (Berglund, 1960) it was found that those sheep

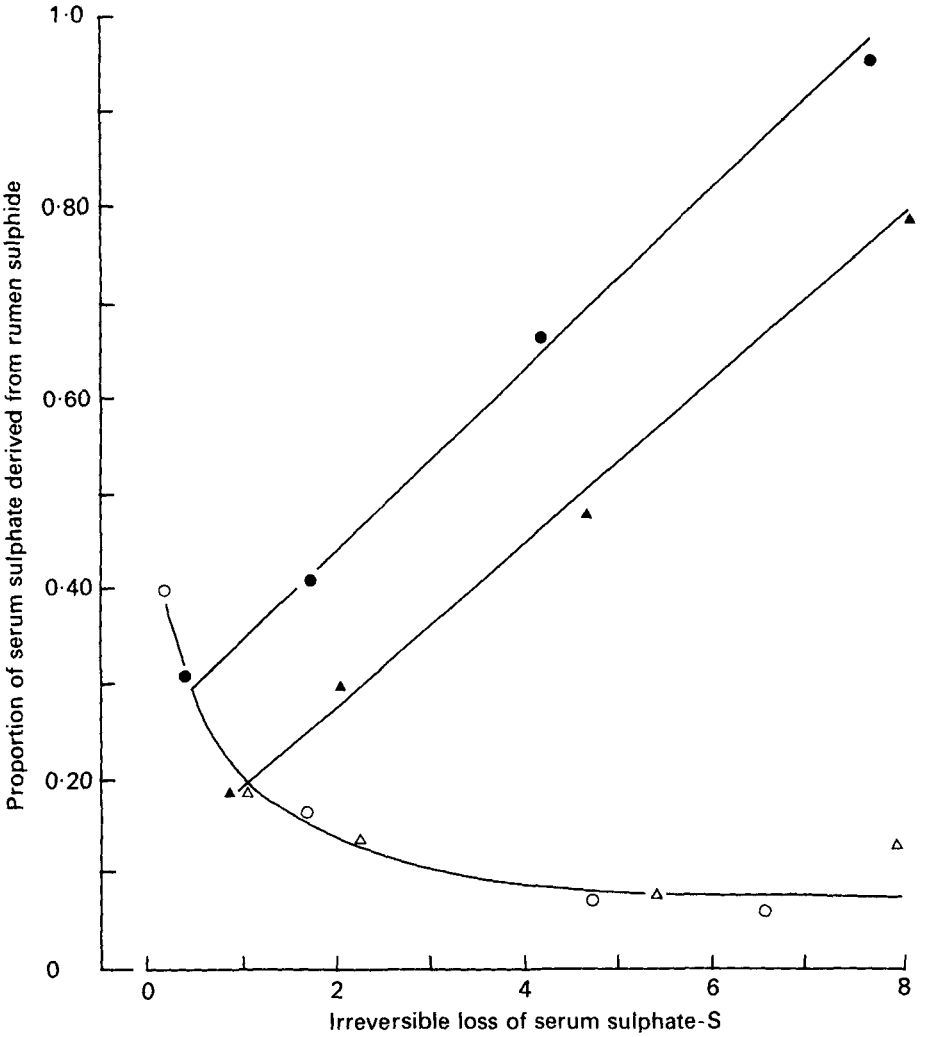

Fig. 3. Relationship between the proportion of serum sulphate derived from rumen sulphide and the irreversible loss of serum sulphate (g sulphur/d) in sheep given brome grass (*Bromus inermis*); (○), (△), sheep nos. 1, 2 (intra-abomasal infusion); (●), (▲), sheep nos. 3, 4 (intraruminal infusion). For details of procedures, see p. 66.

(nos. 1, 2 and 3) which exhibited a maximal concentration of serum inorganic sulphate of approximately 35 mg S/l had values for T_m of approximately 0·71, 0·82 and 0·69 mmol S/l glomerular filtrate respectively, compared with corresponding values of 46 mg S/l and 1·1 mmol S/l glomerular filtrate for sheep no. 4.

Movement of S through the rumen

Digestibility of dietary S in the stomach of sheep given lucerne was 0·54–0·72, in contrast to values of 0·11–0·33 in the same sheep given brome grass in the absence of infused sulphate (Table 3). The rates of irreversible loss and absorption of sulphide from the rumen were also greater for sheep given lucerne than for sheep given brome grass (Table 3). There was an increase in microbial S synthesized when sulphate was infused into the rumen of sheep nos. 3 and 4 (Fig. 7), but not when sulphate was infused into the abomasum (sheep nos. 1

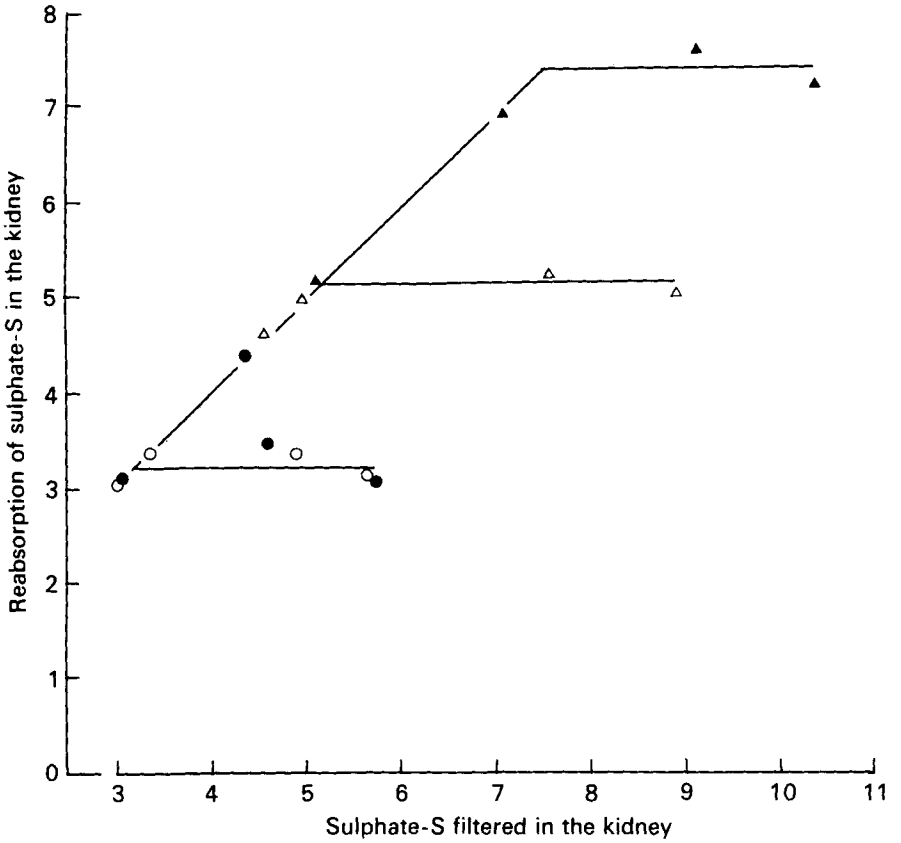

Fig. 4. Relationship between reabsorption of sulphate in kidney (g sulphur/d) and filtered sulphate load (g S/d) in the kidney in sheep given brome grass (*Bromus inermis*); (○), (△), sheep nos. 1, 2 (intra-abomasal infusion); (●), (▲), sheep nos. 3, 4 (intraruminal infusion). For details of procedure, see p. 66.

and 2). When sulphate was infused into the rumen (sheep nos. 3 and 4), there was an increase in both sulphide concentration and irreversible loss of sulphide (Figs 5, 6).

Absorption accounted for 0·9 or greater of movement of sulphide from the rumen, since only small amounts flowed from the rumen in digesta (Table 3). The absorption of sulphide from the rumen was highly correlated (*r* 0·91–0·99) with the concentration of sulphide in the rumen and to the quantity of serum sulphate which was derived from sulphide (Figs 8, 9).

In sheep given brome grass 0·52–0·67 of the bacterial S was obtained from sulphide and the values were approximately 0·45 in the same sheep given lucerne (Table 3). The analytical method used for the sulphide determination measured both extracellular and intracellular sulphide. Approximately 0·9 of protozoal S may have been derived from bacterial S for sheep given both diets (Table 3). Infusion of sulphate did not influence the proportion of S in isolated, washed bacterial cells that was derived from sulphide.

Transfer of serum sulphate-S to rumen sulphide

The proportions of rumen sulphide derived from serum inorganic sulphate for both diets are given in Table 3 and Fig. 10. The transfer of serum sulphate to the rumen was estimated as 45–127 mg S/d for sheep given the basal brome grass diet, and 127–159 mg S/d for these sheep when given lucerne. At the highest level of sulphate infusion (experimental period 4),

Table 3. *Estimates of digestibility of dietary sulphur, and of the flow from the rumen, transfer of sulphate from blood to the rumen, and of irreversible loss, absorption (mg S/d) and concentration of sulphide in the rumen (mg S/l), and of the proportion of bacterial and protozoal S derived from sulphide and bacterial S respectively for sheep given brome grass (Bromus inermis) pellets or lucerne (Medicago sativa) pellets*

(Means with their standard errors for sheep given brome grass and receiving infusions of sodium sulphate (0, 0·5, 2 or 4 g S/d) except where indicated)

	Brome grass								Lucerne			
Sheep no. ...	1		2		3		4					
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	1	2	3	4
S leaving rumen												
Organic S	1352	28	2372	102	1358	118	2562	133	1185	2832	1201	2827
Microbial S	497	52	814	70	321*		835*		698	1306	643	1213
Sulphide	14	3	13	5	19	15	56	69	10	24	12	33
Sulphate-S	20	8	38	15	26	9	50	29	4	7	4	6
Bacterial S from sulphide	0·646	0·125	0·520	0·184	0·661	0·049	0·674	0·085	0·455	0·450	0·426	0·421
Protozoal S from bacterial S†	0·925	0·039	0·887	0·074	0·864	0·081	0·888	0·086	0·877	0·930	0·848	0·880
Digestion of dietary S in the stomach*	251		739		118		670		1267	1984	1197	1896
Digestibility of dietary S in the stomach*	0·23		0·33		0·11		0·30		0·72	0·57	0·68	0·54
Irreversible loss of sulphide from rumen*	499		476		396		570		1302	1754	1080	1586
Absorption of sulphide from rumen*	266		175		220		139		1054	1241	869	1145
Sulphide concentration in rumen*	1·6		1·3		0·85		1·8		1·5	1·5	1·4	2·1
Transfer of sulphate from blood to rumen*	75		127		106		45		134	159	127	152

* Values for basal diet only.
† Calculated assuming incorporation of label by way of bacterial organic S only.

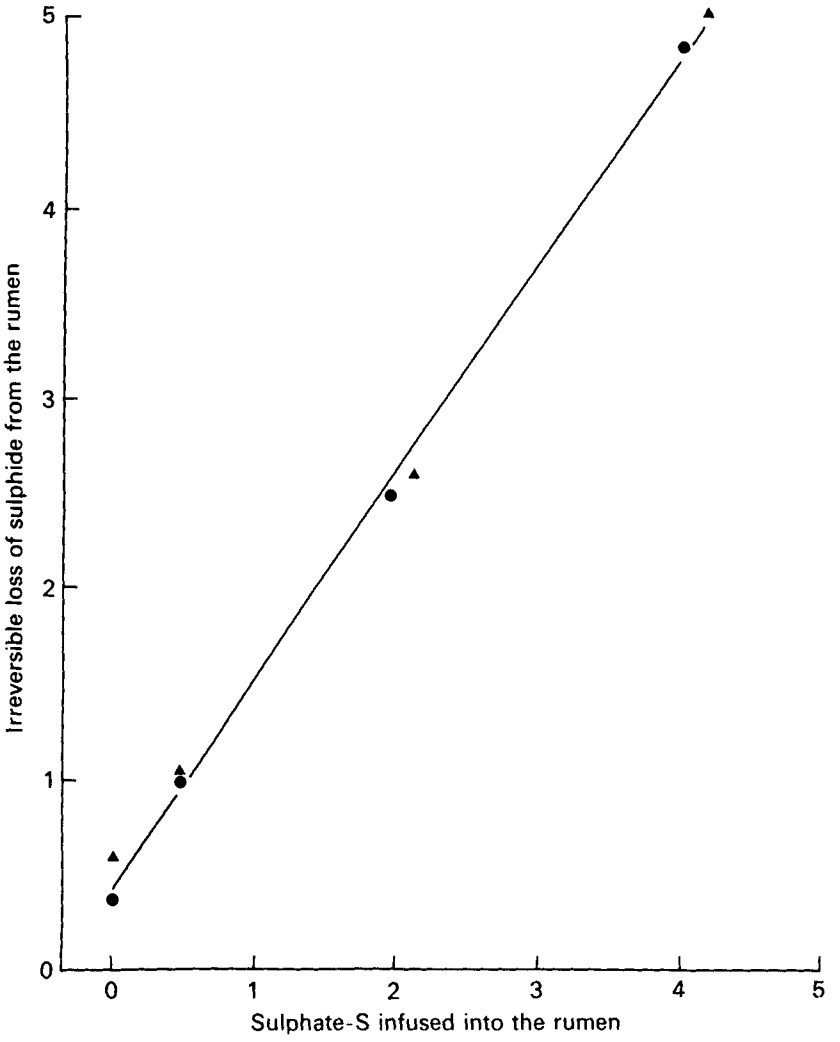

Fig. 5. Relationship between irreversible loss of sulphide (g sulphur/d; Y) and the level of sulphate infusion (g S/d; X) into the rumen; (●), (▲), sheep nos. 3, 4.

$$Y = 1{\cdot}09X + 0{\cdot}44, \quad r\ 0{\cdot}99.$$

For details of procedures, see p. 66.

this transfer (mean ± SEM) was 133 ± 13 mg S/d. Using values from the present study and from other sources (Kennedy *et al.* 1975; Kennedy, Hogan *et al.* 1976; Kennedy, unpublished results), a relationship between transfer of sulphate to the rumen (Y; mg S/d per kg body-weight) and concentration of inorganic sulphate in serum (X; mg S/l) (Fig. 11) in sheep and cattle was obtained according to the equation:

$$Y = 0{\cdot}0577X + 0{\cdot}176 \quad (r\ 0{\cdot}82, \text{residual SD } 0{\cdot}585).$$

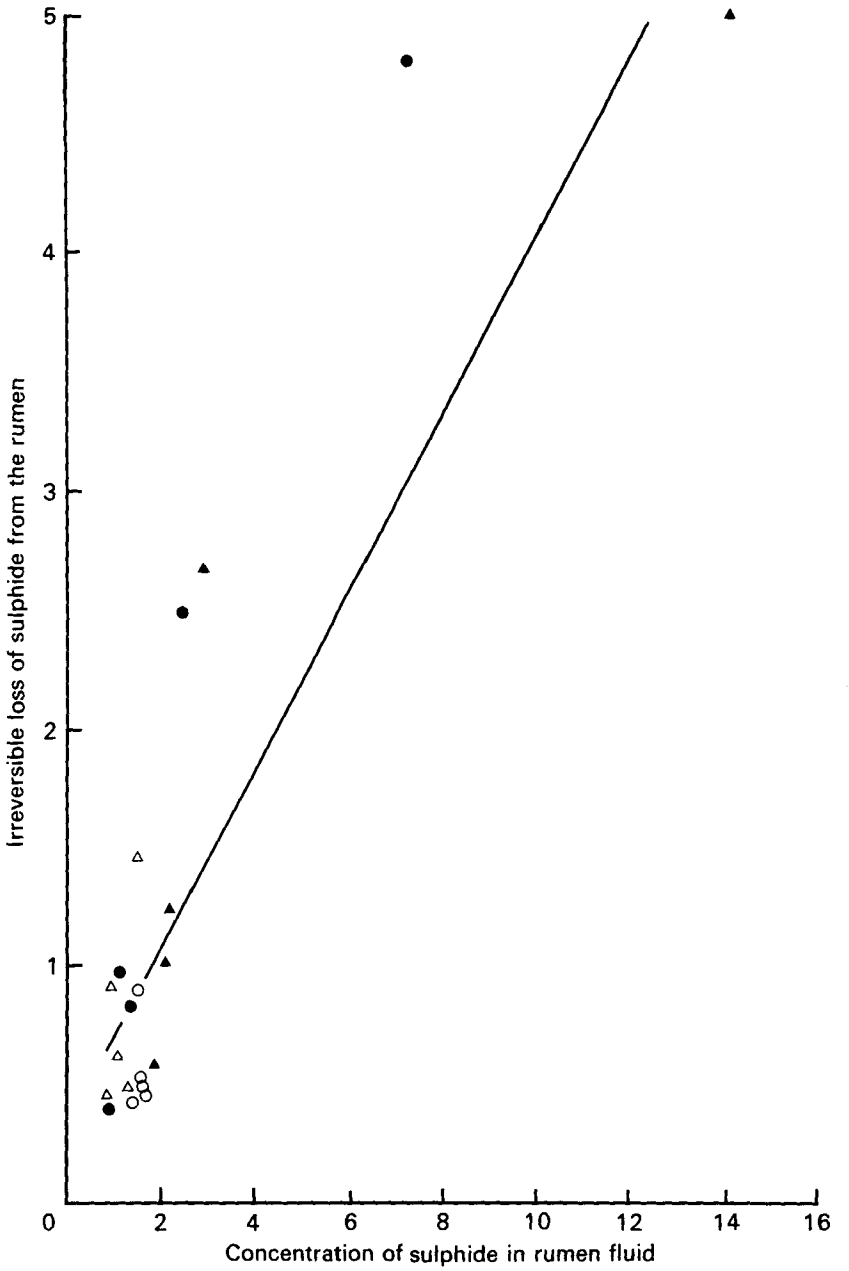

Fig. 6. Relationship between irreversible loss of sulphide (g sulphur/d; Y) and sulphide concentration (mg S/l; X) in the rumen of sheep given brome grass (*Bromus inermis*) or lucerne (*Medicago sativa*).

$$Y = 0{\cdot}393X + 0{\cdot}374, \quad r\ 0{\cdot}87.$$

(○), (△), sheep nos. 1, 2 (intra-abomasal infusion); (●), (▲), sheep nos. 3, 4 (intraruminal infusion). For details of procedure, see p. 66.

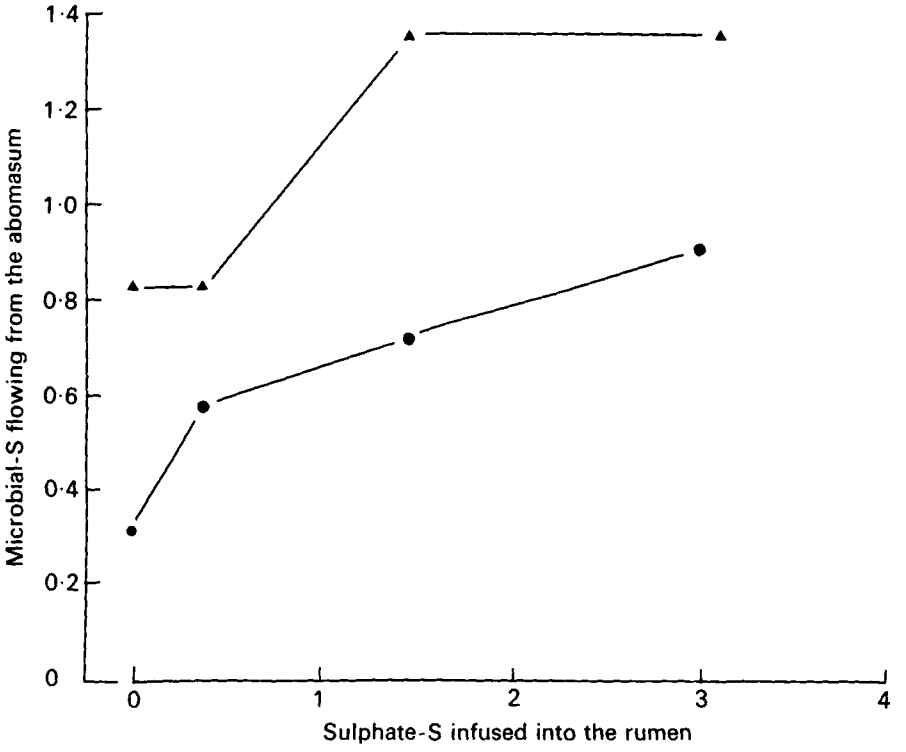

Fig. 7. Effects of sulphate infusion (g sulphur/d) into the rumen on the production of microbial S (g S/d) in sheep given brome grass (*Bromus inermis*); (●), (▲), sheep nos. 3, 4. For details of procedures, see p. 66.

Summary of S metabolism in the body

A schematic diagram of the apparent movements of organic S, microbial S, sulphate and sulphide in the rumen is presented in Fig. 12 for sheep given brome grass or lucerne pellets.

DISCUSSION

Sulphate infused into the abomasum was efficiently absorbed into the serum inorganic sulphate pool, with the increase in the rate of irreversible loss of serum inorganic sulphate representing 0·87–0·90 of the amount of infused sulphate in three sheep (Fig. 1). However, the capacity of the intestines to absorb sulphate was apparently exceeded in one sheep receiving 4 g sulphate-S/d infused into the abomasum, where the increase in irreversible loss of serum inorganic sulphate accounted for only approximately 0·4 of the increment from 2 to 4 g S as infused sulphate/d. Bird & Moir (1971) found that the absorptive capacity of the intestines of sheep was approximately 5 g sulphate-S/d.

Sulphate infused into the rumen was largely reduced to sulphide, the increase in the rate of irreversible loss of rumen sulphide representing 0·98 of infused sulphate (Fig. 5). At the highest level of sulphate infusion, only approximately 0·02 of the infused sulphate was detected flowing from the rumen in solution or in abomasal digesta. Bird (1971, 1972*a*) reported that only small amounts of sulphate escaped from the stomach of sheep given sulphate in the diet or as an intraruminal infusion. Other authors have described high concentrations of sulphate-reducing bacteria in the rumen of sheep given sulphate as the only S source (Huisingh, McNeill & Matrone, 1974).

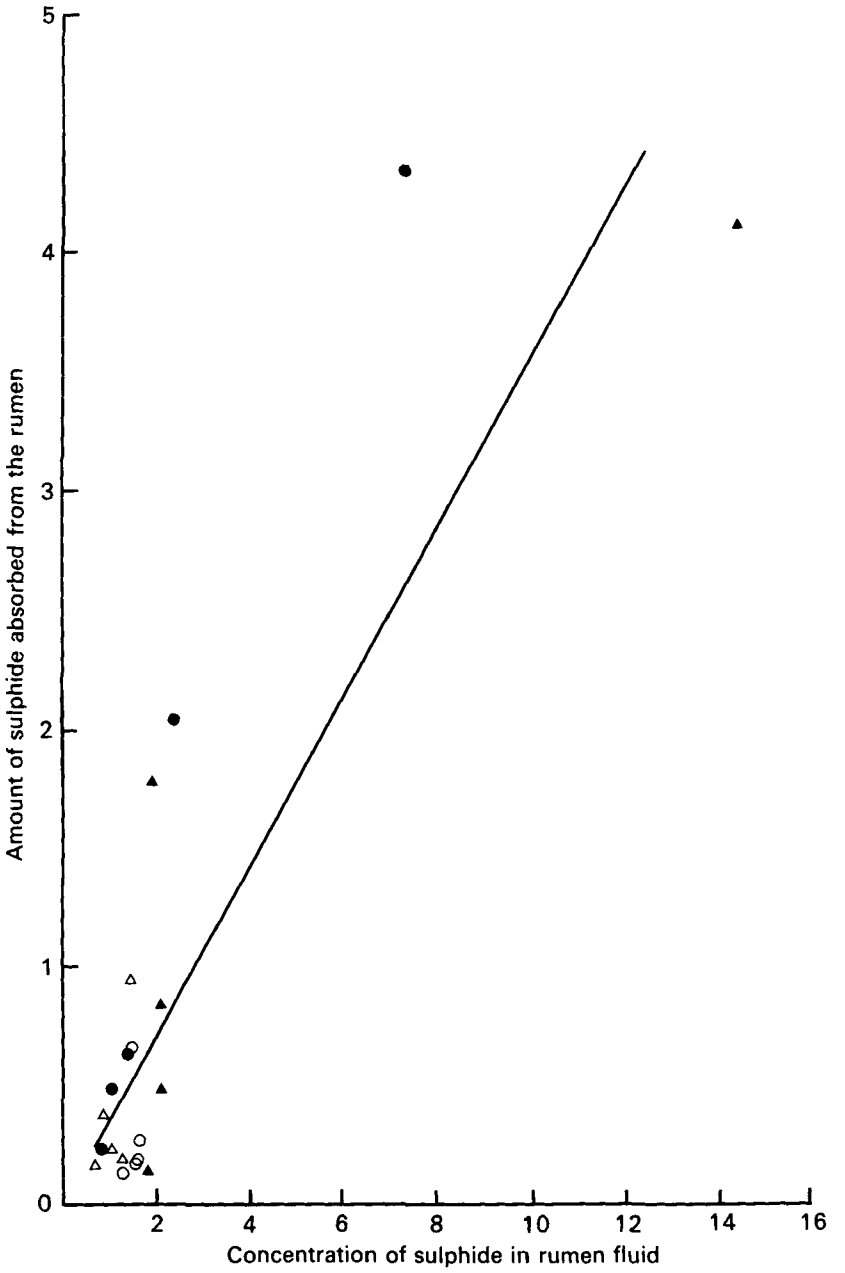

Fig. 8. Relationship between absorption of sulphide (g sulphur/d; Y) and sulphide concentration (mg S/l; X) in the rumen of sheep given brome grass (*Bromus inermis*) or lucerne (*Medicago sativa*).

$$Y = 0{\cdot}356X + 0{\cdot}026, \quad r\ 0{\cdot}91.$$

(○), (△), sheep nos. 1, 2 (intra-abomasal infusion); (●), (▲), sheep nos. 3, 4 (intraruminal infusion). For details of procedures, see p. 66.

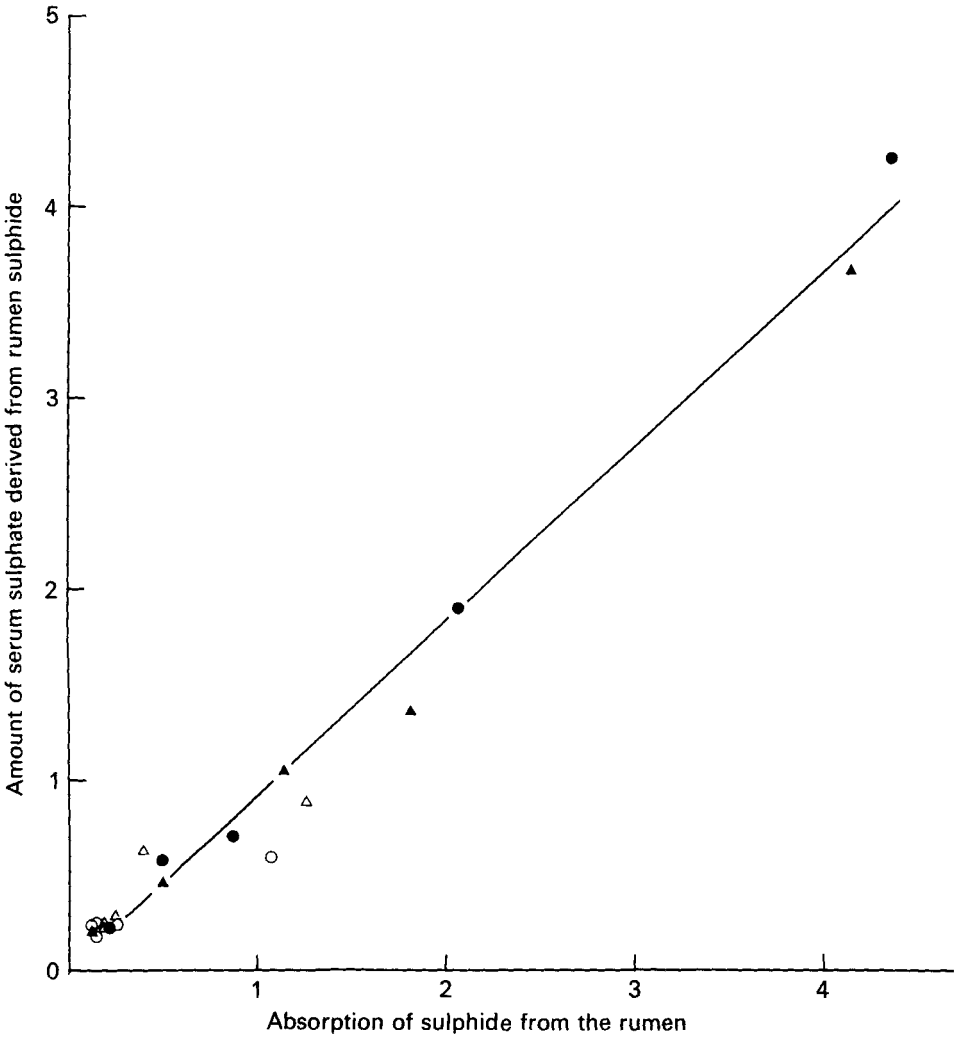

Fig. 9. Relationship between the amount of serum sulphate derived from sulphide (g sulphur/d; Y) and the absorption of sulphide from the rumen (g S/d; X) of sheep given brome grass (*Bromus inermis*) or lucerne (*Medicago sativa*).

$$Y = 0{\cdot}926X + 0{\cdot}001, \quad r\ 0{\cdot}99.$$

(○), (△), sheep nos. 1, 2 (intra-abomasal infusion); (●), (▲), sheep nos. 3, 4 (intraruminal infusion). For details of procedures, see p. 67.

In the present study, both diets appeared to supply adequate quantities of S to maintain microbial production, since infusion of sulphate into the rumen did not increase the flow of non-ammonia-N from the abomasum, in contrast to the situation with sheep given semi-purified diets with a wide range of values for N:S where a reduction in microbial growth may occur (Hume & Bird, 1970; Bird, 1972*b*). Other measurements using ^{15}N as a microbial marker support this conclusion (Kennedy & Milligan, unpublished results), in seeming contradiction to the observation that the amount of microbial S as indicated by organic ^{35}S flowing from the abomasum was increased by intraruminal infusion of sulphate to the

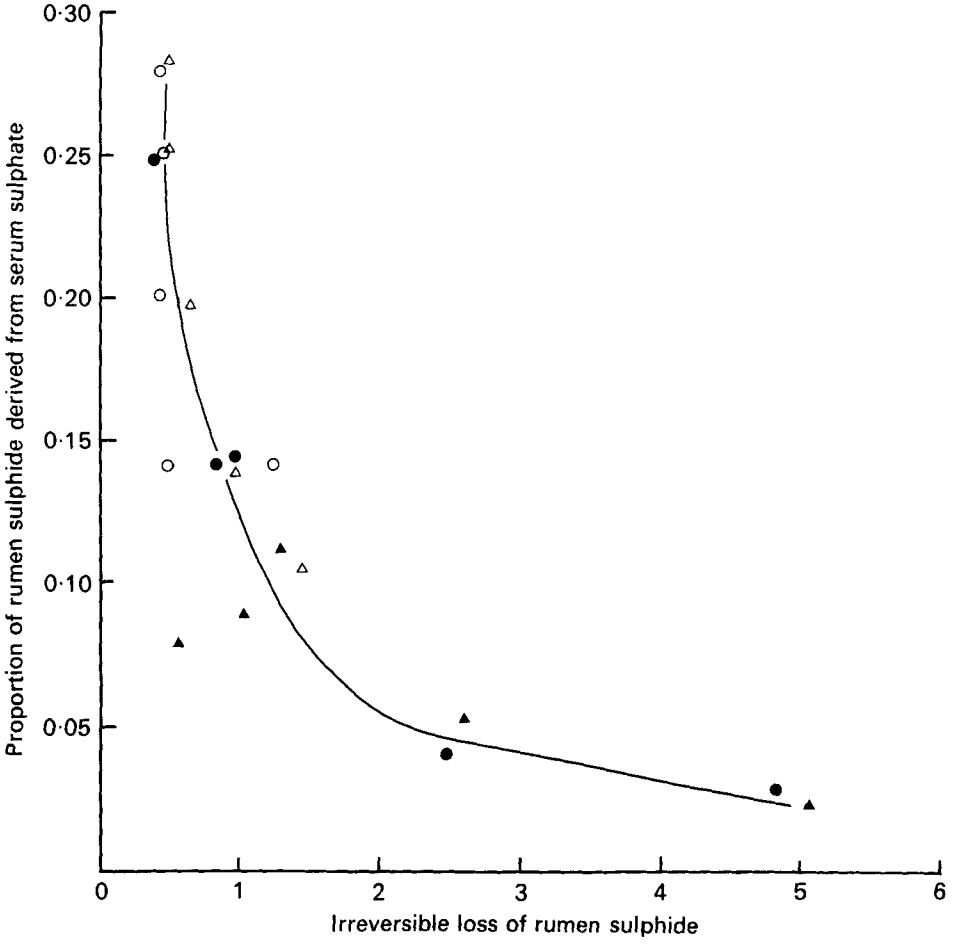

Fig. 10. Relationship between the proportion of ruminal sulphide derived from serum sulphate and irreversible loss of sulphide (g sulphur/d) of sheep given brome grass (*Bromus inermis*) or lucerne (*Medicago sativa*); (○), (△), sheep nos. 1, 2 (intra-abomasal infusion); (●), (▲), sheep nos. 3, 4 (intraruminal infusion). For details of procedures, see p. 67.

extent of approximately 0·1 of the quantity of sulphate-S infused (Fig. 7). Since no concurrent increase in the concentration of organic S in bacteria or protozoa isolated from the rumen was noted, the increase in microbial ^{35}S flowing from the abomasum was probably due to an increased S content of microbes associated with food particles escaping the rumen, to an artifact caused by the secretion of endogenous ^{35}S-labelled organic S into the omasum or abomasum, or to secretion of glycoprotein by rumen bacteria (Cheng, Akin & Costerton, 1976). It should be noted that Bird (1971) and Kennedy (1974) found that intraruminal infusion of sulphate increased faecal excretion of organic S but not of N, and Bray & Till (1975) observed that the value 14:1 for non-ammonia-N:non-sulphate-S of digesta reaching the intestines was narrower than many values reported for rumen micro-organisms.

The absorption of sulphide from the rumen appeared to be related to sulphide concentration in the rumen (Fig. 8). Such a relationship is in agreement with the results of Anderson (1956) and Bray (1969*a*), who found that the absorption of a dose of sulphide introduced into the rumen was described by first-order kinetics, as occurs for ammonia (Hogan, 1961).

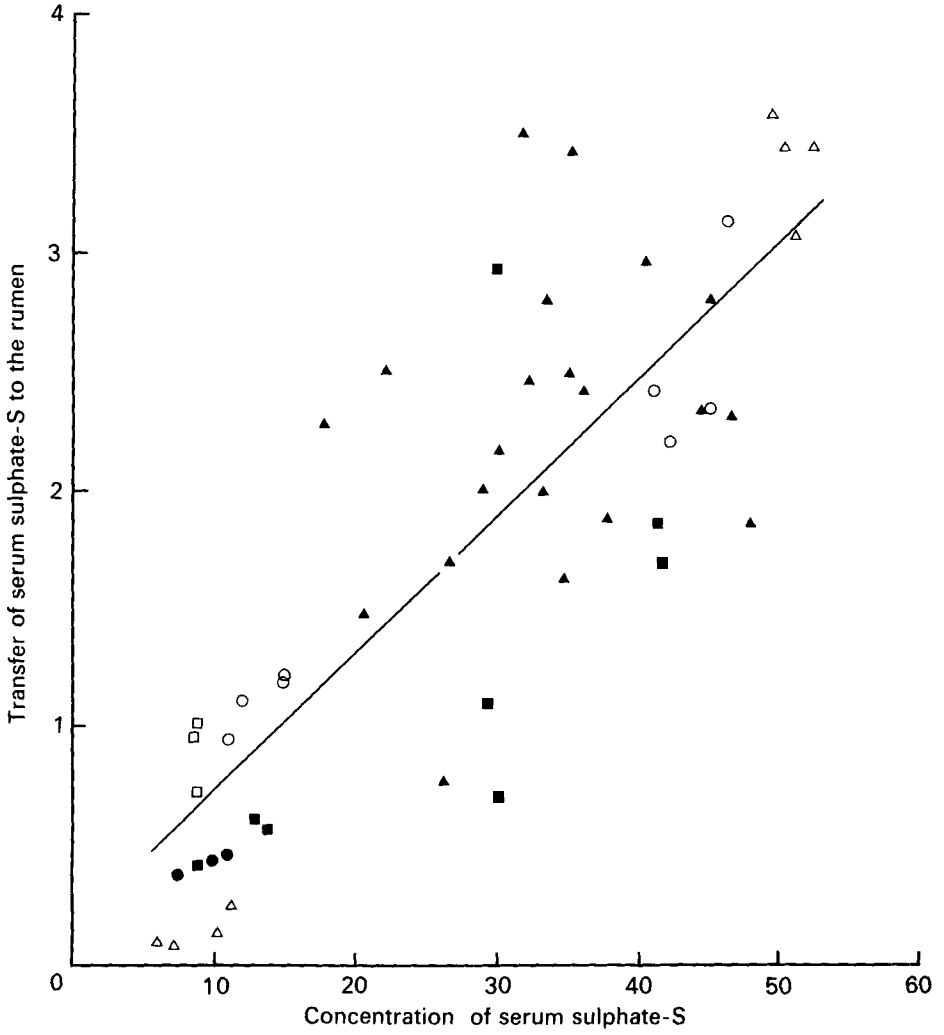

Fig. 11. Relationship between transfer of serum sulphate to the rumen (mg sulphur/d per kg body-wt; Y) and the concentration of serum sulphate (mg S/l; X).

$$Y = 0{\cdot}0577X + 0{\cdot}176, \quad r\,0{\cdot}82.$$

Sheep: (▲), diet of brome grass (*Bromus inermis*), lucerne (*Medicago sativa*) pellets (present experiment); (△), diet tropical grass, lucerne (Kennedy *et al.* 1975); (□), semi-purified diet (Kennedy, Hogan *et al.* 1976); (■), tropical grass–lucerne mixtures (Kennedy, unpublished results); Cattle: (○), diet tropical grass, lucerne (Kennedy *et al.* 1975); (●), tropical grass–lucerne mixtures (Kennedy, unpublished results).

Since 0·93 of sulphide absorbed from the rumen entered the serum inorganic sulphate pool (Fig. 9), it may be concluded that either incorporation of S from sulphide into esterified sulphate-S in the liver (Dziewiatkowski, 1949) is minor, or the turnover of such compounds is sufficiently rapid that equilibration of ^{35}S in ester sulphate and serum inorganic sulphate is essentially complete within 3–4 d.

The regulation of excretion of sulphate via the kidney of sheep resembles that in other mammals. When filtered load exceeds the T_m of sulphate, excretion of inorganic sulphate in

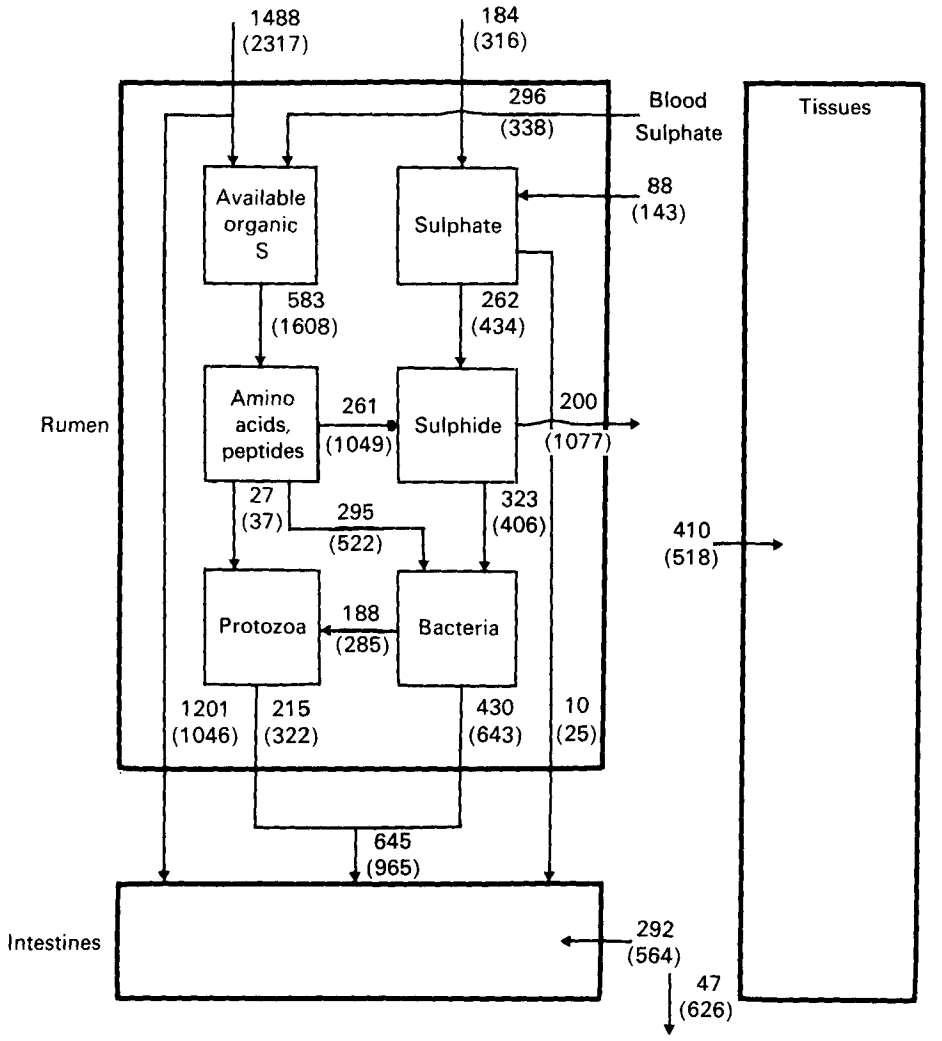

Fig. 12. A model of sulphur metabolism showing the movement of S (mg S/d) between pools in the rumen and blood in sheep given brome grass (*Bromus inermis*) or lucerne (*Medicago sativa*) pellets. Values in parentheses are for the lucerne diet.

the urine sharply increases (Berglund, 1960). A marked variability of T_m between individuals as noted for dogs (Lotspeich, 1947; Berglund, 1960) and sheep (present experiment) may account for differences between maximum serum sulphate concentrations in this experiment and published values of approximately 60 mg S/l for sheep (Whiting, Slen, Bezeau & Clark, 1954; Peirce, 1960; Bray & Hemsley, 1969).

Sulphate from the serum inorganic sulphate pool is used in the body for esterification reactions and in the gut, with postruminal secretion of mucoprotein (Clarke, Ellinger & Phillipson, 1966; Bird & Thornton, 1972; Hecker, 1973), bile and pancreatic secretions (Bird, 1972*a*) and entry of ester sulphates associated with plasma proteins (Bray & Till, 1975) probably accounting for the majority of gut utilization. The extent to which bacteria in the hind-gut would derive S from sulphate secreted into the caecum and colon from the blood (Bird & Thornton, 1972) compared with S derived from breakdown of mucoprotein is

unknown, but certainly mucus isolated from the sheep colon is rich in sulphate (Kent & Marsden, 1963).

The reliance of bacteria on sulphide to provide 0·42–0·67 of bacterial organic S is comparable to the values of Gawthorne & Nader (1976), who found that 0·52–0·57 of the S of S amino acids in rumen micro-organisms was synthesized de novo from the sulphide pool, but lower than values from an in vitro experiment in which 0·99 of cystine-S and 0·89 of methionine-S of rumen bacteria was derived from sulphide (Nader & Walker, 1970). For purposes of comparison published results have shown that nitrogenous precursors other than ammonia are also utilized extensively in microbial synthesis, with 0·36–0·78 of bacterial N being derived from ammonia, and 0·56–0·96 of protozoal N derived from bacteria (Pilgrim, Gray, Weller & Belling, 1970; Mathison & Milligan, 1971).

In contrast to the results obtained in the present experiment, Gawthorne & Nader (1976) stated that in sheep receiving an intraruminal infusion of 2 g sulphate-S/d, of the 3·0 g S/d that passed through the sulphate pool in rumen fluid, 1·17 g S/d entered the sulphide pool, and 1·94 g S/d left the rumen by an unidentified pathway. If the latter pathway is quantitatively significant, the estimates of irreversible loss of sulphide in the present experiments would be invalid, since it is implicit in our estimate that infused ^{35}S that did not pass from the rumen as sulphate was reduced to sulphide. Because it has been demonstrated (Bray, 1969*b*) that sulphate is not absorbed from buffer solutions placed in the rumen, and that the sulphate content of abomasal digesta can be attributed to dissolved sulphate flowing from the rumen (present results), a pathway as indicated by Gawthorne & Nader (1976) would require release and absorption in the omasum and abomasum of sulphate attached to large plant particles or in sparingly-soluble sulphates. Also the values (640–880 mg S/d) calculated for the transfer of endogenous sulphate into the rumen of sheep by Gawthorne & Nader (1976) are high compared to the more direct estimates reported in the present paper. However, the calculations of Gawthorne & Nader (1976) are not directly accepted by us because they depend on the correct estimation of the pool size of rumen sulphide, a substantial proportion of which is intracellular (Bray & Hemsley, 1969), and on the isolation of inorganic sulphate from ester sulphates in rumen fluid to calculate irreversible loss of inorganic sulphate.

The transfer of endogenous S to the rumen may be of major importance to the nutrition of rumen micro-organisms, especially for ruminants given forage diets (Bray & Hemsley, 1969; Hume & Bird, 1970) and may result in a net gain in the stomach of up to 0·44 g S/d (Bird & Hume, 1971). The contribution of sulphate from blood to rumen sulphide appears to be relatively minor although measurements using ^{35}S have been limited to grass and legume diets. If it is assumed that the transfer of sulphate from the blood directly across the rumen wall may reach a maximum of 20 mg S/d (Bray, 1969*b*), then 110–140 mg S/d may enter the rumen via salivary secretions, since the highest values for total transfer of sulphate measured in the present experiments were 130–160 mg S/d. The concentration of total sulphate in mixed saliva may approach 0·5 of the inorganic sulphate concentration in blood (Bray, 1969*b*; Kennedy & Hogan, unpublished results), therefore the return of 110–140 mg sulphate-S/d to the rumen would require secretion of approximately 7 l saliva/d. Such rates are easily attained in sheep (Kay, 1960). Suggestions that the rate of transfer of sulphate from blood to the rumen is related to sulphate concentrations in blood and saliva (Bray & Hemsley, 1969; Moir, 1970; Kennedy *et al.* 1975) are supported by the linear relationship observed between sulphate transfer and serum inorganic sulphate concentration (Fig. 11). The regression accounted for 0·67 of the variance with individual differences in salivary flow probably accounting for much of the remaining variance.

The entry of endogenous organic S into the sheep's rumen may be calculated if it is assumed that entry of S in feed and endogenous organic S and sulphate equals the absorption

of sulphide from the rumen plus flow of total S from the abomasum. Estimates of 296 mg S/d and 338 mg S/d for sheep given brome grass and lucerne pellets respectively indicate that the entry of endogenous organic S into the rumen may exceed sulphate entry by a factor of at least 2–3. Hogan (1975) reached a similar conclusion about the relative entry rates of organic N and urea N in sheep. Furthermore, entry of endogenous S into the rumen accounted for (mean ± SEM) 0·45 ± 0·16 and 0·24 ± 0·03 of S digested in the rumen of sheep given brome grass and lucerne pellets, assuming that all endogenous S was degraded in the rumen and all non-microbial organic S flowing from the abomasum was undegraded food S. Thus the rumen bacteria may derive a significant proportion of their S from endogenous sources, especially when the diet of the sheep contains low levels of S.

The authors gratefully acknowledge financial assistance from the National Research Council of Canada.

REFERENCES

Anderson, C. M. (1956). *N.Z. Jl Sci. Technol.* **37**, 379.
Beever, D. E., Harrison, D. G., Thomson, D. J., Cammell, S. B. & Osbourn, D. F. (1974). *Br. J. Nutr.* **32**, 99.
Berglund, F. (1960). *Acta Physiol. scand.* **49**, Suppl. 172.
Bird, P. R. (1971). *Aust. J. biol. Sci.* **24**, 1329.
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. & Fountain, R. D. (1970). *Analyst, Lond.* **95**, 98.
Bird, P. R. & Hume, I. D. (1971). *Aust. J. agric. Res.* **22**, 443.
Bird, P. R. & Moir, R. J. (1971). *Aust. J. biol. Sci.* **24**, 1319.
Bird, P. R. & Thornton, R. F. (1972). *Aust. J. biol. Sci.* **25**, 1299.
Blackburn, T. H. & Hobson, P. N. (1960). *Br. J. Nutr.* **14**, 445.
Bray, A. C. (1969*a*). *Aust. J. agric. Res.* **20**, 739.
Bray, A. C. (1969*b*). *Aust. J. agric. Res.* **20**, 749.
Bray, A. C. & Hemsley, J. A. (1969). *Aust. J. agric. Res.* **20**, 759.
Bray, A. C. & Till, A. R. (1975). In *Digestion and Metabolism in the Ruminant* [I. W. McDonald and A. C. I. Warner, editors]. Armidale, Australia: University of New England Publishing Unit.
Cheng, K.-J., Akin, D. E. & Costerton, J. W. (1976). *Fedn Proc. Fedn Am. Socs exp. Biol.* **36**, 193.
Clarke, E. M., Ellinger, G. M. & Phillipson, A. T. (1966). *Proc. Roy. Soc.* B **166**, 63.
Dziewiatkowski, D. D. (1949). *J. biol. Chem.* **178**, 389.
Faichney, G. J. (1975). In *Digestion and Metabolism in Ruminants* [I. W. McDonald and A. C. I. Warner, editors]. Armidale, Australia: University of New England Publishing Unit.
Gawthorne, J. M. & Nader, C. J. (1976). *Br. J. Nutr.* **35**, 11.
Hecker, J. F. (1973). *J. agric. Sci., Camb.* **80**, 63.
Hogan, J. P. (1961). *Aust. J. biol. Sci.* **14**, 448.
Hogan, J. P. (1975). *J. Dairy Sci.* **58**, 1164.
Huisingh, J., McNeill, J. J. & Matrone, G. (1974). *Appl. Microbiol.* **28**, 489.
Hume, I. D. & Bird, P. R. (1970). *Aust. J. agric. Res.* **21**, 315.
Kay, R. N. B. (1960). *J. Physiol., Lond.* **150**, 515.
Kent, P. W. & Marsden, J. C. (1963). *Biochem. J.* **87**, 38P.
Kennedy, P. M. (1974). *Aust. J. agric. Res.* **25**, 1015.
Kennedy, P. M., Christopherson, R. J. & Milligan, L. P. (1976). *Br. J. Nutr.* **36**, 231.
Kennedy, P. M., Hogan, J. P., Lindsay, J. R. & Hogan, R. M. (1976). *Aust. J. biol. Sci.* **29**, 525.
Kennedy, P. M., Williams, E. R. & Siebert, B. D. (1975). *Aust. J. biol. Sci.* **28**, 31.
Lotspeich, W. D. (1947). *Am. J. Physiol.* **151**, 311.
Mathison, G. W. & Milligan, L. P. (1971). *Br. J. Nutr.* **25**, 351.
Moir, R. J. (1970). In *Symposium: Sulfur in Nutrition* [O. H. Muth and J. E. Oldfield, editors]. Westport, Conn., USA: AVI Publishing Co.
Nader, C. J. & Walker, D. J. (1970). *Appl. Microbiol.* **20**, 677.
Nolan, J. V. & Leng, R. A. (1972). *Br. J. Nutr.* **27**, 177.
Nolan, J. V., Norton, B. W. & Leng, R. A. (1976). *Br. J. Nutr.* **35**, 127.
Peirce, A. W. (1960). *Aust. J. agric. Res.* **11**, 548.
Pilgrim, A. F., Gray, F. V., Weller, R. A. & Belling, C. B. (1970). *Br. J. Nutr.* **24**, 589.
Postgate, J. R. (1965). *Bact. Rev.* **29**, 425.

Shipley, R. A. & Clark, R. E. (1972). *Tracer Methods for in vivo Kinetics*. New York and London: Academic Press.
Stacy, B. D. & Thorburn, G. D. (1966). *Science, N.Y.* **152**, 1076.
Tan, T. N., Weston, R. H. & Hogan, J. P. (1971). *Int. J. appl. Radiat. Isotopes* **22**, 301.
Walker, D. J. & Nader, C. J. (1975). *Aust. J. agric. Res.* **26**, 689.
Whiting, F., Slen, S. B., Bezeau, L. M. & Clark, R. D. (1954). *Can. J. agric. Sci.* **34**, 261.

Printed in Great Britain