

Sulfur and Methionine Metabolism in Sheep.

I. First Approximations of Sulfur Pools in and Sulfur Flows from the Reticulo-rumen

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Abstract

Sulfur pools in the rumen and sulfur flows from the rumen were investigated in two experiments with sheep on a diet containing equal parts of oaten and lucerne chaffs. The diet was fed at two levels, either chopped or pelleted, and with intraruminal DL-methionine supplements. Ruminal fluid volumes and fluid flows to the omasum were measured.

None of the treatments influenced ruminal fluid volume. Fluid flow to the omasum, however, was increased by increasing dry matter intake (DMI), and was further enhanced by feeding chaffed hay rather than the same materials ground and pelleted; the DL-methionine supplement had no effect.

First approximations of the ruminal sulfur pools and of sulfur flows to the omasum were derived from the concentration of sulfur in true digesta and the ruminal fluid volume or fluid flow. Increasing DMI from 500 to 1000 g/day resulted in larger ruminal pools of total (1096 *v.* 792 mg), neutral (1016 *v.* 731 mg) and protein (479 *v.* 419 mg) sulfur, but the reducible sulfur pools were not affected by the level of DMI. Infusions of DL-methionine increased the ruminal sulfide sulfur pool irrespective of level of DMI.

The first approximation of total sulfur flow was increased by 1660 mg/day at the higher level of DMI, due mainly to increases of 710 mg S/day as protein sulfur and 859 mg S/day as non-protein neutral sulfur. Flows of inorganic sulfate and ester sulfate sulfur, although small in comparison with organic sulfur flows, increased with level of DMI. Sulfide sulfur flows were also increased at the higher level of DMI, and were almost doubled by intraruminal infusions of DL-methionine.

Introduction

Prior to the advent of digesta markers interpretation of digestive events in ruminants depended on the concentrations of nutrients and metabolites usually determined in ruminal fluid strained through several layers of gauze or bolting silk (e.g. Moir and Harris 1962; Hemsley and Moir 1963). With the introduction of markers permitting determination of fluid flow (e.g. Hyden 1961; Downes and McDonald 1964), the concentration of nutrients, metabolites and markers was still largely determined in strained ruminal fluid.

Carrol and Hungate (1954) realized the difficulty of obtaining representative samples of total rumen contents and tried to overcome the problem by the use of large samples. For ensuing work, Hungate's group very specifically attempted to reconstitute or collect 'true ruminal digesta' in a number of their experiments (e.g. Hungate *et al.* 1961; El-Shazly and Hungate 1965). Recent advances in the methodology of digesta markers (see MacRae 1974; Faichney 1975) present the opportunity to study quantitatively the flow of different nutrients and metabolites through the gastrointestinal tract of ruminants.

Previous estimates of sulfur flows to the omasum have been based on fluid flow estimates and the concentration of sulfur in collected ruminal digesta (Bird and Moir 1972). True digesta flow from the rumen cannot be estimated by combining fluid and digesta, either mathematically or physically (see Faichney 1975), since rumen samples contain particles that have not been reduced to the dimensions required for passage from the rumen (Becker *et al.* 1963; Hungate 1966).

In order to clarify and extend previous results two experiments were devised to determine ruminal sulfur pools and sulfur flows to the omasum that approach true pools and flows.

Materials and Methods

Experimental Animals

Twenty Merino wethers, each weighing c. 38 kg and fitted with a permanent ruminal cannula (Jarrett 1948) and a simple T-piece cannula in the proximal duodenum, were used. In addition eight of the wethers were fitted with omasal cannulae (Willes and Mendel 1964; Hume *et al.* 1970) (experiment 1); the remaining 12 had simple T-piece cannulae inserted in the terminal ileum (experiment 2).

Experimental Diets

The components of the basal diet (moist weight basis) were: chopped oaten hay (oaten chaff) (49%), chopped lucerne hay (lucerne chaff) (49%) and minerals (2%). Different batches of oaten and lucerne chaffs were used in each experiment. The composition of the mineral mix is described by Hume and Bird (1970).

The diet was fed either in the form of mixed chaffs (*C*) or was ground and pelleted (*P*). For pelleted diets the chaffs were hammer-milled through a 4-mm screen, then mixed with the minerals in a rotary mixer, and pelleted through a 16-mm die.

Rations supplying 540 and 900 g dry matter (experiment 1) and 500 and 1000 g dry matter (experiment 2) per day were offered to sheep in 12 equal portions at 2-h intervals by means of automatic feeders. Both chaff and pelleted diets were used in experiment 1, and pelleted diets only in experiment 2. The rations contained on average 0.17% S and 1.99% N (experiment 1) and 0.18% S and 2.03% N (experiment 2). No feed refusals occurred during collection periods in either experiment.

Tap water was available to sheep *ad libitum* in stainless steel troughs; the daily intake was measured during collection periods. The sulfur content of tap water prior to experiment 1 was extremely low, and analysis of the sulfur content of water was not carried out during the experiments.

Infusion Solutions

DL-Methionine (commercial grade) was continually infused by a peristaltic pump into the rumen at the rate of 3.0 g/day in c. 860 ml water (experiment 1) and 4.5 g/day in c. 760 ml water (experiment 2).

The water-soluble marker polyethylene glycol 4000 (PEG) was used to estimate ruminal fluid volumes and rates of flow of fluid out of the rumen as described by Hyden (1961), Hogan (1964) and Weston and Hogan (1967) in experiment 1.

In experiment 2, ^{51}Cr -EDTA and ^{103}Ru tris(1,10-phenanthroline)ruthenium(II)chloride (^{103}Ru -phen) [prepared from $^{103}\text{RuCl}_3$ by the method of Tan *et al.* (1971)] were used. The isotopes were obtained from the Radiochemical Centre, Amersham, England. The two markers were administered as a continuous infusion (c. 6.6 μCi ^{103}Ru -phen and 32.8 μCi ^{51}Cr -EDTA per day) into the rumen over periods of 4 days after a priming dose (4.0 μCi ^{103}Ru -phen and 20.0 μCi ^{51}Cr -EDTA) of the markers had been injected.

The theory behind the use of these markers and the calculations made are outlined on pages 53–54.

Sampling Methods

Ruminal liquor (experiments 1 and 2) was obtained through a tube extending through the cannula bung into the rumen contents as described by Bird (1972a). Ruminal digesta samples (experiment 2)

were taken 10–15 min after these samples from the ventral region of the rumen by aspiration into a 13-mm diameter polythene tube inserted through the cannula. Liquor samples were analysed immediately for sulfide sulfur; the remaining liquor and the digesta samples were stored at -20°C for subsequent analyses.

Sampling schedules were designed to cover variations between 2-h feeds and diurnal variations in the flow pattern by taking 11 samples over 3 days, each sample following a different feeding time, consecutive samples being taken approximately 30, 60 or 90 min after a feed.

Marker analysis was carried out on individual samples in both experiments. The remaining material was bulked for subsequent analyses. Ruminal digesta (experiment 2) was separated into particulate (A) and fluid (B) fractions by centrifuging at 100 g for 20 min in a refrigerated centrifuge. The supernatant was decanted off and the process repeated twice on the resulting fluid. The particulate matter from each centrifugation was resuspended in 0.9% (w/v) NaCl, bulked, and centrifuged. The supernatant from two such washings was decanted off and bulked with the fluid fraction.

Faeces and urine samples were collected and treated as described by Hume *et al.* (1970). ^{51}Cr -EDTA and ^{103}Ru -phen analysis was carried out on daily samples.

Calculation of Ruminal Fluid Volume, Fluid Flow from the Rumen, Ruminal Fluid Sulfur Pools, Flow of Sulfur in Fluid to the Omasum, the Flow of True Digesta, and the Concentration of Sulfur in True Digesta

(i) *Fluid phase calculations*

A mathematical study of the movement of particles and solutes by Warner (1966) and a digesta flow study by Weston and Hogan (1967) indicate that in a 'steady state' system

$$F = 0.693 V/T, \quad (1)$$

where F is the rate of fluid flow from the rumen, V is the volume of liquid in the rumen (ruminal fluid volume), and T is the time for the equivalent of half of the liquid in the rumen to be transferred to the omasum. When a water-soluble marker (PEG or ^{51}Cr -EDTA) is infused continuously into the rumen it may be shown that

$$F = I/C \quad (2)$$

and

$$\begin{aligned} R &= V/F \\ &= 1.44 T, \end{aligned} \quad (3)$$

where I is the rate of infusion of marker into the rumen, C is the concentration of marker in the liquid leaving the rumen, and R is the mean retention time of a population of marker molecules in the rumen.

In experiment 1 ruminal fluid sulfur pools were calculated from the concentration of sulfur in fluid multiplied by the ruminal fluid volume (from equation 1). The flow of sulfur in fluid was estimated by multiplying the concentration of sulfur in ruminal fluid by the fluid flow to the omasum (from equation 2).

(ii) *Reconstitution of true digesta composition, and composition and approximations of the flow of constituents of true digesta*

The criteria of the ideal marker have been listed by Faichney (1975). However, none of the available markers satisfy all these criteria (Engelhardt 1974). Consequently selection of a marker must be made with due consideration given to the errors that might arise. In the present experiments the digesta markers used were PEG (experiment 1), and ^{51}Cr -EDTA and ^{103}Ru -phen (experiment 2). Previous studies have shown that PEG (Hyden 1955) and ^{51}Cr -EDTA (Downes and McDonald 1964; Hogan 1964) are suitable liquid-phase markers.

Sampling digesta through a cannula presents problems in obtaining samples containing not only particulate matter, but also dissolved substances in the same proportions as are present in digesta flowing past the cannula (Hogan 1964; Hogan and Weston 1967). Similarly any single marker may not be present in a sample in the same concentration as in digesta flowing past the cannula. Hogan and Weston (1967) suggest that the problem can be solved by the use of two markers, one of which remains in solution while the other is intimately associated with particulate matter. However, the flow of each phase can be accurately estimated only if the markers associate exclusively with

and are distributed uniformly throughout one or other phase. ^{103}Ru -phen is not distributed uniformly throughout or associated exclusively with the particulate phase (see Tan *et al.* 1971). Therefore this marker cannot be used to estimate particulate flows or particulate volume. This difficulty of separating the flow of the two phases of digesta can be avoided if the true composition of digesta passing the sampling point is determined from the marker concentrations (Faichney 1975), a method that does not require that each marker associate exclusively with one phase.

The reconstitution of true digesta in experiment 2 was carried out by the method of Faichney (1975), using the following formulae. If x is a quantity of digesta (D), y is a quantity of fluid (F) which when added to or removed from x reconstitutes true digesta (TD), S_D , S_F , and S_{TD} are concentrations of the solute marker, and P_D , P_F , and P_{TD} are concentrations of the particulate marker, then

$$xS_D + yS_F = xP_D + yP_F,$$

so that

$$\begin{aligned} y/x &= (P_D - S_D)/(S_F - P_F) \\ &= R, \end{aligned} \quad (4)$$

where R is the reconstitution factor, i.e. the number of units of fluid that must be added to (or removed from) one unit of digesta to obtain true digesta. Then

$$\begin{aligned} (S_D + RS_F)/(1 + R) &= S_{TD} \\ &= (P_D + RP_F)/(1 + R) \\ &= P_{TD}. \end{aligned} \quad (5)$$

The concentration of any constituent of true digesta, e.g. dry matter, sulfur, etc., can be calculated by substituting its concentration for that of the marker in equation (5).

The flow of true digesta (TD) is given by

$$\begin{aligned} \text{flow of TD} &= 1/S_{TD} \\ &= 1/P_{TD}. \end{aligned} \quad (6)$$

Estimations of true digesta flows were made only in regions of the gastrointestinal tract beyond the stomach for reasons which will be discussed. In these regions the true flow of any constituent of true digesta can be calculated by multiplying its true concentration (from equation 5) by the flow of true digesta (from equation 6).

As it is possible to estimate the true concentration of sulfur in ruminal fluid, approximations of sulfur pools in and sulfur flows from the rumen were made. These were termed first approximations and were derived by multiplying the concentration of sulfur in true ruminal digesta (from equation 5) by the ruminal fluid volume (from equation 1) or the fluid flow to the omasum (from equation 2). As dry matter determinations were not carried out on ruminal digesta or the fractions of this digesta it is not possible to estimate the true volume of rumen contents.

Analytical Methods

Total nitrogen, dry matter and organic matter in feeds and faeces were determined by the procedures given by Hume *et al.* (1970). Feed total sulfur was determined by the method of Bird and Fountain (1970), and feed reducible sulfur by their method described for dried faeces.

Analyses of total sulfur and protein sulfur in ruminal fluid and digesta samples were made by the methods described by Bird and Fountain (1970) and Bird (1972a, 1972b). Total reducible sulfur and ester sulfate sulfur in total ruminal digesta and the particulate fraction were determined after extraction with 2 M HCl.

PEG was determined by the method of Hyden (1955). Samples containing ^{51}Cr -EDTA and ^{103}Ru -phen were prepared for radioassay as described by Tan *et al.* (1971), and counted in a 1185 Series automatic gamma counter.

Experimental Designs

In experiment 1 eight treatments involving two levels each of three factors [feed intake (L_1 , L_2), form of feed (C , P), and methionine supplementation (S_1 (0 g), S_2 (3 g))] were arranged in two 4×4 latin squares (see Table 1) in such a way that the second-order interaction was confounded

with squares. Analysis of variance was carried out according to the methods of Steel and Torrie (1960); the difference between means was tested using Duncan's multiple range method.

All animals received *c.* 720 g dry matter per day for 28 days before the four treatment periods I-IV (28 days each). Digesta sampling was carried out on days 15-17 and 25-27 of each treatment period.

Table 1. Design of experiment 1, showing tag numbers of sheep

The treatment factors are as follows. Level of intake: $L_1 = 540$ g dry matter per day, $L_2 = 900$ g dry matter per day. Form of intake: *C* = chaff, *P* = pellets. Methionine supplementation: $S_1 = 0$ g DL-methionine, $S_2 = 3.0$ g DL-methionine

Latin square 1 treatment	Treatment period				Latin square 2 treatment	Treatment period			
	I	II	III	IV		I	II	III	IV
$L_1 CS_1$	38	32	40	34	$L_2 CS_1$	4	3	33	37
$L_2 CS_2$	34	40	32	38	$L_1 CS_2$	37	33	3	4
$L_2 CS_2$	32	38	34	40	$L_1 PS_1$	3	4	37	33
$L_1 PS_2$	40	34	38	32	$L_2 PS_2$	33	37	4	3

For experiment 2, twelve wethers were allocated at random to three groups (1, 2 and 3) of four sheep in a simple crossover design (see Table 2). Measurements on the three groups were started 3 weeks apart to facilitate sampling. Analyses of variance were carried out according to the methods of Steel and Torrie (1960), and treatment comparisons were made according to Cochran and Cox (1957).

Table 2. Design of experiment 2, showing tag numbers of sheep

Treatment	Group:		1		2		3	
	Period:		I		I		I	
			I	II	I	II	I	II
500 g			22	20	15	1	2	3
500 g + methionine			20	22	1	15	3	2
1000 g			17	18	6	4	7	5
1000 g + methionine			18	17	4	6	5	7

All sheep in experiment 2 received 750 g dry matter per day for 28 days prior to the experiment. This was followed by two treatment periods I and II of 49 days separated by, and each followed by, 14-day periods in which individual sheep received their treatment level of intake with intraruminal infusions of water.

Results

Dry Matter Intake, Water Intake, Ruminal Fluid Volume and Fluid Flow to the Omasum (Table 3)

Water intake (water drunk + water infused) increased with level of dry matter intake (DMI) ($P < 0.05$) and with chaffed hay compared to ground and pelleted hay ($P < 0.01$).

There were no treatment effects on ruminal fluid volume, but this did vary with sheep ($P < 0.001$) in experiment 1. Increasing DMI did increase fluid flow from the rumen ($P < 0.001$) and this was further enhanced ($P < 0.05$) by the chaffed hay in experiment 1. The amino acid supplement had no effect.

Sulfur Concentrations in Fractions A and B of Ruminal Digesta and Ruminal Sulfur Pools (Table 4)

In experiment 1 ruminal fluid sulfur pools were estimated from the concentration of sulfur in strained ruminal fluid and the fluid volume of the organ. Neither level of intake nor form of the feed affected total, neutral, protein, inorganic sulfate, ester sulfate or sulfide sulfur pools in this fluid. The methionine supplement increased ruminal fluid pools of inorganic sulfate ($P < 0.001$), ester sulfate ($P < 0.05$) and sulfide ($P < 0.001$) sulfur.

Table 3. Dry matter intake, water intake, ruminal fluid volume, and fluid flow in sheep in experiments 1 and 2

Significant differences between treatment means are indicated by dissimilar superscripts: a, b, c, d, e, ($P < 0.05$); x, y ($P < 0.001$)

Parameter									
		(a) Experiment 1							
Treatment:		L ₁ CS ₁	L ₁ CS ₂	L ₁ PS ₁	L ₁ PS ₂	L ₂ CS ₁	L ₂ CS ₂	L ₂ PS ₁	L ₂ PS ₂
DMI (g/day)		539 ^b	539 ^b	539 ^b	539 ^b	898 ^a	898 ^a	897 ^a	897 ^a
Water intake (l/day)		3.414 ^a	2.247 ^c	2.012 ^c	3.203 ^a	3.073 ^a	3.307 ^a	2.911 ^a	2.519 ^a
Rumen volume (l)		3.66 ^a	3.29 ^a	3.34 ^a	3.77 ^a	2.91 ^a	3.39 ^a	3.29 ^a	3.23 ^a
Ruminal flow (l/day)		8.261 ^{bcd}	7.107 ^{cde}	6.411 ^{de}	6.054 ^e	9.267 ^{abc}	10.998 ^a	9.459 ^{ab}	8.808 ^{abc}
		(b) Experiment 2							
Treatment:		500 g	500 g + Met	1000 g	1000 g + Met				
DMI (g/day)		482 ^y	500 ^y	1000 ^x	997 ^x				
Water intake (l/day)		1.829 ^b	2.363 ^b	3.455 ^a	3.584 ^a				
Rumen volume (l)		3.43 ^a	3.43 ^a	4.17 ^a	3.56 ^a				
Ruminal flow (l/day)		6.767 ^y	6.370 ^y	11.048 ^x	11.097 ^x				

In Table 4 the total sulfur concentrations are given independently for the particulate (A) and fluid (B) fractions of total digesta. The total sulfur concentration in the fluid portion of the digesta is very much less than that of the particulate fraction.

Increasing the DMI resulted in larger ($P < 0.05$) defined pools (i.e. first approximations) of total, neutral and protein sulfur. DL-Methionine infusions given to sheep fed 500 g dry matter tended to increase these sulfur pools towards those in sheep receiving 1000 g dry matter/day. The amino acid supplement also resulted in larger ($P < 0.05$) sulfide sulfur pools irrespective of level of DMI; it is clear that the supplement made a substantial contribution to this pool, particularly at the lower level of DMI.

Neither the level of feed consumed nor the DL-methionine infusion affected the inorganic sulfate or ester sulfate sulfur pools.

First Approximations of Sulfur Flows to the Omasum (Table 5)

First approximations of sulfur flow to the omasum in experiment 2 were calculated using concentration data of sulfur in calculated true digesta and the fluid flow from the rumen. Paralleling the sulfur pool data, increasing DMI increased the flow of total, neutral and protein sulfur ($P < 0.001$) and also sulfide sulfur ($P < 0.05$). Intraruminal infusions of DL-methionine almost doubled ($P < 0.05$) sulfide sulfur flow from the reticulo-rumen. Flows of ester sulfate and inorganic sulfate increased ($P < 0.05$) with level of intake, but not with DL-methionine infusions.

Table 4. Ruminal fluid sulfur pools (experiment 1), the concentration of sulfur in fractions A and B of ruminal digesta, and first approximations of ruminal sulfur pools (experiment 2)

Significant differences between treatment means are indicated by dissimilar superscripts: a, b ($P < 0.05$); x, y, z ($P < 0.001$)

Parameter		(a) Experiment 1							
Treatment:		L ₁ CS ₁	L ₁ CS ₂	L ₁ PS ₁	L ₁ PS ₂	L ₂ CS ₁	L ₂ CS ₂	L ₂ PS ₁	L ₂ PS ₂
Ruminal fluid sulfur pools (mg):									
Total		180 ^{ab}	202 ^{ab}	178 ^{ab}	186 ^{ab}	139 ^b	158 ^{ab}	173 ^{ab}	213 ^a
Protein		105 ^{ab}	97 ^{ab}	111 ^a	77 ^{ab}	71 ^b	69 ^{ab}	85 ^{ab}	114 ^a
Inorganic sulfate		4 ^b	12 ^a	8 ^{ab}	11 ^a	5 ^b	8 ^{ab}	5 ^b	13 ^a
Ester sulfate		5 ^a	5 ^{ab}	3 ^{ab}	5 ^{ab}	3 ^b	5 ^a	3 ^{ab}	5 ^a
Sulfide		8 ^b	22 ^a	8 ^b	20 ^a	7 ^b	17 ^a	7 ^b	15 ^a
Treatment:		(b) Experiment 2							
Sulfur concentration (µg/g) ^A :		500 g	500 g + Met		1000 g	1000 g + Met			
Total A		209	256		239	270			
B		39	39		37	41			
Protein A		139	170		169	189			
B		17	17		15	14			
Total reducible A		6	7		6	7			
B		4	4		3	3			
Ester sulfate A		4	5		5	5			
B		1	1		1	1			
Ruminal sulfur pools (mg):									
Total		728 ^b	856 ^{ab}		1132 ^a	1060 ^a			
Neutral		675 ^c	787 ^{bc}		1059 ^a	973 ^{ab}			
Protein		390 ^b	449 ^{ab}		591 ^a	467 ^{ab}			
Inorganic sulfate		32 ^a	49 ^a		47 ^a	41 ^a			
Ester sulfate		21 ^a	20 ^a		23 ^a	17 ^a			
Sulfide		11 ^{bz}	26 ^{ax}		14 ^{byz}	21 ^{axy}			

^A Sulfur concentrations are expressed as micrograms per gram of total digesta sampled.

Table 5. First approximations of sulfur flows in digesta from the rumen (experiment 2)

Significant differences between treatment means are indicated by dissimilar superscripts: a, b, c, d ($P < 0.05$); x, y, z ($P < 0.001$)

Parameter	Treatment:			
	500 g	500 g + Met	1000 g	1000 g + Met
Sulfur flow (mg/day):				
Total	1382 ^y	1576 ^y	3019 ^x	3260 ^x
Neutral	1280 ^y	1453 ^y	2826 ^x	3045 ^x
Protein	739 ^y	822 ^y	1532 ^x	1449 ^x
Total reducible	102 ^{by}	123 ^{bxy}	193 ^{ax}	215 ^{ax}
Inorganic sulfate	64 ^c	87 ^{bc}	129 ^{ab}	145 ^a
Ester sulfate	38 ^b	36 ^b	63 ^a	60 ^a
Sulfide	20 ^{dz}	49 ^{bxy}	36 ^{cy}	64 ^{ax}

The amounts of the various sulfur fractions in the fluid leaving the rumen (experiment 1) (derived from the values in Tables 3 and 4) represent only a small proportion of the total sulfur flowing to the omasum. However, paralleling the results in experiment 2, increasing DMI increased ($P < 0.05$) total, neutral and protein sulfur flows in the fluid, but neither form of feeding nor intraruminal infusion of DL-methionine had any effect on these components.

Discussion

The reliability of estimates of ruminal flow, of fluid volume and of all derived values depends upon the suitability of the markers used in the stomach and gastrointestinal tract, the achievement of 'steady state' conditions, and obtaining, reconstituting, synthesizing or calculating a representative digesta sample. The usefulness of the markers PEG, ^{51}Cr -EDTA and ^{103}Ru -phen with regard to specific functions has been discussed on pages 53-54.

Although estimates of true digesta flow can be made (see Faichney 1975) at certain sites in the digestive tract, the method cannot be applied to the reticulo-rumen as many of the particles sampled are much too large to pass into the omasum. The particle size distribution in relation to rumen samples has been described before (see Becker *et al.* 1963; Hungate 1966). It must be agreed, as Faichney (1975) states, that use of reconstituted flow data for the rumen would introduce errors of unknown magnitude into the estimation. However, Faichney's method was used to estimate the concentration of sulfur in true digesta at a sampling point within the reticulo-rumen.

Comparison of the sulfur concentrations in the particulate and fluid fractions of ruminal digesta samples in experiment 2 (see Table 4) indicates that the sulfur content in the fluid is only a small proportion (c. 15%) of the total. This also applies when the sulfur concentration in true digesta is calculated. The small contribution made by the sulfur content of the fluid and the possible sequestration of some sulfur compounds (discussed later) emphasize the necessity to use concentrations in true digesta. In this regard the data of experiment 1 differ in important detail from the first approximations of ruminal sulfur pools in experiment 2 in that concentrations and volumes are based only on strained ruminal fluid and therefore represent ruminal fluid pools only. These are gross underestimates of true ruminal sulfur pools.

In the first approximations of sulfur pools in and sulfur flows from the reticulo-rumen it has been assumed that the fluid volume and fluid flow equate to the 'actual' values. These assumptions necessarily underestimate true digesta volume and flow of true digesta as no provision is made for the size or flow of particulate material in these estimates. Other errors may also arise as saliva does not always fully mix with the entire contents of the reticulo-rumen (see Engelhardt 1974), and the composition of the reticular contents may differ from that of digesta in the ventral region of the rumen (Hauffe 1972; Engelhardt and Hauffe 1975b). Real estimates of these variations are not readily obtainable.

Increasing DMI from 482 to 1000 g/day meant that the animals consumed an extra 946 mg S/day (881 *v.* 1827 mg S/day). However, the estimated total sulfur pool increased by only 404 mg S (728 *v.* 1132 mg S), due to increases in the protein (201 mg S) and non-protein organic (183 mg S) sulfur pools. Intraruminal supplements of DL-methionine at 4.5 g/day (c. 970 mg S/day) did not influence ruminal

total sulfur pools at the low [728 (without supplement) *v.* 856 mg S] or high [1132 (without supplement) *v.* 1060 mg S] level of feed intake. The supplement did result in a substantial increase in the ruminal sulfide sulfur pool, particularly at the low level of intake (11 *v.* 26 mg S). This indicates that the supplement was largely degraded to sulfide.

As recorded previously (Weston and Hogan 1967) increasing the level of DMI of roughage rations resulted in greater fluid flow from the reticulo-rumen. Grinding of the ration resulted in a decreased rate of fluid flow from the organ. Possible reasons for this, such as reduced saliva secretion and reticular activity and contraction, have been discussed by Weston and Hogan (1967). Despite these differences, large variations occurred between estimates of fluid or digesta flows in sheep receiving the same treatments. MacRae (1975) pointed out that a large part of the variation in estimates of digesta flow, with both continuous and spot sampling procedures, is associated with a between-animal component rather than with a component between samplings within the same animal. Our experience in experiments 1 and 2 strongly supports the view that true biological variability is important in studies on digestion.

A block diagram (Fig. 1) has been used to illustrate first approximations of the different sulfur pools in the reticulo-rumen, the interactions between them and first approximations of sulfur flows to the omasum. No attempt has been made to distinguish between dietary and microbial protein, nor to subdivide the non-protein neutral sulfur fraction into peptide sulfur, free cyst(e)ine and methionine or other organic sulfur compounds present. Further, the reducible sulfur pools and sulfur flows are small in comparison with the sulfur in organic fractions. In this regard the model places quantitative values on the relative contributions of different pools, without overlooking the potential importance of small rapidly changing pools.

The first approximations of protein sulfur flows to the omasum are subject to errors. Overestimates occur as the sulfur content of particles in the rumen too large to flow through the reticulo-omasal orifice have been included in the estimated sulfur concentration of true digesta. Another error is that introduced by using fluid flow which is an underestimate of true flow. In addition, it might be expected that the non-protein neutral sulfur and the reducible sulfur forms present in ruminal digesta should all be capable of passing through the reticulo-omasal orifice in the fluid fraction. However, sequestration of these compounds occurs. A substantial part of the α -amino nitrogen of ruminal contents is associated with microbial cells (Annison 1956), and a proportion of free sulfur amino acids is precipitated by trichloroacetic acid (Bird and Hume 1971). It seems likely therefore that apparent non-protein organic sulfur flows from the reticulo-rumen will be inflated by these ruminal retentions. Concentrations of total reducible sulfur and ester sulfate sulfur in particulate and fluid fractions of the ruminal digesta indicate that inorganic sulfate was evenly distributed in the two fractions, while ester sulfate, despite several washings, was contained largely in the particulate material and may be sequestered amongst the solids in the rumen.

The first approximation of true flow of neutral sulfur from the rumen of sheep fed 1000 g dry matter and infused intraruminally with 4.5 g/day of DL-methionine was substantially higher (3045 *v.* 1616 mg S/day) than the flow in the experiment of Bird and Moir (1972) where sheep were fed 900 g dry matter and received 2 g/day of DL-methionine. The values reported by Bird and Moir are calculated from the sulfur concentration in ruminal digesta as collected and from the fluid flow from the

rumen. Differences in sulfur intake in the two experiments (2790 mg/day compared to 1897 mg/day for Bird and Moir's experiment) account for a large proportion of the difference in non-protein neutral and protein sulfur flows. However, even when

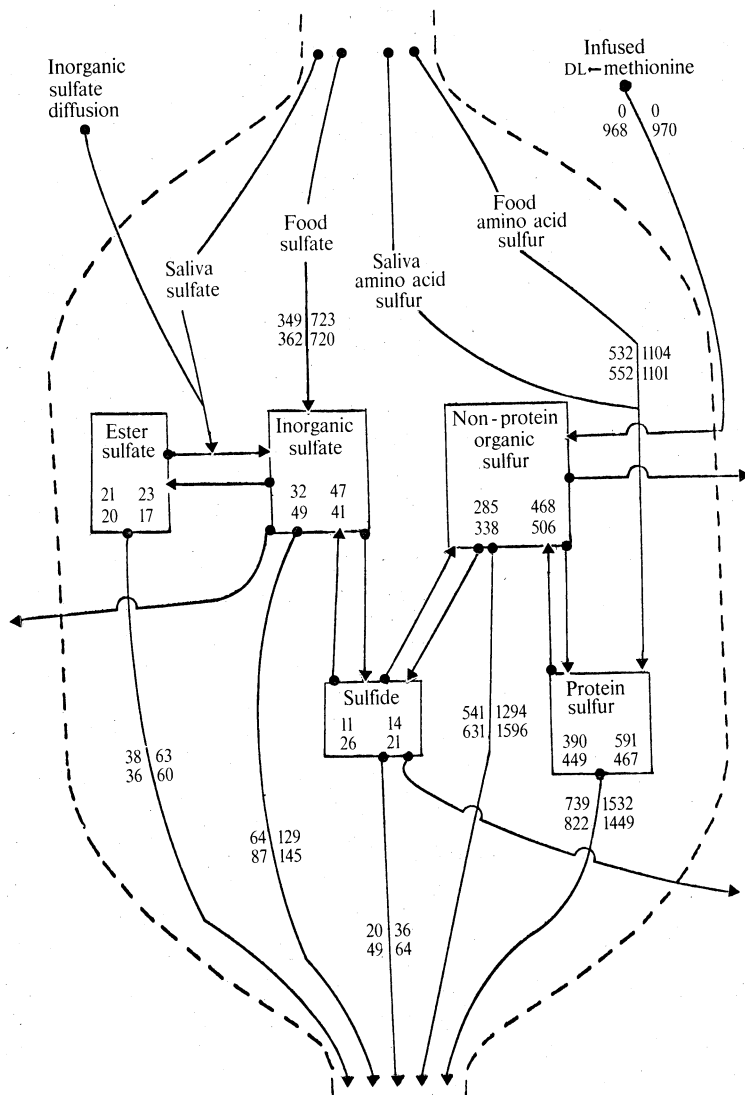


Fig. 1. Sulfur inputs (mg S/day) into the reticulo-rumen, first approximations of sulfur pools (mg S) and of sulfur flows to the omasum (mg S/day). The pool sizes and flows illustrated are the means for each of the four treatments in experiment 2. The convention used within each set of data is related to the treatments as follows:

500 g	1000 g
500 g + Met	1000 g + Met

1000 g dry matter was fed without DL-methionine supplementation (sulfur intake 1827 mg/day) there was a greater (2826 v. 1616 mg/day) estimated flow of neutral sulfur to the omasum than in the earlier work of Bird and Moir (1972). There were

also large differences in non-protein neutral sulfur flows indicating that either the method of estimating sulfur concentration in the rumen has a large influence on the estimated sulfur flows, or that there were large differences in the metabolism of sulfur between the two experiments, or both. As sequestration of non-protein nitrogen compounds occurs in the rumen (discussed earlier) the methods used to chemically and mathematically determine organic sulfur concentrations in the protein and non-protein fractions will influence estimates of the various sulfur flows from the rumen.

Differences in reducible sulfur flow values between the two studies are due entirely to differences in ester sulfate sulfur flows (60 mg S/day compared to 18 mg S/day in Bird and Moir's experiment), largely resulting from determining concentrations of this component in the liquid or HCl-extracted digesta fractions in our work. As sequestration of ester sulfate occurs in the reticulo-rumen, the estimates of ester sulfate flow made in experiment 2 may be high.

Increasing the level of DMI from 500 to 1000 g/day doubled the first approximations of total, neutral and protein sulfur flows to the omasum. Although there was considerable variation between these estimates in supplemented and unsupplemented sheep, it appeared that DL-methionine infusions did not increase the flow of organic sulfur from the reticulo-rumen. The amino acid did, however, double estimates of sulfide sulfur flow from the organ—an indication that a large proportion of the supplementary sulfur passed through the ruminal sulfide pool. This is contrary to the findings of Bird and Moir (1972).

The data of experiment 2 and the model illustrate the importance of using true digesta in assessing pools in the reticulo-rumen of particular metabolites and the flows of these. Dry matter measurements would further improve estimates of pool size, as the concentration of dry matter in true digesta would enable estimates to be made of true digesta volume. However, such measurements were not possible as the increased size of samples would have placed the animals under undue stress. First approximations of sulfur flow illustrate that doubling the level of DMI approximately doubled the flow of sulfur to the omasum, and that a very large proportion of the sulfur flowing from the reticulo-rumen was in an organic form, of which only half was protein sulfur.

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