SULPHUR METABOLISM AND EXCRETION STUDIES IN RUMINANTS

XI.* CYCLING OF ^{35}S TO THE CAECUM AND COLON AND THE INFLUENCE OF THE HINDGUT ON SULPHUR EXCRETION

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[Manuscript received 23 May 1972]

Abstract

The flow of sulphur to the ileum and the transfer of sulphur from the blood to the intestinal contents and its relationship with urinary and faecal sulphur excretion were studied in sheep fitted with re-entrant ileal cannulae.

In four sheep the mean daily flow of total sulphur in the digesta passing into the caecum was 685 (±17·4) mg. The composition of sulphur in the ileal digesta (mean of 16 observations ±S.E.M.) was: protein sulphur 59·9 (±1·27)%; soluble organic sulphur 23·1 (±1·33)%; ester sulphur 9·3 (±0·44)% and inorganic sulphur 6·7 (±0·76)% of the total sulphur.

After single intravenous infusions of $Na_2{}^{35}SO_4$ the mean recovery of ^{35}S in the ileal digesta, after 48 hr, was 24·5 (±1·25)%. The composition of the ^{35}S was: protein ^{35}S, 49·4 (±1·30)%; soluble organic ^{35}S, 10·1 (±2·37)%; reducible ^{35}S, 40·5 (±1·04)%. It was estimated that *c.* 40% of the infused ^{35}S was cycled to the rumen and incorporated into microbial protein.

Infusions of glucose (30–90 g/day) into the distal ileum increased the faecal excretion of sulphur (up to 110 mg/day) and decreased the urinary excretion of sulphur by similar amounts. Comparing the basal (no glucose) with daily infusions of 90 g of glucose, the percentage of the infused ^{35}S recovered in faeces and urine over 96 hr was 11·5 (±2·43) *v.* 22·7 (±0·18)% and 79·1 (±5·55) *v.* 63·4 (±1·65)%, respectively. When 60–90 g of glucose was infused daily, more organic sulphur or organic ^{35}S was excreted in the faeces than passed the terminal ileum. Up to 24% of the faecal ^{35}S could have resulted from the transfer of ^{35}S from the blood into the large intestine.

The transfer of sulphate from the blood to the fermentative areas of the alimentary tract is apparently the pre-emptive pathway of excretion in ruminants.

I. Introduction

Infusion of glucose into the distal ileum of sheep has been shown to increase the output of faecal nitrogen and to decrease the urinary nitrogen output by similar amounts (Thornton *et al.* 1970). These effects were attributed to cycling of urea nitrogen

* Part X, *Aust. J. biol. Sci.*, 1972, **25**, 1087–98.

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to the hindgut (caecum and colon) and its incorporation into microbial protein which was largely excreted in the faeces.

Sulphur is an essential nutrient requirement for microbial protein synthesis (Roberts *et al.* 1955) and the addition of sulphur to diets low in sulphur but adequate in nitrogen has been shown to increase the production of microbial protein in the rumen (Hume and Bird 1970; Bird 1972*c*). An increased production of microbial protein in the hindgut, as suggested by Thornton *et al.* (1970), indicates that the supply of sulphur to this region was adequate. Little inorganic sulphur is secreted into the duodenum via pancreatic–biliary fluids (Bird 1972*b*) while inorganic sulphate is readily absorbed from the small intestine (Bird and Moir 1971), therefore to meet this demand for sulphur, transfer of sulphate from the blood to the digesta in the hindgut may have been required.

In the present experiments the transfer of endogenous sulphur from the blood to the alimentary tract and its relationship(s) with urinary and faecal sulphur excretion was studied with sheep fitted with re-entrant ileal cannulae.

II. Methods

(*a*) *Experimental Plans*

Experiment I

In conjunction with the experiment described by Thornton *et al.* (1970), where glucose was infused into the distal ileum of sheep in order to change the fermentative activity in the hindgut, $Na_2{}^{35}SO_4$ was infused intravenously and the output of ^{35}S and of sulphur in ileal digesta, faeces, and urine followed to establish cycling of sulphur to the gut tract prior and distal to the terminal ileum and net transformation of sulphur within the digesta.

Details of the experimental animals, diets, treatments, and sampling procedures have been given by Thornton *et al.* (1970). Briefly, in a 4×4 Latin square design experiment, four Merino wethers each fitted with a ileal re-entrant cannula were fed once daily 800 g dry matter of a ration containing oat hulls (60%), lucerne chaff (36%), and minerals (4%), and supplying, on average, 9·5 g nitrogen/day and 1·30 g sulphur/day (0·4 g as added sodium sulphate sulphur). The treatments imposed were four levels of glucose infused at the rate of 0, 30, 60, or 90 g/day into the distal ileum (treatments 1, 2, 3, and 4, respectively). After 13 days for treatment adjustment faeces and urine were collected for 6 days. On the 20th day of each period a single intravenous injection of carrier-free $Na_2{}^{35}SO_4$ (450 μCi) was given to each sheep after feed presentation and ileal digesta was continuously collected for 48 hr thereafter. Samples (10 g) of the ileal digesta collected at intervals of 2–4 hr were stored separately, while a further aliquot of the 4-hourly collections (10% of total) were combined on a daily basis, frozen, and stored. The remaining digesta, together with the appropriate amount of glucose solution, was returned to the sheep during the next 4 hr.

Urine and faeces were collected quantitatively at 4-hourly intervals during the first day, 8-hourly during the second day, and daily for a further 2 days.

Experiment II

Three weeks after completion of experiment I, two of the sheep (010 and 013) were each given a single intravenous injection of $Na_2{}^{35}SO_4$ (407 μCi) and ileal digesta was continuously collected for 60 hr from them and from the other two sheep (014 and 011). At 4-hourly intervals a sample (5% of total) of the ileal digesta collected from each sheep was taken and separately stored. The digesta remaining from sheep 010 and 013 was infused into the ileum of sheep 014 and 011, respectively. Conversely, the digesta from sheep 014 and 011 was infused into the ileum of sheep 010 and 013, respectively.

Urine and faeces were quantitatively collected at 4–24-hr intervals over a 96-hr period (see Fig. 4).

This experiment provided an assessment of the apparent digestibility of ^{35}S in ileal digesta in the large intestine, the cycling of ^{35}S back to the ileum, the retention of ^{35}S in the body, and the entry of ^{35}S into the large intestine from the blood.

The quantity and composition of the ration fed was the same as in experiment I.

(*b*) *Chemical Analysis*

The methods described by Bird and Fountain (1970) and Bird and Hume (1971) were used for the determination of total sulphur in rations, and total sulphur, inorganic sulphur, ester sulphur, organic sulphur, total ^{35}S, reducible ^{35}S, and organic ^{35}S fractions in ileal digesta, urine, and faeces.

Protein in duplicate 20-g samples of ileal digesta was precipitated with 20 ml of 20% trichloroacetic acid. Portions of the precipitates were analysed for sulphur by the method of Bird and Fountain (1970) and for nitrogen by the Kjeldahl technique.

(*c*) *Statistical Analysis*

The results of experiment I were analysed by analysis of variance to account for variation due to treatment, sheep, and period of treatment.

III. Results

(*a*) *Experiment I*

There were no differences between treatments in the intake of sulphur (Table 1), but there were differences between successive periods ($P < 0{\cdot}001$) due to changes in the sulphur content of the dietary lucerne; the mean daily intakes in periods 1, 2, 3,

Table 1

SULPHUR INTAKE AND EXCRETION IN URINE AND FAECES (EXPERIMENT I)

Treatments 1, 2, 3, and 4 represent infusion per ileum of 0, 30, 60, and 90 g glucose/day, respectively. Values are means. Statistical differences due to treatment are indicated thus: n.s., not significant; *, $P < 0{\cdot}05$. Significant differences between treatments are indicated by similar superscripts: a, $P < 0{\cdot}001$; b, $P < 0{\cdot}01$; c, $P < 0{\cdot}05$

Parameter	Treatment				S.E.M.	Statistical significance
	1	2	3	4		
Sulphur intake (mg/day)	1295	1303	1307	1307	5·4	n.s.
Sulphur balance (mg/day)	182	177	196	171	23·1	n.s.
Urinary sulphur (mg/day)						
Total	612^{c,c1}	565	505^{c}	502^{c1}	21·7	*
Neutral	53	53	68	66	13·8	n.s.
Ester	199	182	182	167	11·3	n.s.
Inorganic	334^{c}	331^{c1}	255^{c,c1}	269	19·1	*
Faecal sulphur (mg/day)						
Total	526^{b,c}	561^{c1}	606^{c}	635^{c1,b}	17·7	*
Neutral	486^{c,c1}	511	550^{c}	556^{c1}	14·6	*
Ester	35^{b,c}	39^{c1}	49^{c}	56^{b,c1}	3·5	*
Inorganic	8^{c}	11	8	22^{c}	4·1	n.s.
Faecal N/S ratio	8·02	8·54	8·15	8·20	0·28	n.s.

and 4 were 1169, 1303, 1221, and 1520 mg/day, respectively. The urinary and faecal excretions of ^{35}S on the basal treatment in these respective periods was 72·6, 86·2, 67·1, 90·6% and 15·4, 7·9, 16·1, 6·8% of the infused dose, respectively. Over all

treatments the urinary excretion of total sulphur was also affected by period of treatment ($P < 0{\cdot}01$); more was excreted in period 4 than in other periods ($P < 0{\cdot}01$) and less in period 1 than in other periods ($P < 0{\cdot}05$). Similarly, urinary ^{35}S output ($P < 0{\cdot}05$), faecal ^{35}S output ($P < 0{\cdot}05$) and ^{35}S retention ($P < 0{\cdot}05$) was affected by period of treatment. Comparing period 4 with period 1, more ^{35}S was excreted in the urine ($P < 0{\cdot}01$), less was excreted in the faeces ($P < 0{\cdot}01$), and less was retained ($P < 0{\cdot}01$).

Faecal sulphur excretion was increased and the urinary output of sulphur decreased by the ileal infusion of glucose (Table 1). Sulphur balances were not changed (Table 1). The increased output of organic ("neutral") sulphur and of ester sulphur fractions in the faeces largely accounted for the increased faecal total sulphur output. The concomitant decrease in urinary sulphur excretion was due mainly to a decreased output of reducible (inorganic plus ester) sulphur ($P < 0{\cdot}05$) (Table 1). The ratio of nitrogen to sulphur excreted in the faeces was not altered by treatment (Table 1).

The flow of ileal digesta was not affected by treatment nor day of collection (see Thornton *et al.* 1970). The daily flow of dry matter in one sheep (014) was less than in the other animals ($P < 0{\cdot}01$) and the dry matter collected daily at the ileum was substantially less than collected daily in the faeces prior to the ileal collection period. Dietary lignin was used as a marker to assess possible bias resulting from the quantitative collection or sampling of ileal digesta or both in this and the other sheep (see Thornton *et al.* 1970). The results presented in the present paper (Tables 2 and 3) for the four sheep include data based on the ileal digesta collected and the adjusted values based on the flow of lignin to the ileum and the output of lignin in the faeces. Results are also given for the mean ileal flow of total sulphur and of ^{35}S for sheep excluding 014. The non-isotopic ileal digesta data for each sheep were based on the means of the 2 days collection.

Different treatments did not affect the sulphur composition of the ileal digesta collected, however, for sheep 013 protein sulphur formed a smaller proportion (54·2% compared with 61·2–62·1% for the other sheep) ($P < 0{\cdot}05$) and soluble organic sulphur a larger proportion (28·3% compared with 19·5–22·6% for the other sheep) ($P < 0{\cdot}01$) of the total sulphur in the digesta. The mean composition of sulphur in the digesta was: neutral sulphur 84·3 ($\pm$0·72)%, protein sulphur 59·9 ($\pm$1·27)%, soluble organic sulphur 23·1 ($\pm$1·33)%, ester sulphur 9·3 ($\pm$0·44)%, and inorganic sulphur 6·7 ($\pm$0·76)% of total sulphur.

With the lignin adjusted values there was a significant effect of glucose infusion on the flow to the ileum of total sulphur, neutral sulphur, and protein nitrogen (Table 2). More ester sulphur ($P < 0{\cdot}05$) and more soluble organic sulphur ($P < 0{\cdot}01$) was found in the ileal digesta of sheep 013 than in any other sheep.

On the unadjusted data, significantly less sulphur in any form, or protein, was found in the daily flow of digesta to the ileum of sheep 014 than any other sheep.

The ratio of protein nitrogen to protein sulphur in the digesta did not vary with treatment (Table 2). The overall mean value was 6·94 which was similar to the comparable ratio of total nitrogen (from Thornton *et al.* 1970) to total sulphur of 7·05.

Ileal infusions of glucose resulted in an apparent gain of sulphur to the digesta during transfer from the ileum to the faeces mainly as a result of a "gain" in organic ("neutral") sulphur compounds (Table 2).

TABLE 2

FLOW OF SULPHUR AND PROTEIN TO THE DISTAL ILEUM AND THE LOSS OF SULPHUR FROM THE HINDGUT DIGESTA (EXPERIMENT I)

Treatments 1–4 as in Table 1. Values are means. Statistical differences due to treatment are indicated thus: n.s., not significant; *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$. Significant differences between treatments are indicated by similar superscripts: a, $P < 0{\cdot}001$; b, $P < 0{\cdot}01$; c, $P < 0{\cdot}05$

Parameter	Treatments 1	2	3	4	S.E.M.	Statistical significance
Ileal digesta sulphur (mg/day):						
Total						
Collected	661	641	582	614	36·1	n.s.
Adjusted	741^{b,c}	725^{c1,c2}	642^{c,c1}	630^{b,c2}	19·1	*
Collected†	696	673	675	651	17·6	
Adjusted†	749	710	641	616	21·8	
Neutral						
Collected	552	542	491	516	30·3	n.s.
Adjusted	618^{b,c}	613^{c1,c2}	544^{c,c1}	531^{b,c2}	15·9	*
Protein‡						
Collected	396	375	345	369	19·4	n.s.
Adjusted	444^{c,c1}	429	385^{c1}	378^{c}	17·0	n.s.
Soluble organic						
Collected	150	154	142	142	11·0	n.s.
Adjusted	161	169	159	145	7·9	n.s.
Ester						
Collected	57	63	57	60	4·6	n.s.
Adjusted	63	70	64	59	3·9	n.s.
Inorganic						
Collected	53	37	35	39	7·6	n.s.
Adjusted	61	43	40	40	7·1	n.s.
Ileal digesta nitrogen:						
Protein (g N/day)‡						
Collected	2·68^{c}	2·75^{b}	2·33^{c,b}	2·55	0·08	*
Adjusted	2·99^{c,c1}	3·11^{b,b1}	2·60^{b,c}	2·61^{b1,c1}	0·08	**
Protein N/S ratio	6·73	7·34	6·75	6·92	0·27	n.s.
Total N/S ratio	6·94	7·05	7·03	7·27		
Ileum to faeces sulphur loss (mg/day)§:						
Total						
Collected	135^{c,c1}	80	−24^{c}	−21^{c1}	44·0	n.s.
Adjusted	215^{b,b1}	164^{c,b2}	36^{b,c}	−5^{b1,b2}	25·5	**
Collected†	171	116	79	12	23·2	
Adjusted†	224	154	45	−23	31·7	
Organic						
Collected	66^{c,c1}	31^{c2}	−58^{c,c2}	−40^{c1}	24·4	*
Adjusted	132^{b,b1}	102^{b2,b3}	−5^{b,b2}	−25^{b1,b3}	20·4	**
Ester						
Collected	21	24	8	4	10·4	n.s.
Adjusted	28^{c}	31^{c1}	15	3^{c,c1}	6·9	n.s.
Inorganic						
Collected	46	26	26	17	7·1	n.s.
Adjusted	54^{c}	32	31	18^{c}	8·6	n.s.

† Values excluding sheep 014.

‡ Protein precipitated with trichloroacetic acid.

§ Negative values indicate a gain in sulphur during transit.

TABLE 3

RECOVERY OF ^{35}S IN ILEAL DIGESTA, URINE, AND FAECES AFTER A SINGLE INTRAVENOUS INFUSION OF $Na_2{}^{35}SO_4$ (EXPERIMENT I)

Treatments 1–4 as in Table 1. Values are means. Statistical differences due to treatment are indicated thus: n.s., not significant; *, $P < 0{\cdot}05$; ***, $P < 0{\cdot}001$. Significant differences between treatments are indicated by similar superscripts: a, $P < 0{\cdot}001$; b, $P < 0{\cdot}01$; c, $P < 0{\cdot}05$

	Treatments				S.E.M.	Statistical significance
	1	2	3	4		
Ileal digesta ^{35}S						
Recovery 0–48 hr (% of dose):						
Collected	20·8	23·3	22·2	22·7	1·79	n.s.
Adjusted	23·4	26·4	25·0	23·1	1·07	n.s.
Collected†	20·9	24·2	25·3	24·9	1·64	
Adjusted†	22·5	25·4	24·0	23·5	1·57	
Composition (% of total):						
Protein ^{35}S	50·5	49·4	49·4	48·5	0·77	n.s.
Soluble organic ^{35}S	4·9	12·0	14·3	9·3	4·02	n.s.
Reducible ^{35}S	42·5	39·5	38·4	41·7	2·08	n.s.
Faecal ^{35}S						
Recovery (% of dose):						
0–24 hr	3·8	5·3	5·3	5·8	0·62	n.s.
0–48 hr	$8{\cdot}9^{a,a1,c}$	$11{\cdot}3^{b,b1,c}$	$15{\cdot}0^{a,b}$	$16{\cdot}5^{a1,b1}$	0·65	***
0–96 hr	$11{\cdot}5^{a,b}$	$14{\cdot}2^{a1,b1}$	$19{\cdot}8^{b,b1}$	$22{\cdot}7^{a,a1}$	0·99	***
Composition (% of total):						
Organic ^{35}S	91·1	$93{\cdot}0^{c}$	$92{\cdot}4^{c1}$	$88{\cdot}8^{c,c1}$	0·80	*
Reducible ^{35}S	8·9	$7{\cdot}0^{c}$	$7{\cdot}6^{c1}$	$11{\cdot}2^{c,c1}$	0·80	*
Ileum to faeces ^{35}S loss (% of dose)‡§:						
Total ^{35}S						
Collected	$7{\cdot}1^{b,c}$	$6{\cdot}8^{b1,c1}$	$0{\cdot}2^{c,c1}$	$-2{\cdot}3^{b,b1}$	1·66	*
Adjusted	$9{\cdot}8^{a,b}$	$9{\cdot}9^{a1,b1}$	$3{\cdot}0^{b,b1,b2}$	$-1{\cdot}9^{a,a1,b2}$	0·91	***
Collected†	8·6	8·1	5·1	−0·2	1·36	
Adjusted†	10·2	9·4	3·8	−1·5	1·68	
Organic ^{35}S						
Collected	$0{\cdot}1^{b,c}$	$-0{\cdot}2^{b1,c1}$	$-6{\cdot}5^{c,c1}$	$-8{\cdot}3^{b,b1}$	1·40	*
Adjusted	$1{\cdot}6^{b,c}$	$1{\cdot}9^{b1,c1}$	$-3{\cdot}7^{c,c1}$	$-8{\cdot}1^{b,b1}$	1·59	*
Reducible ^{35}S						
Collected	6·9	7·3	6·2	6·0	0·59	n.s.
Adjusted	$8{\cdot}1^{c,c1}$	8·5	$7{\cdot}4^{c}$	$6{\cdot}2^{c1}$	0·48	n.s.
Urinary ^{35}S						
Recovery (% of dose):						
0–24 hr	$65{\cdot}4^{c,c1}$	62·0	$56{\cdot}0^{c}$	$53{\cdot}2^{c1}$	2·70	n.s.
0–48 hr	$75{\cdot}3^{b,c}$	$71{\cdot}5^{c1}$	$65{\cdot}4^{c}$	$60{\cdot}2^{b,c1}$	2·54	*
0–96 hr	$79{\cdot}1^{b,c}$	$75{\cdot}1^{c1}$	$68{\cdot}9^{c}$	$63{\cdot}4^{b,c1}$	2·49	*
^{35}S retained 0–96 hr (% of dose)	7·3	8·3	9·1	13·2	1·80	n.s.

† Values excluding sheep 014.

‡ Negative values indicate a gain in ^{35}S during transit.

§ Allowing for loss of sulphur in ileal digesta samples.

The recovery of ^{35}S in ileal digesta following the single intravenous infusion of $Na_2{}^{35}SO_4$ was not affected by treatment; in 24 hr the mean percentage recovery, on the basis of collected and adjusted values, was 18·3 (±1·56) and 19·9 (±1·03)%, respectively. Data for the 48-hr period are shown in Table 3. There were differences between sheep ($P < 0·01$) and between period of treatment ($P < 0·05$). Over the 48-hr post-infusion period, the percentage of dose ^{35}S recovered (mean of four adjusted treatment values) from sheep 010, 011, 014, and 013 was 20·9 (±2·83), 21·7 (±0·89), 26·3 (±1·70), and 29·0 (±2·21)%, respectively. The cumulative recovery of ^{35}S (as percentage of dose) in the ileal digesta after the intravenous infusion of $Na_2{}^{35}SO_4$ is shown in Figure 1. The values plotted are the unadjusted mean values for each sheep (four observations per sheep).

TABLE 4

RECOVERY OF ^{35}S IN ILEAL DIGESTA, URINE, AND FAECES AFTER INTRAVENOUS INFUSION OF $Na_2{}^{35}SO_4$ (SHEEP 010 AND 013) OR ILEAL INFUSION OF ILEAL DIGESTA LABELLED *IN VIVO* WITH ^{35}S (SHEEP 014 AND 011) (EXPERIMENT II)

Over a 60-hr period ileal digesta was collected, sampled, and cross-infused between sheep 010 and 014, and 013 and 011, respectively. Urine and faeces were collected over 96 hr

	Sheep 010	Sheep 014	Sheep 013	Sheep 011
^{35}S infused (μCi)				
Intravenous	407·0	—	407·0	—
Ileum	12·6	79·9	10·9	101·4
^{35}S recovered (μCi)				
Ileal digesta	84·1	11·6	106·8	13·3
Urine	277·4	15·9	281·7	16·1
Faeces	16·3	42·8	15·9	27·3
^{35}S recovered (as % of dose)*				
Ileal digesta	20·7	14·3	26·2	13·1
Urine	68·2	19·9	69·2	15·8
Faeces	4·0	53·6	3·9	26·9
	(2·8)†		(2·8)†	
^{35}S retained (as % of dose)*	7·2	12·2	0·6	44·2

* As % of intravenous ^{35}S dose (sheep 010 and 013) or of ileal digesta ^{35}S infusion (sheep 014 and 011).

† Corrected for the faecal excretion of an estimated 40% of the ^{35}S infused with ileal digesta.

There was no effect of treatment, or period of treatment, on the composition of ^{35}S in ileal digesta, but there were differences between sheep ($P < 0·001$). In sheep 013, protein ^{35}S comprised 41·6% of the total ^{35}S in the ileal digesta collected, compared with 50·4–53·6% for the other three sheep. Overall, protein ^{35}S accounted for 49·4%, reducible ^{35}S 40·5%, and soluble organic ^{35}S 10·1% of the total ^{35}S in ileal digesta (Table 3).

Faecal excretion of ^{35}S was increased and the urinary output decreased by the ileal infusion of glucose (Table 3). The retention of ^{35}S was not affected by treatment (Table 3). There were differences between sheep in retention of ^{35}S ($P < 0·05$). The cumulative excretion of ^{35}S (as percentage of dose) in faeces (Fig. 2) and in urine (Fig. 3)

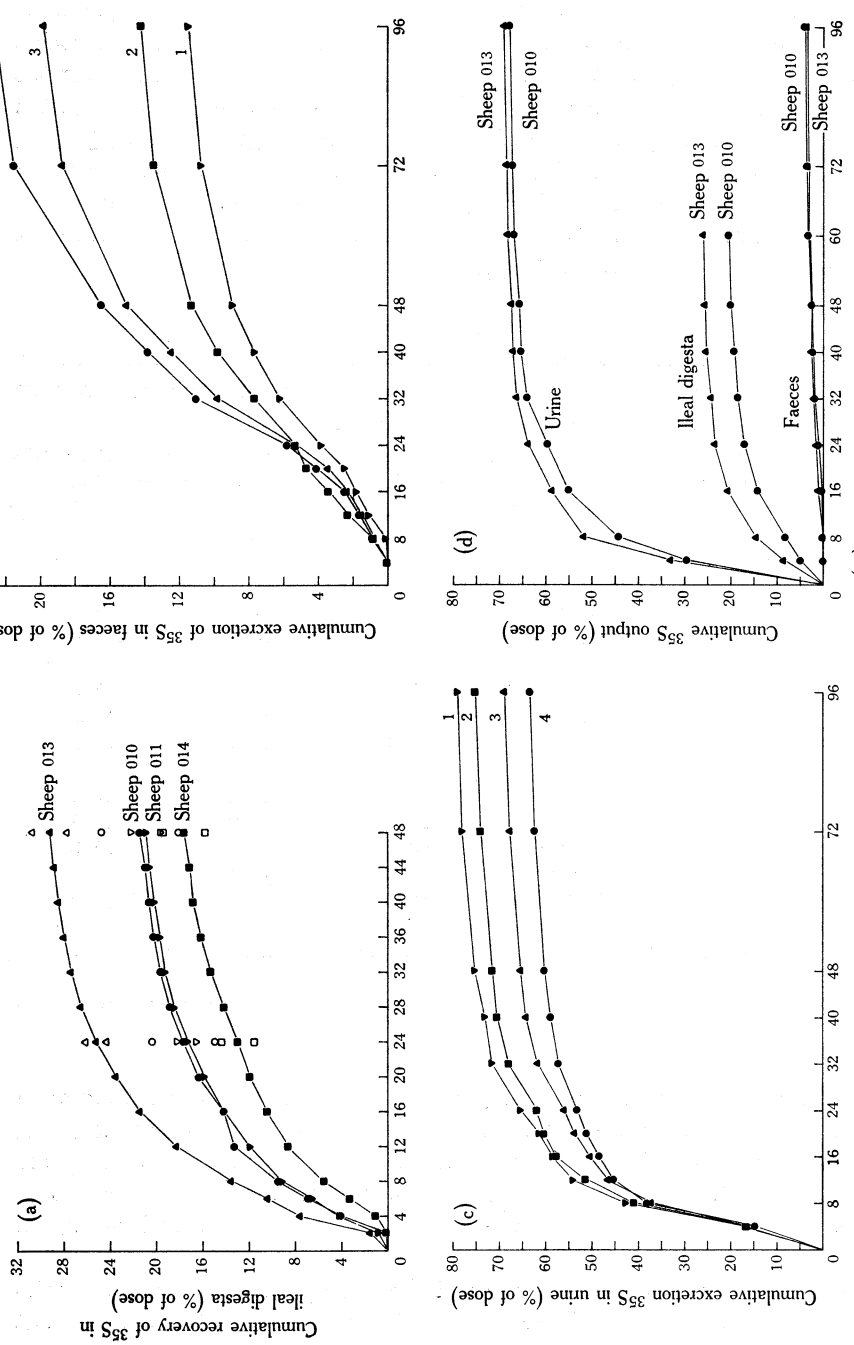

Fig. 1.—(*a*)–(*c*) Cumulative recovery of ^{35}S in ileal digesta (*a*) and cumulative excretion of ^{35}S in faeces (*b*) and in urine (*c*) after single intravenous infusions of $Na_2{}^{35}SO_4$ (experiment I). In (*a*) each point represents the mean of four unadjusted values per sheep. The S.E.M. at 24 and 48 hr for each sheep are shown by open symbols. In (*b*) and (*c*), treatment 1 is the control, and treatments 2, 3, and 4 are infusions per ileum of 30, 60, and 90 g glucose per day; each point represents the mean of four values per treatment. (*d*) Cumulative recovery of ^{35}S in ileal digesta, urine, and faeces after single intravenous infusions of $Na_2{}^{35}SO_4$ and cross-infusion of collected ileal digesta with digesta from control sheep (experiment II).

following the intravenous infusion of $Na_2{}^{35}SO_4$ is shown in Figures 2 and 3. The treatment values plotted are means (four observations per treatment).

Ileal infusions of glucose resulted in a net gain of ^{35}S to the digesta during transfer from the ileum to the faeces as a result of a "gain" of organic ^{35}S compounds (Table 3). There was a net loss of reducible ^{35}S compounds (i.e. inorganic or ester ^{35}S) from the ileal digesta on all treatments. Total ^{35}S and reducible ^{35}S "gains" differed between sheep ($P < 0{\cdot}05$).

(*b*) *Experiment II*

When single intravenous infusions of $Na_2{}^{35}SO_4$ were given to sheep 010 and 013, 68·2–69·2% of the infused ^{35}S was apparently excreted in the urine, 3·9–4·0% in the faeces, and 20·7–26·2% was recovered in the ileal digesta (Fig. 4). When this ^{35}S-labelled digesta was infused into sheep 014 and 011, 15·8–19·9% of the infused ^{35}S was excreted in the urine, 13·1–14·3% was recycled back into ileal digesta, and 12·2–44·2% was retained in the body (Table 4). From 26·9 to 53·6% was excreted in the faeces, therefore from 46·4 to 73·1% (mean 59·8%) of ^{35}S from ileal digesta labelled *in vivo* with $Na_2{}^{35}SO_4$ was absorbed from the hindgut.

Since sheep 010 and 013 received some ^{35}S by way of the ileal digesta transferred from sheep 014 and 011, respectively (Table 4), then the estimated recovery of ^{35}S as a percentage of the intravenous dose given to these sheep has been slightly overestimated. Assuming that 59·8% of the ^{35}S infused via the ileum into sheep 010 and 013 was absorbed in the hindgut, then, in either sheep 010 or 013, only 2·8% of the intravenously infused ^{35}S was excreted in the faeces after diffusing across the walls of the large intestine.

IV. Discussion

With increased intakes of sulphur more ^{35}S was excreted in urine, less was retained, and less was cycled to the ileal digesta and excreted in the faeces. This was apparently due to greater dilution of the intravenously infused $^{35}SO_4^{2-}$, with consequently a smaller proportion of the ^{35}S being secreted into the gut tract or utilized in sulphation reactions.

Comparing the basal treatment with the infusion of 90 g of glucose per day, the mean percentage ^{35}S excreted in faeces or urine, or retained, was 11·5 *v.* 22·7%, 79·1 *v.* 63·4%, and 7·3 *v.* 13·2%, respectively. Hansard and Mohammed (1968) found that yearling ewes ingesting 900 mg sulphur per day excreted a mean 16% of ^{35}S from oral or intravenous doses of $Na_2{}^{35}SO_4$ in the faeces, 75% in the urine, and retained 9% over a 7-day period. Johnson, Goodrich, and Meiske (1971), however, claimed retention of 56% of ^{35}S from oral doses of $Na_2{}^{35}SO_4$, with 20% being excreted in the urine and 22% in the faeces. In that experiment wether lambs (average weight 28·6 kg) were used and high activity of ^{35}S in nose and tracheal cartilage was found, suggesting that in these immature animals, as in growing birds (see Almquist 1970), absorbed inorganic ^{35}S was extensively esterified and incorporated into mucopolysaccharides of cartilage and bone and other structural tissues. It is probable, therefore, that the amount of sulphur ingested, the extent of fermentation in the rumen or hindgut or both, and the age of the animal will each affect the retention of infused $^{35}SO_4^{2-}$ and the pathway of ^{35}S excretion.

Bray (1969*a*) reported the faecal recovery of 12 and 19% of ^{35}S from intravenous doses of $Na_2{}^{35}SO_4$ and suggested that intravenously infused $^{35}SO_4^{2-}$ was secreted primarily into the post-ruminal tract in the form of sulphate esters or inorganic sulphate, and excreted as such in the faeces, but the results of the present experiment (Table 3) show that these reducible sulphur or ^{35}S fractions in the faeces were of minor significance. Bray's conclusion that little of the ^{35}S excreted could have been derived from the cycling of $^{35}SO_4^{2-}$ into the rumen and incorporation into microbial protein which is incompletely digested (Bird 1972*a*) is open to doubt. The protein ^{35}S that reached the ileum appears to be largely derived from ^{35}S cycled through the rumen because:

(1) A mean 24·5% of ^{35}S from intravenous infusions of $Na_2{}^{35}SO_4$ was recovered in the ileal digesta compared with only 6–8% in bile–pancreatic secretion (Bird 1972*b*), which constitute a major input of sulphur into the intestine. Further, the composition of ^{35}S in the bile–pancreatic secretions compared with ileal digesta was: protein ^{35}S, 0·5–0·7 *v.* 49·4%; soluble organic ^{35}S, 18–19 *v.* 10·1%; reducible ^{35}S, 80–81 *v.* 40·5%, respectively.

(2) The ability of mammalian tissues to synthesize organic sulphur compounds from inorganic sulphate (e.g. Houvinen and Gustafsson 1967) is extremely limited. The overall apparent digestibility of ^{35}S from ^{35}S-labelled ruminal microorganisms is *c.* 71% (Bird 1972*a*); therefore, since *c.* 12% of the infused ^{35}S was found in the ileal digesta protein, *c.* 40% of the infused dose could have been cycled to the rumen and incorporated into protein. However, this value would undoubtedly alter with changes in blood sulphate concentration and ruminal sulphide availability (both reflecting sulphur intake) and with changes in ruminal microbial growth potential (e.g. energy and nitrogen limitations). Changes in sulphur intake would alter the dilution of ^{35}S, while the trapping of cycled ^{35}S would depend upon microbial growth activity.

Sulphate is secreted in saliva and also may diffuse from the blood into the rumen (Bray 1969*b*). Mucin-secreting glandular cells in the colon, caecum, small intestine, and stomach actively secrete sulphate in an ester form and as inorganic sulphate (e.g. Belanger 1954; Dziewiatkowski 1956; Jennings and Florey 1956; Kent *et al.* 1956; Pasternak and Kent 1958). In the sheep, the uptake of $^{35}SO_4^{2-}$ by the colon and lower intestinal regions and incorporation into mucin is far greater than occurs in the duodenal or gastric areas of the foregut (Pasternak, Kent, and Davies 1958). Since little reducible sulphur normally passes from the rumen to the omasum (e.g. Bird and Hume 1971) or into the duodenum from the abomasum (Bird and Moir 1971) the major portion of the reducible ^{35}S found in the ileal digesta is probably derived from the bile-pancreatic secretions (Bird 1972*b*) and possibly from secretions into the ileum.

Glucose infusions decreased the flow to the ileum of total sulphur, organic sulphur, and protein, suggesting that increased plasma concentrations of glucose, or products thereof, in some way decreased the intestinal secretion of these endogenous compounds. From 531 to 618 mg of organic sulphur, or 378 to 444 mg of protein sulphur flowed daily to the terminal ileum. From Clarke, Ellinger, and Phillipson's (1966) data up to 6·9 mM cystine (i.e. 440 mg sulphur) flowed daily to the same region, therefore it is probable that much of the organic sulphur found in the ileal digesta is in the form of cystine, either in microbial protein, animal protein (e.g. trypsin) or, as Clarke, Ellinger,

and Phillipson suggest, in mucoprotein. Mucoprotein may not be precipitated by trichloroacetic acid (see Bird and Hume 1971) and might therefore account for much of the 142–154 mg of soluble organic sulphate which flowed daily to the ileum in the present experiment.

The ratio of protein nitrogen to protein sulphur in the ileal digesta (6·94) is narrower than found in omasal digesta (Bird and Hume 1971; Bird 1972*c*), in ruminal microorganisms (Walker and Nader 1968), or in animal tissue proteins (see Garrigus 1970) and indicates either the secretion of relatively indigestible sulphur-rich proteins into the abomasum or intestine or the absorption from microbial cells of a greater proportion of nitrogen than of sulphur. The ratio of nitrogen to sulphur in the ileal digesta (7·05) was similar to the protein nitrogen to sulphur ratio and therefore, for faeces, this latter ratio may be similar to the total nitrogen to sulphur ratio which was 8·02–8·54.

The extent to which protein ^{35}S in the ileal digesta was degraded and the sulphide ^{35}S either absorbed from the hindgut or converted into microbial protein cannot be ascertained from the results of this experiment. With treatments 1 and 2, as a percentage of the dose injected, 1·6–1·9% less organic ^{35}S was excreted in the faeces than left the ileum. This difference might have arisen from the absorption of ^{35}S from tauro-conjugated bile acid rather than from protein. These organic sulphur compounds can be labelled to some extent by duodenal infusions of $Na_2{}^{35}SO_4$ and cycling to the intestines occurs but little of the sulphur is excreted (Bird 1972*b*). Endogenous secretions of pancreatic protein are also efficiently absorbed (e.g. Snook and Meyer 1964).

The infusion of glucose into the distal ileum increased the faecal excretion of total sulphur by up to 110 mg/day. This was compensated by a decreased urinary excretion of sulphur, up to 109 mg/day, without affecting the retention of sulphur. Similar compensatory changes in faecal and urinary excretion of ^{35}S may be seen in Figures 2 and 3. Comparing the basal treatment with the infusion of 90 g of glucose/day, the mean percentage of the intravenously infused ^{35}S recovered in the faeces and urine was 11·5 *v.* 22·7% and 79·1 *v.* 63·4%, respectively. Increases in the faecal output of sulphur and ^{35}S were due mainly to an increased output of organic sulphur and organic ^{35}S, while the decrease in urinary sulphur excretion was due largely to a decreased output of inorganic sulphate. These results for sulphur correspond with those for nitrogen (Thornton *et al.* 1970), where infusions of glucose increased the faecal nitrogen output while there was a corresponding decrease in the output of total nitrogen and urea nitrogen in the urine.

The kidney is generally regarded as having the sole responsibility for regulating the concentration of sulphate and of most anions found in the blood (e.g. McClean 1960). In dogs, plasma inorganic sulphate is completely filtrable by the glomerular membrane (Goudsmit, Power, and Bollman, 1939) but is efficiently reabsorbed from the tubules when plasma concentrations are less than *c.* 1·5 mmoles/litre (Lotspeich 1947). However, changes in the filtration rate and in the concentrations of other electrolytes can affect the tubular maximum reabsorptive capacity for sulphate (e.g. Berglund and Lotspeich 1956). The results of the present experiment indicate that in ruminants the cycling of sulphate from the blood to the alimentary tract and incorporation into microbial protein may contribute to the regulation of blood sulphate levels.

There was a net transformation of reducible ^{35}S to organic ^{35}S forms in the hindgut when glucose was infused (Table 3) and more organic sulphur and ^{35}S was excreted in the faeces than passed the terminal ileum. Under the same circumstances more nitrogen was excreted in the faeces than passed the terminal ileum (Thornton *et al.* 1970). The results of both experiments indicate the entry and incorporation of endogenous sulphur and nitrogen into the digesta of the hindgut. The isotope data for sulphur also indicate that inorganic sulphur cycled to the hindgut, either via the small intestine or directly into the large intestine, was incorporated into microbial protein in increasing amounts when fermentative activity in that region was increased by the addition of glucose.

The relative contribution made towards the faecal excretion of ^{35}S by diffusion of $^{35}SO_4^{2-}$ directly into the hindgut was assessed in two ways. Firstly, when ileal infusions of glucose were given, up to 1·9% more of the infused ^{35}S was excreted in the faeces then passed the terminal ileum (Table 3). This must represent ^{35}S secreted into the large intestine and is *c.* 8·4% of the faecal ^{35}S. If, in the hindgut, relatively more of the ^{35}S is absorbed from the ileal digesta than from ^{35}S secreted into the hindgut then this estimate is minimal. When $^{35}SO_4^{2-}$ was infused intravenously into sheep in which the ileal digesta was diverted (experiment II, Table 4) an estimated 2·8% of the infused ^{35}S was excreted in the faeces, or *c.* 24·4% of the ^{35}S excreted in the faeces on the comparable basal treatment of the first experiment. Although ileal infusions of glucose would have increased the incorporation of cycled $^{35}SO_4^{2-}$ into hindgut digesta and the excretion of ^{35}S in the faeces, presumably the proportion of the faecal ^{35}S derived from secretions of ^{35}S into the large intestine would not change under those circumstances.

As with the excretion of urea (Thornton *et al.* 1970) it is concluded that the supply and availability of nutritional requirements for microbial growth in the fermentative regions of the gut tract exert a substantial influence on the proportion of endogenous sulphur excreted via the alimentary tract.

V. Acknowledgments

Technical assistance from Messrs. D. H. Drakes and W. N. Forward, Mrs. J. Madras, and Miss L. K. Jowett and helpful criticism by Professor R. J. Moir and Dr. M. Somers is acknowledged. Financial support was provided by the Australian Meat Research Committee and the Sulphur Institute.

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