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Sulfur–Selenium Studies in Sheep. II.* Effect of a Dietary Sulfur Deficiency on Selenium and Sulfur Metabolism in Sheep Fed Varying Levels of Selenomethionine

C. L. White

Department of Animal Science and Production, Institute of Agriculture, University of Western Australia, Nedlands, W.A. 6009; present address: Division of Animal Production, CSIRO, Private Bag, P.O., Wembley, W.A. 6014.

Abstract

The effect of a sulfur deficiency on the metabolism of selenium and sulfur was investigated in eight merino wethers. The sheep were fed high-sulfur (2 g S/kg) or low-sulfur (0·5 g S/kg) diets for two periods of 35 days each, and received selenium as selenomethionine at dietary concentrations of 0·02, 0·06, 0·09 and 0·67 mg Se/kg.

Sheep fed the low-sulfur diet had reduced feed intake, reduced nitrogen, sulfur and selenium balance, but elevated plasma and wool selenium concentrations.

Selenium concentrations in organs and tissues of slaughtered animals paralleled the selenium intake of the animal, with the renal cortex containing the highest concentration and bone the lowest.

The effect of the 0·5 g S/kg diet on feed intake is in contrast with the results from the previous experiment (White and Somers 1977) using 0·7 g S/kg. It is this difference in feed intake which was responsible for many of the effects on selenium metabolism observed in this experiment. Once the feed intake effects are accounted for, the implications for sulfur–selenium interactions remain as before, i.e. more selenium is incorporated into wool and plasma protein when dietary sulfur is limiting than when it is not.

Introduction

In a previous study (White and Somers 1977) it was shown that a reduction in dietary sulfur concentration from 2 to 0·7 g/kg resulted in increased wool and plasma selenium concentrations. Likewise, Pope *et al.* (1979) have shown that decreased dietary sulfate resulted in decreased urinary excretion and increased apparent retention of ^{75}Se in sheep dosed with radioactive selenate. In the former experiment, feed intake was not significantly influenced by sulfur treatment, and in the latter, pair feeding was undertaken.

In the light of these two reports, and others concerning the effects of sulfur on selenium requirements of sheep (Paulson *et al.* 1966; Whanger 1970), the purpose of the experiment described below was to examine the effects of a severe sulfur deficiency on selenium metabolism, as well as on nitrogen and sulfur balance in sheep which were not pair-fed.

Materials and Methods

Experimental Animals

Eight mature Merino wethers weighing between 34 and 40 kg at the start of the experiment were individually housed in metabolism crates consisting of stainless steel mesh flooring, jarrah uprights and black polythene pipe-coated iron. Deionized water was provided *ad libitum* in stainless steel troughs. All sheep were fed daily at 1000 h.

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Experimental Design

A 4 by 2 split-plot design was adopted in each of two 35-day periods separated by a 60-day readjustment interval. Diets containing 2 g S/kg were fed in period 1, and those containing 0·5 g S/kg were fed in period 2. The sheep were randomly allocated to each treatment in the first period, and the same concentration of dietary selenium was fed to the same sheep in both periods.

Prior to the start of both periods 1 and 2, the sheep were fed for 60 days on a standard maintenance ration of cereal chaff, lucerne chaff and commercial sheep cubes. This diet contained 2 g S/kg and 0·13 mg Se/kg. The last 5 days in each of the 60-day intervals were used to obtain an estimate of between-sheep variability in plasma selenium concentrations.

Diets

Solutions containing sodium sulfate and DL-selenomethionine (Calbiochem) were added to a diet containing 59% (w/w) wheaten straw, 25% (w/w) cornflour, 9% (w/w) sucrose, 4% (w/w) urea and 3% (w/w) mineral mix as described by Hume and Bird (1970), except that H_2SeO_3 was omitted. Analytical grade sucrose, minerals and urea were used. The basal diet contained 22 g N/kg, 0·5 g S/kg and 0·02 mg Se/kg. The feed was mixed as a slurry in a stainless steel paddle churner and dried at 49°C for 48 h. The four selenium treatments were 0·02, 0·06, 0·09, 0·67 mg Se/kg.

Collection of Samples

Daily blood samples (20 ml) were taken in heparinized tubes from the jugular vein immediately prior to feeding on the last 5 days of each 60-day interval preceding the introduction of sheep to the experimental diets. Plasma selenium levels from these collections were used in an analysis of covariance with treatment plasma selenium as a means of removing pretreatment sheep variability. These and all other plasma and whole blood samples were stored at −20°C prior to analysis. Blood samples were also taken on the last 5 days of each experimental period.

Over each 10-day collection period, dry matter intakes and urine and faeces outputs were measured daily. The urine was collected, subsampled and stored using the techniques of Bird and Moir (1971). A 10% aliquot of each daily faecal collection was bulked, stored at −20°C, and at the end of each 10-day collection freeze-dried before analysis commenced.

An area on the neck was clipped 20 days prior to the last day of each collection when part of the area was reclipped for wool samples to analyse for sulfur and selenium (i.e. the samples represented wool grown over 20 days). The neck was chosen for wool sampling because it provided an area consistent between sheep, and because it had to be clipped for blood sampling.

Tissue samples were taken from sheep slaughtered immediately after experiment 1 (White and Somers 1977) and this experiment (i.e. sheep had received diets containing 0·7 and 0·5 g S/kg, respectively, for 35 days prior to slaughter). The results were pooled for each selenium treatment since analysis of variance revealed no effects of sulfur.

Assay Procedure

The total nitrogen content of diets, feed residues, faeces and urine was determined by the Kjeldahl method of McKenzie and Wallace (1954). The total sulfur content of all samples, and plasma sulfate-sulfur were determined by the method of Bird and Fountain (1970). Wool samples were scoured in Shell X-4 (Bird and Moir 1971) prior to sulfur and selenium analyses. Subsamples of the scoured wool were clipped into lengths of approximately 2 mm and fibre diameter measurements were made using a Lanameter on 250 fibres selected at random. The method of Watkinson (1966) was used for selenium analysis.

Whole blood glutathione peroxidase (EC 1.11.1.9) activity was measured using a modified method of Paglia and Valentine (1967, cited by Paynter 1979).

Statistical Analysis

Data were analysed according to a split-plot analysis of variance. This technique was considered legitimate where analysis revealed a subplot error sum of squares equal to or greater than the main plot error sum of squares. Comparison of selenium treatment effects was by Duncan's multiple range test (Steel and Torrie 1960) using $\log_{10}$ transformed data.

Results

Responses to Sulfur Treatments

A reduction in dietary sulfur concentration from 2 to 0·5 g/kg resulted in a significant decrease in dry matter intake (DMI) and dry matter digestibility (DMD), as well as in nitrogen and sulfur balance (Table 1). Differences in faecal and urinary

Table 1. Responses to sulfur treatments

Each value is the mean ± s.e. for eight sheep. * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$; n.s., not significant

Parameter	Sulfur concn in diet (g/kg) 2·0	0·5	*P*
Dry matter			
Intake (g/day)	706±7	423±62	***
Digestibility (%)	64·4±0·8	56·6±0·8	***
Nitrogen			
Intake (g/day)	15·53±0·16	9·30±1·37	***
Faecal (g/day)	3·70±0·14	2·29±0·29	***
Apparent digestibility (%)	76·15±0·85	74·5±0·92	n.s.
Urinary (g/day)	9·14±0·34	6·90±0·76	***
Balance (g/day)	2·69±0·22	0·11±0·41	***
Sulfur			
Intake (mg/day)	1412±14	212±31	***
Faecal (mg/day)	514±17	184±21	***
Apparent digestibility (%)	64±1	4±8	***
Urinary (mg/day)	699±25	41±4	***
Balance (mg/day)	199±31	−13±13	***
Selenium			
Intake (μg/day)	150±72	81±39	n.s.
Faecal (μg/day)	79±36	59±23	n.s.
Apparent digestibility (%)	33±5	−7±12	n.s.
Urinary (μg/day)	38±14	21±6	n.s.
Balance (μg/day)	32±27	1±10	n.s.
Plasma			
Total sulfur (μg/ml)	813±27	809±14	n.s.
Sulfate-sulfur (μg/ml)	52±2	21±1	***
Whole blood glutathione peroxidase (EU/ml)	375±19	398±26	n.s.
Wool			
Total sulfur (g/kg)	27·6±0·4	27·6±0·4	n.s.
Selenium (μg/kg)	736±224	1261±267	n.s.
Fibre diameter (μm)	20·1±0·4	19·2±0·5	n.s.

nitrogen and sulfur excretion reflected differences in intake. Excretion of sulfur in the urine, as a proportion of total sulfur ingested, declined as sulfur intake fell from 1412 to 212 mg/day. Excretion of sulfur in the faeces, as a proportion of sulfur ingested, increased and sulfur balance was negative in the sheep fed 0·5 g S/kg diets.

Selenium intake and excretion were not shown to be affected by sulfur treatment. This was attributable to the variability induced by the tenfold range in selenium concentrations; Table 2 shows the separate effects more distinctly.

Table 2. Responses to selenium treatments

Each value is the mean ± range for two sheep. Different superscripts within each sulfur treatment represent differences at the 5% level of significance (Duncan's multiple range test)

Parameter	Dietary sulfur concn (g/kg)	Dietary selenium concn (mg/kg) 0·67	0·09	0·06	0·02
Selenium intake (μg/day)	2·0	481 ± 0[a]	61 ± 5[b]	41 ± 0[c]	17 ± 0[d]
	0·5	251 ± 97[a]	34 ± 48[b]	26 ± 16[b]	12 ± 1[b]
Faecal selenium excretion (μg/day)	2·0	234 ± 21[a]	41 ± 4[b]	28 ± 2[bc]	15 ± 1[cd]
	0·5	159 ± 55[a]	31 ± 37[b]	32 ± 24[b]	17 ± 3[b]
Apparent selenium digestibility (%)	2·0	51 ± 4[a]	33 ± 1[b]	32 ± 5[b]	15 ± 6[c]
	0·5	37 ± 3[a]	−2 ± 36[ab]	−20 ± 18[b]	−43 ± 14[b]
Urinary selenium excretion (μg/day)	2·0	100 ± 0[a]	18 ± 4[b]	17 ± 2[b]	15 ± 2[b]
	0·5	49 ± 6[a]	12 ± 8[b]	11 ± 2[b]	10 ± 0[b]
Selenium balance (μg/day)	2·0	147 ± 21[a]	2 ± 7[b]	−4 ± 4[b]	−13 ± 1[b]
	0·5	43 ± 36[a]	−9 ± 3[b]	−17 ± 10[b]	−15 ± 2[b]
Plasma selenium concn (ng/ml)	2·0	149 ± 12[a]	110 ± 9[b]	89 ± 9[b]	75 ± 5[cd]
	0·5	188 ± 19[a]	103 ± 3[b]	88 ± 3[b]	85 ± 7[b]
Wool selenium concn (μg/kg)	2·0	1745 ± 115[a]	519 ± 60[b]	380 ± 24[b]	300 ± 12[b]
	0·5	2465 ± 5[a]	1000 ± 70[b]	920 ± 40[b]	660 ± 20[c]
Whole blood selenium concn (ng/ml)	Combined	360 ± 22[a]	292 ± 10[b]	262 ± 21[b]	272 ± 15[b]

Sulfur intake had no influence on plasma total sulfur concentration, but plasma sulfate level showed a direct response to treatment (Table 1). Wool total sulfur concentration and fibre diameter were all unaffected by dietary sulfur. Wool selenium concentration, however, tended to increase as sulfur intake fell. Regression analysis (Fig. 1) showed this more clearly.

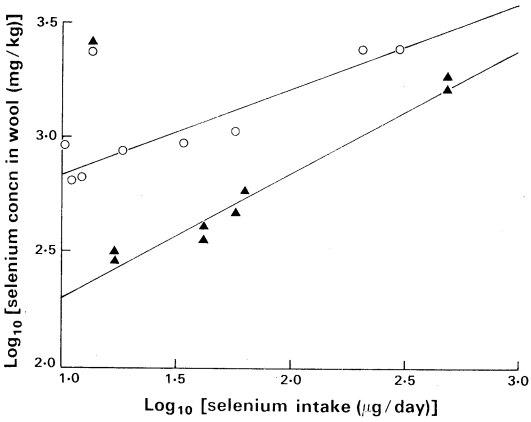

Fig. 1. Relationship between selenium concentration in wool and selenium intake.
○ 0·5 g S/kg; $y = 2{\cdot}46 + 0{\cdot}37x$, $r = 0{\cdot}95$.
▲ 2·0 g S/kg; $y = 1{\cdot}75 + 0{\cdot}55x$, $r = 0{\cdot}99$.

Responses to Selenium Treatment

Although not shown in Table 2, level of selenium in the diet had no significant effect on DMI, DMD, nitrogen or sulfur balance, nor on plasma or wool sulfur concentrations. Selenium intakes and excretion were directly related to dietary

selenium concentrations and selenium intake at both levels of sulfur, and this was reflected in differences in selenium balance (Table 2). As selenium intake fell, the amount excreted via the faeces increased as a proportion of that ingested. For example, faecal excretion expressed as a percentage of the daily intake changed from 49 to 85% as selenium intake went from 481 to 17 μg/day in the high-sulfur animals (Table 2). The corresponding excretion of selenium via the urine went from 21 to 88% of that ingested.

Plasma, whole blood and wool selenium concentrations responded directly to changes in selenium intake. Decreasing sulfur intake also resulted in increased selenium levels in wool and plasma (Fig. 1 and Table 2 respectively).

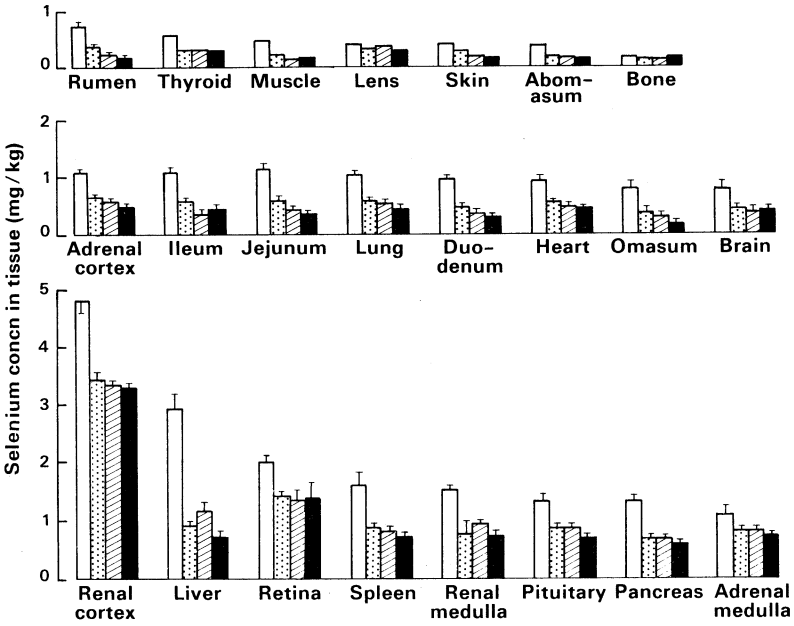

Fig. 2. Response in tissue concentrations of selenium to changes in concentration of selenium in the diet. Values are means ± s.e. for four sheep killed after receiving 0·5 or 0·7 mg S/kg in the diet.

The concentration of selenium in tissues varied with selenium intake and the tissue analysed (Fig. 2). In all cases, tissues from sheep fed the diet containing 0·67 mg Se/kg contained the highest concentration of selenium. In general, differences in tissue selenium concentration at the other three levels of selenium intake were not significant, but did reflect changes in selenium intake. The renal cortex contained the highest level of selenium, followed by the liver, retina and spleen. Skeletal muscle (femoral) and heart muscle (left ventricle) contained relatively low concentrations of selenium, although these tissues responded to changes in selenium intake.

Discussion

The results confirm and extend those reported previously (White and Somers 1977). The relationship between dietary sulfur concentration and DMI was more clearly established, with a marked reduction in intake occurring when sheep were fed 0·5 g

S/kg diet compared with 0·7 or 2·0 g S/kg. This reduced intake was associated with a significant decrease in DMD, a result not observed in sheep fed 0·7 g S/kg. Furthermore, the relative increase in the proportion of ingested nitrogen appearing in the urine of sheep fed 0·5 g S/kg compared with 2 g S/kg supports the findings of Bray and Hemsley (1969) and Hume and Bird (1970), and indicates that a greater proportion of nitrogen is escaping from the rumen as ammonia when sulfur is limiting. In this context, the failure to observe any differences in apparent nitrogen digestibility is to be expected since nitrogen supplied as urea is readily absorbed from the rumen as ammonia and excreted in the urine.

Sulfur metabolism differs from nitrogen metabolism in that excess sulfur promotes the growth of the ruminal bacteria *Desulphovibrio* spp., with the resulting sulfide being absorbed across the rumen wall and showing up in elevated plasma sulfate levels. For example, Bird and Hume (1971) reported that 36% of ingested sulfate-sulfur was absorbed as sulfide through the rumen wall when sulfur intake was 1940 mg/day. The majority of this appeared in the urine as ester and inorganic sulfate. This supports the results in Table 2 relating to changes in the relative importance of faecal and urinary excretory pathways for sulfur and nitrogen. Sheep fed the 2 g S/kg diet excreted 50% of the daily sulfur intake in the urine and 36% in the faeces. Respective values for the low-sulfur sheep were 19 and 96%. At low levels of sulfur intake, the origin and chemical nature of sulfur in faeces is not clear, although Bird (1971) showed that faecal sulfur was largely organic with some as insoluble metal sulfides.

The absence of any effect of sulfur treatment on wool sulfur levels agrees with previous results. Values for total sulfur content per kilogram of wool of 27·6 g were at the lower end of the range of 27 and 42 g S/kg cited by Reis (1965) for the normal range in sulfur content of wool. Reis estimated that the sulfur amino acid requirement for obtaining maximal sulfur levels in wool and maximal wool growth was approximately 2 g/day. The sheep fed the high- and low-sulfur treatments were in positive nitrogen balance, but the relatively low levels of sulfur in wool, irrespective of differences in sulfur intake, suggested that the supply of essential sulfur-containing amino acids may have been inadequate, even on the high-sulfur diet, to permit a full expression of potential for wool growth.

Both the total amount of selenium excreted, and the proportion of selenium excreted in the urine and faeces, were shown to be a function of selenium intake. It was evident that endogenous sources of selenium contributed significantly to both urinary and faecal selenium excretion when selenium intakes were low. For example, 49 and 20% of the ingested selenium was excreted in the faeces and urine, respectively, of sheep receiving the diet containing 0·67 mg Se/kg and 2 g S/kg. This was compared with 88% excreted in both the faeces and urine of sheep receiving the diet containing 0·02 mg Se/kg (Table 2).

Given adequate to luxury levels of selenium intake, the routes of excretion of selenium appear to differ from those of sulfur when sulfur is also in adequate supply. For example, the majority of ingested selenium appears in the faeces, whereas the majority of ingested sulfur appears in the urine (Tables 1 and 2). This difference in route of excretion has been used by Peterson and Spedding (1963) to indicate differences in the metabolism of sulfur and selenium in ruminants. Since there have been no measurements on the metabolism of sulfur compounds administered at

levels comparable to those of selenium, it is not possible to differentiate between effects of differences in level and differences in form on the excretion of the two elements.

The results from Table 1 show that the primary effect of sulfur on selenium excretion in urine and faeces is via a change in DMI. In other experiments, where DMI has been kept constant at different levels of sulfur intake, additional sulfate has been shown to increase selenium excretion in the urine when control diets contained less than 1 g S/kg (White and Somers 1977; Pope *et al.* 1979). However, this experiment shows that if intake is allowed to fall as a consequence of reduced dietary sulfur concentration, then sheep are unable to sufficiently compensate for the reduction in selenium intake, and selenium balance is adversely affected (Table 2). In spite of this drop in intake, however, wool and plasma selenium concentrations were maintained or were elevated, respectively, suggesting that selenium was being used in place of sulfur compounds for protein synthesis.

Wool selenium concentrations showed a significant linear correlation with selenium intake, and the changes in wool selenium concentration were more sensitive than plasma selenium to changes in selenium intake. Leonard and Burns (1955) found no correlation between blood and wool selenium content in sheep grazing pastures with high or low selenium concentrations. However, Hidiroglou *et al.* (1965) reported a positive correlation between pasture selenium levels and the selenium content of hair in cattle. The effect of sulfur on selenium incorporation into wool (Fig. 1) is similar to that observed in the previous experiment (White and Somers 1977) and perhaps explains some of these reported inconsistencies.

Tissue selenium concentrations (Fig. 2) showed a similar ranking to those for rats and chickens (Taussky *et al.* 1965; Hopkins *et al.* 1966). The significant differences in selenium levels between the renal cortex and renal medulla suggest the occurrence of a distinct partitioning of selenium within the kidney, perhaps on the basis of chemical form. In this context, Oh *et al.* (1976) reported a higher ratio of total selenium to glutathione peroxidase activity in the kidney compared with other organs, suggesting renal selenium may be largely in an inactive form. The high concentration of selenium in the retina is also interesting considering the photo-electric properties of selenium.

The failure of sulfur to influence tissue selenium concentration (Fig. 2) should be considered with caution. The samples were taken from only two sheep at each level of sulfur, and the sheep were killed immediately following the previous experiment (White and Somers 1977) or immediately after this experiment (i.e. after being fed diets containing 0·7 and 0·5 g S/kg, respectively). The results were pooled for four sheep when analysis revealed no differences between sulfur treatment, and should be used only to show the effect of selenium on tissue selenium concentration.

In summary, the results show that sulfur can influence selenium metabolism both directly (as indicated by elevated wool selenium levels in low-sulfur sheep) and indirectly via changes in feed intake. The plasma and wool selenium responses to sulfur suggest that a reduction in sulfur intake will result in an overall increase in selenium retention, despite a reduced feed, and hence selenium, intake. This possibility was not supported by data on selenium balance, but this could be due to carryover effects related to high endogenous selenium levels prior to the start of

treatment, and the relatively short treatment period. The results show that 35 days is adequate for comparing nitrogen and sulfur balance, but perhaps not for selenium balance studies.

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