Aust. J. Biol. Sci., 1977, **30**, 47–56

Sulphur–Selenium Studies in Sheep
I. The Effects of Varying Dietary Sulphate and Selenomethionine on Sulphur, Nitrogen and Selenium Metabolism in Sheep

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Abstract

Sulphur, selenium and nitrogen metabolism were studied in Merino wethers fed for 35-day periods on semipurified diets in which the sulphur content was increased to either 0·07 or 0·20% by the addition of sodium sulphate. At both levels of sulphur, additions of selenium as DL-selenomethionine increased the basal level of selenium (0·02 μg/g) to 0·06, 0·09 and 0·67 μg/g.

Both levels of dietary sulphur supported positive sulphur balances but a reduction in sulphur intake *per se* resulted in a significant depression in dry matter digestibility ($P < 0·05$), apparent nitrogen digestibility ($P < 0·05$), nitrogen balance ($P < 0·01$), sulphur balance ($P < 0·05$) and plasma sulphate-sulphur ($P < 0·001$). Sheep fed the diet with 0·07% sulphur also had significantly higher plasma ($P < 0·05$) and wool ($P < 0·01$) selenium levels. Selenium balance was not affected by differences in sulphate-sulphur intake.

Selenium balances ($P < 0·001$), plus the selenium levels in plasma ($P < 0·001$), and wool ($P < 0·001$) were significantly different at the different levels of selenium supplementation. A positive selenium balance was achieved when the selenium intake was approximately 37 μg/day, regardless of sulphur treatment.

The validity of using plasma and/or wool selenium levels as indices of the selenium status of sheep is questioned.

Introduction

Although there is evidence of sulphur antagonizing the uptake of chemically similar selenium analogues in plants (Hurd-Karrer 1937; Fleming 1962; Pratley and McFarlane 1974), in microorganisms (see review by Shrift 1973), and in rats (Halverson and Monty 1960; Ganther and Baumann 1962; Halverson *et al.* 1962), evidence of such an antagonism operating in ruminants is equivocal (see review of Whanger 1970). Paulson *et al.* (1966) failed to show any antagonistic effect of 0·5% sulphur as sulphate on ^{75}Se excretion or tissue ^{75}Se levels in lactating ewes fed a basal diet containing 0·282% total sulphur, when orally administered a dose of [^{75}Se]-selenate. Pope *et al.* (1968), however, did show a sulphate–selenate interaction in sheep fed diets containing 0·05, 0·10, 0·15 and 0·20% sulphur, whereby blood ^{75}Se activity was elevated, and urinary ^{75}Se output reduced in those sheep fed the diet containing the lowest sulphur level compared with the other three levels. It is probable that the basal sulphur level (0·05% S) was deficient for optimum energy and nitrogen utilization at both the rumen and the tissue level (Moir *et al.* 1967–68; Bray and Hemsley 1969; Hume and Bird 1970; Bird 1972). These findings imply that changes in selenium metabolism, due to sulphate supplementation, may be due solely to changes in microbial and/or tissue metabolism associated with a change from a deficient

to an adequate dietary sulphur supply. If so, then this could provide a rational explanation of the inconsistencies between reports in the literature dealing with the effects of sulphur supplements on the incidence of white muscle disease in ruminants (see Whanger 1970).

In view of the evidence that (1) selenomethionine is one, if not the main source of selenium ingested by grazing ruminants (Peterson and Butler 1962; Jenkins and Hidiroglou 1967; Olson *et al.* 1970), (2) the metabolism of selenomethionine by rumen bacteria differs from that of inorganic forms of selenium (Paulson *et al.* 1968), as is true of animal tissues (Fuss and Godwin 1975), and (3) because selenomethionine has not previously been fed as a source of selenium in sulphur–selenium studies on sheep, our purpose was to examine the effects of varying the dietary levels of sulphate, and of selenomethionine, on nitrogen, sulphur and selenium metabolism in Merino wethers. The range in dietary selenium (four levels), and sulphur (two levels) was selected to represent those found in many pastures.

Materials and Methods

Experimental Animals

Eight mature Merino wethers weighing between 34 and 40 kg at the start of the experiment were used. Each was individually housed in a metabolism crate made of high density polyethylene and stainless steel in order to minimize contamination. Likewise feed bins and water troughs were made of stainless steel and deionized water was provided *ad libitum* for drinking. Throughout the whole experiment all sheep were fed once daily at 1000 h.

Experimental Design

A 4 × 2 split plot design was adopted in each of the two periods during which the experimental diets were fed and the collection of samples made. Diets containing the 0·20% level of sulphur were fed in period 1, and those containing the lower level (0·07%) were fed in period 2. The sheep were randomly allocated to the diets in the first period, and although the level of dietary sulphur was decreased in period 2, each pair was fed the same level of dietary selenium as in period 1 (namely 0·02, 0·06, 0·09 and 0·67 μg/g).

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 0·2% S and 0·13 μg Se/g. The last 5 days in each of the 60-day intervals were used to obtain an estimate of between sheep variability in plasma selenium levels.

Following the 60-day equilibration intervals, experimental diets were fed for 35 days, partitioned into a 25-day precollection period and a 10-day collection period.

Diets

Solutions containing sodium sulphate and DL-selenomethionine (Calbiochem) were added to a semisynthetic diet containing 59% hammer-milled wheaten straw, 25% cornflour, 9% sucrose, 4% urea, and 3% of a mineral mix as described by Hume and Bird (1970), except that the H_2SeO_3 was deleted. Analytic Reagent grade sucrose, minerals and urea were used. This diet contained 2·20% nitrogen, 0·049% sulphur, and 0·024 μg Se/g. The sodium sulphate or the selenomethionine, or both, in 30 litres of deionized water was sprayed onto 30 kg of the semisynthetic diet during paddle-churning in an all-stainless-steel feed mixer. After thorough mixing the diets were thinly spread on stainless steel trays and dehydrated for 48 h at 49°C. Rations containing 750 g were weighed out as each batch of diet was removed from the dehydrator and at the same time samples were taken for dry matter determinations. Each ration contained an average of 713 g dry matter.

Table 1 shows the concentrations of sulphur and selenium in the diets (as determined analytically) and the order in which the diets were fed (as shown by the treatment number).

Collection of Samples

Daily blood samples (20 ml) were taken in heparinized tubes from the jugular vein immediately before feeding on the last 5 days of each 60-day interval which preceded introducing the sheep to the

experimental diets. Plasma selenium levels from these collections were used in an analysis of covariance with treatment plasma selenium levels as a means of removing individual pretreatment sheep variability. These and all other plasma samples were stored at −20°C while awaiting analysis. Blood samples were also taken on the last 5 days of each experimental period and the plasma fractions treated in the manner described.

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 collecton freeze-dried before analyses commenced.

Table 1. Sulphur and selenium concentrations of the experimental diets and the order in which they were presented

Period	Treatment No.	S in diet (%)	Se in diet (μg/g)
1	1a	0·20	0·67
1	2a	0·20	0·09
1	3a	0·20	0·06
1	4a	0·20	0·02
2	1b	0·07	0·67
2	2b	0·07	0·09
2	3b	0·07	0·06
2	4b	0·07	0·02

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 sulphur 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 in any case.

Chemical Analyses

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

Statistical Analysis

Data were statistically analysed according to a split-plot multiway analysis of variance (Steel and Torrie 1960).

Results

An analysis of covariance using plasma selenium levels from samples collected prior to treatment and during treatment showed that initial between sheep differences in the plasma selenium levels had no significant effect on subsequent plasma selenium levels due to the dietary treatments.

The responses due to differences in the level of dietary sulphur are shown in Table 2. It can be seen from the table that reducing the concentration of dietary

* Ort: morsel left after meal.

Table 2. Effects of the level of dietary sulphur on dry matter intake and digestibility, on nitrogen, sulphur and selenium balances, on sulphur and selenium in plasma and wool and on wool fibre diameter

Each value is the mean ± s.e. for eight sheep

	S in diet (%)		
	0·20	0·07	*P*
Dry matter:			
Intake (g/day)	700±8	657±25	n.s.
Digestibility (%)	65·5±0·8	62·3±0·7	<0·05
Nitrogen:			
Intake (g/day)	15·40±0·18	14·45±0·56	n.s.
Faecal (g/day)	3·28±0·10	3·36±0·12	n.s.
Apparent digestibility (%)	79·0±0·6	76·8±0·5	<0·05
Urinary (g/day)	8·99±0·30	9·55±0·40	n.s.
Balance (g/day)	3·14±0·23	1·56±0·19	<0·01
Sulphur:			
Intake (mg/day)	1387±10	444±17	<0·001
Faecal (mg/day)	456±21	307±18	<0·001
Urinary (mg/day)	746±34	82±10	<0·001
Balance (mg/day)	186±32	55±23	<0·05

	S in diet (%)		
	0·20	0·07	*P*
Selenium:			
Intake (μg/day)	148±72	131±62	n.s.
Faecal (μg/day)	79±35	69±30	n.s.
Urinary (μg/day)	43±21	34±14	n.s.
Balance (μg/day)	25±17	27±19	n.s.
Plasma:			
Total-S (μg/ml)	896±29	851±15	n.s.
Sulphate-S (μg/ml)	62±3	34±1	<0·001
Se (ng/ml)	82±11	107±18	<0·05
Wool:			
Total-S (%)	2·73±0·05	2·81±0·06	n.s.
Se (ng/g)	897±297	1342±418	<0·01
Fibre diameter (μm)	20·3±0·40	20·0±0·40	n.s.

sulphur from 0·20 to 0·07% did not result in a significant difference in dry matter intake (DMI), although dry matter digestibility (DMD) ($P < 0·05$), apparent nitrogen digestibility ($P < 0·05$), and nitrogen balance ($P < 0·01$) were significantly reduced. Similarly, urinary ($P < 0·001$) and faecal ($P < 0·001$) sulphur excretion, and sulphur balance ($P < 0·05$) were reduced in sheep fed the 0·07% sulphur ration, but both the sulphur and nitrogen balances were positive at both levels of sulphur intake.

Sheep on the 0·20% sulphur diet showed elevated plasma sulphate-sulphur levels ($P < 0·001$) compared with those on the 0·07% sulphur diet, but no significant differences due to sulphur treatment were observed in plasma or wool total sulphur levels, or in wool fibre diameter (Table 2). Despite a three-fold difference in the sulphur intake between the sheep fed the high and low levels of sulphur, and in the sulphur balances, neither the selenium intakes, faecal and urinary excretion of selenium (with one exception), nor the selenium balances were significantly influenced by differences in sulphur intake (Table 2). Sheep fed the diet containing 0·20% sulphur and 0·67 μg Se/g excreted significantly ($P < 0·05$) larger amounts of selenium in the urine than sheep on the diet with 0·07% sulphur and 0·67 μg Se/g (Table 3). However, this increased excretion was associated with an increased DMI (713 *v.* 613 g/day), although differences in DMI were not significant when considered together with all other treatments. This resulted in the net retention of selenium being of the same magnitude at the two sulphur levels.

Despite the fact that dietary sulphur treatment had no effect on selenium balance, plasma ($P < 0·05$) and wool ($P < 0·01$) selenium levels were significantly elevated in sheep on the 0·07% sulphur diet (Table 2).

The results relating to the effects of feeding different levels of dietary selenium on sheep responses are shown in Table 3. Since the variance of the data shown in Table 3 increased with the mean, analysis of variance was performed on $\log_{10}$ transformed data, and statistical differences between responses to different levels of selenium supplementation were assessed using least significant differences ($P < 0·01$) on the transformed data.

Although not indicated in Tables 2 or 3, the level of selenium intake had no significant effect on DMI, DMD, nitrogen or sulphur balances, nor on wool fibre diameter.

Differences in the faecal excretion of selenium were significant for the four selenium intakes ($P < 0·01$) (Table 3). At the two lowest levels of selenium intake (8 and 13 μg/day) faecal excretion exceeded intake, but at all other levels of selenium intake there was an apparent net absorption of selenium. Differences in the urinary excretion of selenium were not significant at the two lower levels of selenium intake for the high sulphur treatment, but otherwise the differences were significant between the remaining values and reflected differences in selenium intake (Table 3).

There were significant differences ($P < 0·01$) between selenium balances. As the selenium intake increased from a mean of 11 to 447 μg/day, so did the selenium balance increase from −12 to 109 μg/day, a positive balance being achieved when the mean selenium intake was 37 μg/day.

Both plasma and wool selenium levels were significantly influenced by differences in selenium and sulphur intake (Table 3) but the total sulphur in wool was influenced only by selenium intake.

The highly significant correlation ($r = 0{\cdot}92$, $P < 0{\cdot}001$) between plasma and wool selenium levels suggests that most of the variability in wool selenium concentration can be explained on the basis of changes in plasma selenium level (Fig. 1).

Table 3. Effect of selenium and sulphur treatment on selenium balance and plasma and wool selenium levels

Each value is the mean ± s.e. for two sheep. Different superscripts represent differences at the 1% level of significance

	Dietary sulphur (%)	Dietary selenium (μg/g)			
		0·67	0·09	0·06	0·02
Selenium:					
Intake (μg/day)	0·20	481^{a}	63^{b}	41 ± 1^{c}	8 ± 6^{d}
	0·07	414 ± 51^{a}	63^{b}	33 ± 6^{c}	13 ± 2^{d}
Faecal (μg/day)	0·20	237 ± 2^{a}	38 ± 2^{b}	31 ± 2^{bc}	15 ± 4^{c}
	0·07	206 ± 27^{a}	35 ± 1^{b}	22 ± 4^{bc}	16 ± 2^{c}
Urinary (μg/day)	0·20	140 ± 5^{a}	13 ± 1^{b}	11 ± 2^{b}	8 ± 2^{b}
	0·07	94 ± 18^{a}	21 ± 1^{b}	12 ± 1^{bc}	9 ± 1^{c}
Balance (μg/day)	0·20	104 ± 4^{a}	12 ± 2^{b}	-1 ± 1^{c}	-15 ± 2^{d}
	0·07	114 ± 6^{a}	7 ± 2^{b}	-1 ± 2^{c}	-12 ± 1^{d}
Plasma:					
Selenium (ng/ml)	0·20	132 ± 6^{a}	74 ± 4^{b}	68 ± 2^{b}	56 ± 8^{b}
	0·07	186 ± 23^{a}	93 ± 2^{b}	86 ± 8^{b}	65 ± 16^{b}
Wool:					
Selenium (ng/g)	0·20	2250 ± 50^{a}	593 ± 9^{b}	391 ± 4^{c}	355 ± 53^{c}
	0·07	3220 ± 380^{a}	855 ± 75^{b}	676^{bc}	615 ± 25^{c}
Sulphur (%)	0·20	$2{\cdot}71 \pm 0{\cdot}03^{c}$	$2{\cdot}73 \pm 0{\cdot}05^{a}$	$2{\cdot}83 \pm 0{\cdot}06^{a}$	$2{\cdot}56 \pm 0{\cdot}04^{c}$
	0·07	$2{\cdot}65 \pm 0{\cdot}01^{a}$	$2{\cdot}97 \pm 0{\cdot}07^{b}$	$3{\cdot}07 \pm 0{\cdot}04^{b}$	$2{\cdot}75 \pm 0{\cdot}12^{a}$

Discussion

The results in Table 2 testify to the importance of the level of dietary sulphur upon nitrogen utilization by sheep, particularly when all, or a high proportion of the dietary nitrogen is supplied as urea (see Moir *et al.* 1967–68; Bray and Hemsley 1969; Hume and Bird 1970; Bird 1972). Under these conditions sub-optimal intakes of sulphur have been shown to adversely affect microbial metabolism in the rumen.

Plasma sulphate-sulphur levels reflected changes in sulphur intake (Table 2) (see also Weir and Rending 1954; Bray and Hemsley 1969). However, the concentration of sulphur in wool, and wool fibre diameter were not affected by sulphur intake.

The observed increase in wool selenium concentration in response to a decrease in sulphur intake has not previously been reported. The significant correlation between wool and plasma selenium levels (Fig. 1) suggests that the effect of sulphur intake on wool selenium concentration is largely mediated via an elevated plasma selenium level.

Although the observed responses in plasma selenium due to differences in sulphur intake are in agreement with results reported by Pope *et al.* (1968), corresponding changes in the faecal and urinary excretion of selenium were not observed in the present experiment, apart from the interaction whereby urinary selenium excretion was elevated ($P < 0{\cdot}05$) by the high selenium–high sulphur treatment. Differences in the form and level of selenium and/or the level of sulphur in the diet may account

for the differences in selenium excretion. Paulson *et al.* (1968) showed there was a difference in the way rumen microbia metabolized selenomethionine, selenite and [^{75}Se]selenate. The selenomethionine was largely incorporated unmodified into microbial protein, while very little selenite or selenate was converted to selenoamino acids. Likewise, Hidiroglou *et al.* (1972) reported a difference in the kinetics of metabolism in the blood between selenoamino acids and inorganic selenium salts administered to the rumen of sheep. Ehlig *et al.* (1967) reported increased retention of selenium in lambs administered selenomethionine compared with selenite. These differences in retention were due mainly to an increased urinary selenium excretion in the sheep administered selenite-selenium. Similarly, Fuss and Godwin (1975) reported significantly greater retention of ^{75}Se from [^{75}Se]selenomethionine than from [^{75}Se]selenite when injected into sheep. Our data and those of Pope *et al.* (1968) show that when the availability of sulphur is limiting metabolism, more selenium is incorporated into microbial amino acids and absorbed as such, and this is reflected in elevated plasma and wool selenium levels. In our experiment selenomethionine rather than selenate was given and presumably more organic selenium was absorbed at the higher levels of sulphur intake than occurred in the experiment of Pope *et al.* This could account for the difference in the pattern of selenium excretion.

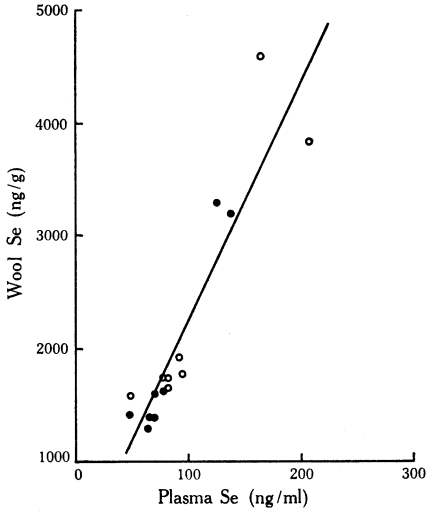

Fig. 1. The effect of plasma selenium concentration on wool selenium concentration. Regression equation is $y = 21x - 831$ ($r = 0{\cdot}92$, $P < 0{\cdot}001$). ● 0·20% S. ○ 0·07% S.

Reference to Table 3 shows that differences in selenium intake resulted in significant differences in the faecal and urinary excretion of selenium and selenium balances. It is of interest to note that the selenium balance became positive (1 μg/day) when the selenium intake was approximately 37 μg/day, corresponding with a dietary level of 0·057 μg/g. Gardiner (1969) suggested that outbreaks of white muscle disease might be anticipated with diets containing 0·05 μg/g or less of selenium.

When the diet containing 0·67 μg Se/g was fed (selenium intake being 447 ± 29 μg/day) approximately 50% of the selenium ingested was excreted in the faeces, and 26% in the urine. As the selenium intake declined, relatively more of the ingested selenium was apparently excreted in the faeces and urine. However, it is probable that a higher proportion of the total selenium excreted was then of endogenous

origin. When the intake of selenium was approximately 11 μg/day, faecal excretion (14 μg/day) alone exceeded intake, and although these sheep were in a negative selenium balance they still excreted 9 μg Se/day in the urine.

Our results pertaining to the faecal excretion of selenium agree with those previously published for sheep (Cousins and Cairney 1961; Butler and Peterson 1963; Peterson and Spedding 1963; Paulson *et al.* 1966; Wright and Bell 1966; Ehlig *et al.* 1967; Hidiroglou *et al.* 1972). The differences in faecal excretion between ruminants and non-ruminants (Wright and Bell 1966), together with the evidence of Hidiroglou *et al.* (1972) concerning abomasal versus ruminal infusions of selenium, suggest that the activities of the microflora in the reticulo-rumen can markedly alter the form in which ingested selenium is presented for absorption, and hence its availability.

The level of selenium in plasma was related to selenium intake (Table 3) at both levels of sulphur intake, although at the lower levels of selenium intake, plasma selenium levels were not significantly different. However, the selenium balances, and thus the selenium status, of the sheep were significantly different. Clearly the plasma selenium level alone may not be an accurate guide in assessing the selenium status of sheep in such short-term experiments.

The regression equation of plasma selenium concentration on wool selenium concentration (Fig. 1) suggests that wool selenium is highly responsive to changes in plasma selenium, and that wool constitutes a significant selenium sink at least when selenomethionine is fed. These results differ from those of Leonard and Burns (1955) who found no correlation between blood and wool selenium levels in sheep grazing pastures with high and low selenium levels. However, Olson *et al.* (1954) and Hidiroglou *et al.* (1965) reported a correlation between pasture selenium levels and the selenium content of hair in cattle.

Accepting that the rate of wool growth did not vary due to differences in sulphur intake, and assuming that the average growth of clean dry wool was 7 g/day, it can be calculated that the selenium incorporated into the wool was approximately 6 μg/day in sheep fed the diet containing 0·20% sulphur, and 9 μg/day in those on the diet with 0·07% sulphur (Table 2). To further stress the role of wool as a selenium sink, it was estimated that even when the sheep were in negative selenium balance, wool was absorbing between 2 (high sulphur) and 4 (low sulphur) μg of selenium per day (Table 3). If plasma and/or wool selenium levels are used as indices of selenium status, our results would indicate that sheep on the diet containing the lower level of sulphur were in a better selenium status than those on the 0·20% sulphur diet. However, there were no differences in selenium balances between the two sulphur treatments, and it is noteworthy that the sheep on the high sulphur diet were in a better selenium status than those on the low sulphur diet because less selenium was entering the wool.

The effect of selenium intake on wool sulphur level ($P < 0·01$) (Table 3) has not previously been reported. It does not appear to be a direct sulphur–selenium relationship as the wool sulphur concentration in sheep fed diets with 0·67 or 0·02 μg Se/g (at both levels of sulphur intake) was lower than for the other two levels of selenium, and there were no differences in fibre diameter due to the levels of selenium fed.

Our observations do not indicate that there is a sulphur–selenium antagonism in sheep consuming physiological levels of selenium as selenomethionine when the

animals are fed sufficient sulphate-sulphur to maintain a positive sulphur balance. If such an antagonism does occur and plays a part in the aetiology of white muscle disease, then it operates at submaintenance levels of sulphur intake or the antagonism operates with other forms of sulphur or selenium, or both of these elements.

Acknowledgments

We are grateful to the Australian Wool Corporation whose funds supported this work, and for the statistical advice given by Dr N. A. Goodchild and Mr C. S. Edwards.

References

Bird, P. R. (1972). Sulphur metabolism and excretion studies in ruminants. IX. Sulphur, nitrogen, and energy utilization by sheep fed a sulphur-deficient and sulphur supplemented, roughage-based diet. *Aust. J. Biol. Sci.* **25**, 1073.

Bird, P. R., and Fountain, R. D. (1970). A method for the determination of sulphur in some biological materials. *Analyst (Lond.)* **95**, 98.

Bird, P. R., and Moir, R. J. (1971). Sulphur metabolism and excretion studies in ruminants. I. The absorption of sulphate in sheep after intraruminal or introduodenal infusions of sodium sulphate. *Aust. J. Biol. Sci.* **24**, 1319.

Bray, A. C., and Hemsley, J. A. (1969). Sulphur metabolism in sheep. IV. The effect of a varied dietary sulphur content on some body fluid sulphate levels and on the utilization of urea-supplemented roughages by sheep. *Aust. J. Agric. Res.* **20**, 759.

Butler, G. W., and Peterson, P. J. (1963). Availability of selenium in forage to ruminants. *Proc. N.Z. Soc. Anim. Prod.* **23**, 13.

Cousins, F. B., and Cairney, I. M. (1961). Some aspects of selenium metabolism in sheep. *Aust. J. Agric. Res.* **12**, 927.

Ehlig, C. F., Hogue, D. E., Allaway, W. H., and Hamm, D. J. (1967). Fate of selenium from selenite or seleno-methionine with or without vitamin E, in lambs. *J. Nutr.* **92**, 121.

Fleming, G. A. (1962). Some factors affecting the uptake of selenium by plants. *Ir. J. Agric. Res.* **1**, 131.

Fuss, C. N., and Godwin, K. O. (1975). A comparison of the uptake of [^{75}Se]selenite, [^{75}Se]selenomethionine and [^{35}S]methionine by tissues of ewes and lambs. *Aust. J. Biol. Sci.* **28**, 239.

Ganther, H. E., and Baumann, C. A. (1962). Selenium metabolism. II. Modifying effects of sulphate. *J. Nutr.* **77**, 408.

Gardiner, M. R. (1969). Selenium in animal nutrition. *Outlook Agric.* **6**, 19.

Halverson, A. W., Gus, P. O., and Olsen, O. E. (1962). Effect of sulphur salts on selenium poisoning in the rat. *J. Nutr.* **77**, 459.

Halverson, A. W., and Monty, K. J. (1960). The effect of dietary sulphate on selenium poisoning in the rat. *J. Nutr.* **70**, 100.

Hidiroglou, M., Carson, R. B., and Brossard, G. A. (1965). Influence of selenium on the selenium content of hair and on the incidence of nutritional muscular disease in beef cattle. *Can. J. Anim. Sci.* **45**, 197.

Hidiroglou, M., Jenkins, K. J., and Quittkat, E. S. (1972). Le sort du radiosélenium adminstré dans le rumen ou dans la caillette du mouton. *Ann. Biol. Anim. Biochim. Biophys.* **12**, 599.

Hume, I. D., and Bird, P. R. (1970). Synthesis of microbial protein in the rumen. IV. The influence of the level and form of dietary sulphur. *Aust. J. Agric. Res.* **21**, 315.

Hurd-Karrer, A. M. (1937). Comparative toxicity of selenates and selenites to wheat. *Am. J. Bot.* **24**, 720.

Jenkins, K. J., and Hidiroglou, M. (1967). The incorporation of ^{75}Se-selenite into dystrophic pasture grass. The chemical nature of the seleno compounds formed and their availability to young ovine. *Can. J. Biochem.* **45**, 1027.

Leonard, R. O., and Burns, R. H. (1955). A preliminary study of selenized wool. *J. Anim. Sci.* **14**, 446.

McKenzie, H. A., and Wallace, H. S. (1954). The Kjeldahl determination of nitrogen: a critical study of digestion conditions—temperature, catalyst, and oxidizing agent. *Aust. J. Chem.* **7**, 55.

Moir, R. J., Somers, M., and Bray, A. C. (1967–68). Utilization of dietary sulphur and nitrogen by ruminants. *Sulphur Inst. J.* **3**, 15.

Olson, O. E., Dinkel, C. A., and Kamstra, L. D. (1954). A new aid in diagnosing selenium poisoning. *S. D. Farm Home Res.* **6**, 12.

Olson, O. E., Novacek, E. J., Whitehead, E. I., and Palmer, I. S. (1970). Investigations on selenium in wheat. *Phytochemistry* **9**, 1181.

Paulson, G. D., Baumann, C. A., and Pope, A. L. (1966). Fate of physiological dose of selenate in the lactating ewe: effect of sulfate. *J. Anim. Sci.* **25**, 1054.

Paulson, G. D., Baumann, C. A., and Pope, A. L. (1968). Metabolism of ^{75}Se-selenite, ^{75}Se-selenate, ^{75}Se-selenomethionine and ^{35}S-sulfate by rumen microorganisms in vitro. *J. Amin. Sci.* **27**, 497.

Peterson, P. J., and Butler, G. W. (1962). The uptake and assimilation of selenite by higher plants. *Aust. J. Biol. Sci.* **15**, 126.

Peterson, P. J., and Spedding, D. J. (1963). The excretion by sheep of ^{75}selenium incorporated into red clover (*Trifolium protence* L). *N.Z. J. Agric. Res.* **6**, 13.

Pope, A. L., Moir, R. J., Somers, M., and Underwood, E. J. (1968). Effects of sulphur on ^{75}selenium absorption in sheep. *J. Anim. Sci.* **27**, 1771 (abst.).

Pratley, J. E., and McFarlane, J. D. (1974). The effect of sulphate on the selenium content of pasture plants. *Aust. J. Exp. Agric. Anim. Husb.* **14**, 533.

Shrift, A. (1973). In 'Organic Selenium Compounds: Their Chemistry and Biology'. (Eds D. L. Klayman and W. H. H. Gunther.) (Wiley-Interscience, N.Y.)

Steel, R. G. D., and Torrie, J. H. (1960). 'Principles and Procedures of Statistics'. (McGraw-Hill: N.Y.)

Watkinson, J. H. (1966). Fluorometric determination of selenium in biological material with 2,3-diaminonaphthalene. *Anal. Chem.* **38**, 92.

Weir, W. C., and Rendig, V. V. (1954). Serum inorganic sulfate sulphur as a measure of the sulfur intake of sheep. *J. Nutr.* **54**, 87.

Whanger, P. D. (1970). Sulphur–selenium relationships in animal nutrition. *Sulphur Inst. J.* **6**, 6.

Wright, P. L., and Bell, M. C. (1966). Comparative metabolism of selenium and tellurium in sheep and swine. *Am. J. Physiol.* **211**, 6.

Manuscript received 5 May 1976