British Journal of Nutrition (1988), **59**, 93–108

Studies in sheep on the absorption of magnesium from a low molecular weight fraction of the reticulo-rumen contents

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(*Received 13 April 1987 – Accepted 28 August 1987*)

1. Six sheep, three animals per diet, were prepared with rumen fistulas and fed on frozen grass or grass–maize pellets to give magnesium intakes of 1·79 and 2·23 g/d respectively. The mean apparent availabilities of Mg in sheep fed on frozen grass and grass–maize pellets were 0·31 and 0·36 respectively.

2. The rumen contents were fractionated by straining the digesta through linen cloth and then differentially centrifuged to give 20000 ***g*** and 100000 ***g*** supernatant fractions.

3. In all sheep, regardless of diet, at 4 and 16 h after a meal, 50 and 60% respectively of the total Mg in the rumen contents was found in strained rumen fluid while 30 and 38% respectively of the total Mg was found in the 100000 ***g*** supernatant fraction.

4. The net absorption of Mg from the temporarily isolated and washed reticulo-rumen was studied using either 100000 ***g*** supernatant fractions of rumen contents from sheep fed on one or other of the two diets, or inorganic buffers containing the same concentration of Mg and other macroelements.

5. The Mg was readily absorbed from the 100000 ***g*** supernatant fraction placed in the rumen with the rate of absorption being 7·3 μmol/l per min (505 mg/d) from the supernatant fraction obtained from sheep fed on frozen grass and 11·3 μmol/l per min (781 mg/d) from the supernatant fraction from sheep fed on grass–maize pellets. In the same sheep, the previously described rates of Mg absorption from the 100000 ***g*** supernatant fraction were similar to those obtained from the comparable inorganic buffers.

6. The effects of varying concentrations of potassium and sodium on the net absorption rate of Mg (as ^{24}Mg) and on the one-way efflux of Mg (as ^{28}Mg) from supernatant fractions or rumen fluid and inorganic buffers were investigated using the temporarily-isolated and washed rumen in three sheep. Although the net absorption rate of ^{24}Mg from supernatant fractions or buffers containing similar K concentrations varied significantly between sheep, a similar percentage decrease in the absorption rates of both ^{24}Mg and ^{28}Mg was found for each sheep as the K concentration was increased.

7. One pair of sheep was fed on the frozen grass and the other pair was fed on the grass–maize pellets. Their daily intakes of K were then increased to 50 g/d for 14 d by intrarumen infusion of potassium chloride. In three of the four sheep the plasma Mg concentration fell within 12 h of the start of the KCl administration. In all sheep urinary excretion of Mg decreased and its faecal output increased. The increased intake of K had no effect on the distribution of Mg in the rumen contents.

8. Gel-filtration chromatography of the 100000 ***g*** supernatant fractions, regardless of the diet, showed that over 90% of the Mg in the 100000 ***g*** supernatant fractions was associated with a low-molecular-weight fraction of about 200 Da which corresponded to the elution volume of magnesium chloride in 0·1 M-sodium chloride.

9. It is concluded that any binding of Mg ions to small organic molecules in the 100000 ***g*** supernatant fraction of rumen contents played no significant role in the restriction of Mg absorption from the reticulo-rumen. The depressent effect of increased K concentration in rumen contents on the net absorption of Mg is via a reduction in the absorptive flux rather than by increased secretion of Mg into the rumen fluid.

The continual absorption of magnesium by a saturable, energy-dependent process from the reticulo-rumen is crucial for the homeostatic control of Mg metabolism in sheep (Martens *et al.* 1978; Martens & Rayssiguier, 1980; Martens, 1983). The main factors controlling Mg absorption appear to be its concentration in the liquid phase of the digesta, and changes in the rate of Mg transport through the rumen wall caused by factors such as dietary constituents, for example, potassium, (Field & Munro, 1977; Field, 1983).

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There is little information on the distribution of Mg in the digesta in the reticulo-rumen, or of the forms of Mg present in the liquid phase (Field, 1983). It has been reported that Mg exists in an ultrafilterable form in the rumen (Storry, 1961), but it is unclear if this Mg is present as the free ion or associated with various molecules. Several binding substances including dietary lipids, bacterial cell walls, and other organic acid complexes such as tricarballylate have been proposed to explain the variation in Mg availability observed with different diets (Russell & van Soest, 1984). Although they are now not considered to be important in the aetiology of acute hypomagnesaemia (Martens & Rayssiguier, 1980; Field, 1983), few direct or quantitative measurements have been undertaken to examine the influence of binding or complexing of Mg in the liquid phase on the absorption of Mg from the rumen.

In the present study the distribution of Mg in reticulo-rumen contents was determined by differential centrifugation. The form of the Mg and the extent of its absorption from the 100000 ***g*** supernatant fractions taken from sheep fed on either frozen grass or a dried-grass and flaked-maize pelleted diet were also investigated. The possible association of Mg with other molecules in the 100000 ***g*** supernatant fraction was examined by gel-filtration chromatography, and the absorption of Mg from 100000 ***g*** supernatant fractions was studied using the temporarily-isolated and washed rumen technique (Martens & Rayssiguier, 1980).

The well-known hypomagnesaemic effect associated with elevation of the intake of K (Sjollema, 1931) is considered to be caused by a reduction in the net absorption rate of Mg from the reticulo-rumen (Tomas & Potter, 1976). Using the temporarily-isolated and washed rumen technique and ^{28}Mg, we have re-examined the conclusion of Care *et al.* (1984) that this inhibitory effect of increased intrarumen K concentration on the net absorption rate of Mg from a rumen pouch was the result of an increased rate of secretion of Mg ions into the pouch rather than a decrease in their rate of absorption.

MATERIALS AND METHODS

Sheep

Six crossbred sheep were each fitted with a rumen fistula. Three animals had a large fistula (120 mm diameter) and were used to measure Mg absorption by the temporarily-isolated and washed rumen technique. The other three animals had a small fistula (40 mm diameter) and were used for the Mg balance studies.

Diet

The frozen grass was harvested in May 1984 by cutting pasture at a height of 90–100 mm and packing into 25-kg bags which were kept at $-20°$. The pelleted dried-grass–maize diet was prepared from a 1:1 mixture of dried grass and flaked maize. The frozen grass, without thawing, and the grass–maize pellets were given twice daily at 09.00 and 17.00 hours to provide a total daily dry matter (DM) intake of 970 and 1070 g respectively. The mean concentrations of Mg, calcium, sodium and K in the frozen grass were 2·0, 5·4, 4·4 and 25·3 g/kg DM respectively, and the corresponding values for the grass–maize pellets were 2·2, 5·6, 6·2 and 11·0 g/kg DM respectively.

Experimental design

The sheep were randomly divided into two groups of three (two 'Bal' sheep and one 'Abs' sheep) so that one group was fed on the frozen grass and the other group the pelleted dried-grass–maize diet. The Bal sheep were used to determine the apparent absorption of Mg as well as providing rumen contents for investigating (a) the fractionation and distribution of Mg in the rumen contents, (b) the changes in the rumen concentrations of

Mg, Ca, Na and K after feeding, and (c) the preparation of the 100000 ***g*** supernatant fraction needed for the Mg absorption studies using the temporarily-isolated and washed rumen technique with the Abs sheep. Likewise the Abs sheep also provided rumen contents for (a) the study on the fractionation and Mg distribution in rumen contents, and (b) the preparation of the 100000 ***g*** supernatant fraction for their Mg absorption studies.

A pre-experimental period of 10 d was followed by (a) a 12 d period when rumen contents were collected on 2 d from all sheep for the fractionation and Mg distribution studies on the rumen contents, as well as to prepare the 100000 ***g*** supernatant fraction for the Mg absorption studies using the washed rumen technique; (b) a 9 d balance study during which the Mg intake and its apparent absorption were determined using the Bal sheep. During this period the two Mg-absorption experiments using the Abs sheep were also carried out.

The previously-described procedures involving first the 12 d and then the 9 d experimental periods were then repeated, except that in this second 12 d period additional small subsamples (400 g) of rumen contents were collected over 3 d for a study on the changes in the concentrations in it of Mg, Na, K and Ca at 4, 8, 12 and 16 h after feeding. A fifth Mg absorption study was carried out at the end of the second 9 d balance study.

Alteration of rumen K concentration by intrarumen infusion of potassium chloride

This was carried out using two Bal sheep per diet. Each sheep acted as it own control for a 25 d period before KCl was continuously infused intraruminally for a 14 d period. On days 6 and 7 of the control period 300–400 ml rumen contents were removed 16 h after feeding to determine the Mg, Ca, Na and K concentrations. At days 8 and 12 about 2 litres of the rumen contents were removed to prepare the 100000 ***g*** supernatant fraction for the Mg absorption studies using the temporarily-isolated, emptied and washed rumen technique. The residue, largely undigested plant material, remaining after the preparation of the supernatant fraction, was added to buffer (rinsing buffer, Table 1), warmed to 39° and returned to the rumen. To increase the supply of the 100000 ***g*** supernatant fraction, rumen contents were also collected from two extra sheep, one fed on frozen grass and the other grass–maize pellets. A 9 d balance study commenced at day 17 of the control period. Blood samples were taken at days 24 and 25 and continued for a further 7 d.

At the end of the control period the K intake was increased to 50 g/d for all sheep by intrarumen infusion of KCl in 2 litres water and continued for 14 d. On days 4 and 5 after the infusion began, 300–400 ml rumen contents were removed to determine the concentrations of Mg, Ca, Na and K, and the distribution of Mg. This was followed by a second 9 d balance study.

Studies on Mg absorption from the rumen

These experiments involved the use of the temporarily-isolated, emptied and washed rumen technique of Martens & Rayssiguier (1980) to study the effect of varying the K concentration in the 100000 ***g*** supernatant fractions, and inorganic buffers, on the net absorption of Mg. Two sheep fed on frozen grass and one fed on grass–maize pellets were used.

Sheep were only used for absorption experiments if their food intake was normal during the previous week, and if there had been no leakage from the large fistulas. Six absorption experiments were conducted on a sheep in 1 d, and the minimum interval between repeating the procedure on the one sheep was 7 d. Before each measurement of Mg absorption, the rumen wall was adapted to the experimental conditions by introducing 2 litres of the experimental solution, warmed to 39°, into the rumen for 20 min. After removal of this solution by suction, another 2 litres of the experimental solution containing ^{51}CrEDTA (Amersham International plc, Amersham, Bucks) or ^{14}C-labelled polyethylene glycol,

Table 1. *Composition of buffer solutions used in the magnesium absorption studies with the temporarily-isolated, emptied and washed rumen of sheep*

Compound	Concentration of compound in buffer solution* (mmol/l)			
	Rinsing buffer	Low-potassium buffer	Medium-K buffer	High-K buffer
NaCl	61	51	31	4
$NaHCO_3$	25	25	15	7
$Na_2HPO_4 \cdot 12H_2O$	2	2	2	2
KCl	10	10	30	55
$KHCO_3$	20	20	30	35
Sodium acetate	30	30	30	30
Propionic acid	10	10	10	10
Butyric acid	5	5	5	5
$MgCl_2 \cdot 6H_2O$	2·5	2·4	2·4	2·4
$CaCl_2 \cdot 6H_2O$	2	2·8	2·8	2·8
Glucose	5	5	5	5
NH_4Cl	—	10	10	10
Na:K	—	100:30	80:60	45:90

* pH after equilibration with carbon dioxide at 39° was 6·6–6·8.

molecular weight 4000 (PEG; Amersham International plc) was introduced. The solution was continually-gassed with carbon dioxide in the rumen via a tube fitted with a diffusing nozzle to maintain pH of the solution within the range 6·6–7·0 during the experiment. After 20 min, a 10 ml sample was taken and this was deemed to be taken at time zero. Further samples were taken 20, 40 and 60 min later.

^{28}Mg (Institute of Nuclear Chemistry, Julich, Federal Republic of Germany) was added (0·5 μCi/l) to each buffer and supernatant fraction so that the Mg efflux from the rumen to the blood could be measured. The disappearance of ^{24}Mg from the rumen reflects the net Mg absorption from this organ after correction for net water movement using ^{14}C-labelled PEG. The influx of Mg from the blood to the rumen can be calculated from the difference in these two variables. Changes in volume of fluid in the rumen were measured in the presence of ^{28}Mg by using the inert marker ^{14}C-labelled PEG at a concentration of 5 μCi/l with unlabelled PEG (1 g/l). In the absence of ^{28}Mg, $^{51}CrEDTA$ (3 μCi/l) was used. Since none of the experimental fluids used was likely to differ appreciably from isotonicity, no significant absorption of CrEDTA from the rumen would be expected (Dobson *et al.* 1976).

Two sheep were used for experiments comparing the absorption of Mg from supernatant fractions with that from inorganic buffers. The order of experimental solutions used in the sheep fed on frozen grass was as follows: E_1, low-K high-Na buffer (Table 1); E_2, high-K low-Na buffer (Table 1); E_3, 100000 ***g*** supernatant fraction from rumen contents of sheep 16 h after feeding frozen grass; E_4, inorganic buffer with similar Mg, Ca, Na and K concentrations to this frozen-grass supernatant fraction; E_5, 100000 ***g*** supernatant fraction from rumen contents of sheep 16 h after feeding grass–maize pellets; E_6, inorganic buffer with similar Mg, Ca, Na and K concentrations to this grass–maize pellet supernatant fraction. In the sheep fed on grass–maize pellets, experimental solutions E_5 and E_6 were examined before E_3 and E_4.

Values were obtained from experiments repeated five times on the sheep fed on frozen grass over a 7 week period, and three times on the sheep fed on grass–maize pellets over a 3 week period. Experimental solutions E_1 and E_2 were used on all sheep when absorption

studies were carried out to act as a standard between studies and to check on the physiological validity of the technique. The rate of Mg absorption from E_2 was always less than that from E_1 as K inhibits Mg absorption. Results from E_3 were compared with E_4 and similarly E_5 with E_6 in an attempt to compare the absorption of Mg in either the presence or absence of potential Mg-binding compounds capable of reducing the absorption rate of Mg.

Three sheep were used for experiments where the effect of increasing K concentration on Mg absorption from experimental 100000 *g* supernatant fractions was examined. As the intrarumen infusion of K had a similar effect, regardless of diet or sheep, on the distribution and concentrations of Mg, Na and K concentrations in the rumen contents and 100000 *g* supernatant fractions, the 100000 *g* supernatant fractions from all sheep were bulked to provide a pool of material for the Mg absorption studies. By addition of water, KCl, or sodium chloride solutions to the bulked 100000 *g* supernatant fractions the experimental 100000 *g* supernatant fractions were prepared to contain K concentrations increasing from 30 to 90 mmol/l and Na concentrations decreasing from 100 to 45 mmol/l.

The compositions of the low-K, high-Na 100000 *g* supernatant fraction and the inorganic buffer were based on the Mg, Ca, Na and K concentrations observed in 100000 *g* supernatant fractions of the rumen contents collected during the control period of the first part of the study. The compositions of the medium-K and medium-Na 100000 *g* supernatant fraction and electrolyte buffers were based on the Mg, Ca, Na and K concentrations observed in the 100000 *g* supernatant fractions of the rumen contents collected during the period when K was being infused into the rumen of the sheep also used in the present study. In addition, a high-K, low-Na supernatant-fraction buffer was prepared to give K and Na concentrations similar to those found by Scott & Dobson (1965) in the rumen contents of sheep which had developed hypomagnesaemic tetany when grazing pastures high in K and low in Na. The experimental 100000 *g* supernatant fractions as well as being made up with the required amounts of Mg, Ca, Na and K were also reconstituted with ammonium acetate, glucose, propionic acid and butyric acid at concentrations similar to the low-, medium- and high-K buffers (Table 1).

The absorption studies on the three experimental 100000 *g* supernatant fractions and matching buffers were carried out over a 12 h interval (six × 2 h periods) in the isolated, washed rumen in three sheep. The order in which the supernatant fractions and buffers was studied was: period 1, low-K high-Na buffer; period 2, low-K high-Na supernatant fraction; period 3, medium-K medium-Na buffer; period 4, medium-K medium-Na supernatant fraction; period 5, high-K low-Na buffer; period 6, high-K low-Na supernatant fraction.

Each solution was warmed to 39°, and gassed with carbon dioxide to bring the pH to between 6·6 and 6·8. A volume of 2 litres was placed in the rumen for 20 min before the zero sample was collected. Further samples were collected at 20, 40 and 60 min. When changing to solutions containing high-K concentrations this solution was placed in the rumen for 30 min to allow the rumen absorptive surface to adapt to the experimental conditions before it was replaced by a fresh volume of high-K buffer or supernatant fraction from which absorption measurements were made.

Net efflux of Mg, A (μmol/l per min), was calculated as follows:

$$A = \frac{C_1 - (C_2 \times M_1/M_2)}{t}$$

where C_1 is Mg concentration (μmol/l) in rumen at time 0; C_2 is Mg concentration (μmol/l) in rumen fluid after time t (min); M_1 is concentration of ^{51}CrEDTA at time 0; M_2 is concentration of ^{51}CrEDTA after t (min).

Fractionation of the rumen contents

About 2 kg of the rumen contents were removed, a 400 g subsample taken and the remaining contents were immediately returned to the rumen. The subsample was first centrifuged at 20000 ***g*** for 0·5 h and the supernatant fraction collected. This was spun at 100000 ***g*** for a further 0·5 h and the supernatant fraction kept. The various fractions were weighed and subsamples taken for Mg analysis.

Preparation of the 100000 g supernatant fraction

About 2 kg of the rumen contents were collected 16 h after feeding from the Bal sheep as well as all the rumen contents from the Abs sheep just before their preparation for the Mg absorption studies. These rumen contents from all the sheep on a particular diet were then bulked and strained through a linen cloth. The residue containing the food particles was then immediately returned to the rumens of the Bal sheep and at the end of the Mg absorption study in the case of the Abs sheep, as reconstituted rumen contents since it was mixed with the rinsing buffer solution (Table 1) to replace the strained rumen fluid, and warmed to 39°. The 100000 ***g*** supernatant fraction was prepared from the strained rumen fluid by centrifuging at 100000 ***g*** for 60 min. The supernatant fraction was kept at 4° if needed within a few days or otherwise stored at −10°.

Gel filtration of the 100000 g supernatant fraction

Sephadex G-15 was packed into a 240 × 15 mm column giving a bed volume (V_t) of 42 ml. The column was eluted with water to give a flow-rate of 30 ml/h (17 ml/cm^2 per h). The void volume (V_0) as determined by blue dextran was 17 ml and the column was calibrated using L-β-phenylalanine (molecular weight 165; elution volume (V_e) 30 ml) and L-tryptophan (molecular weight 204; V_e 26 ml). A 0·5 ml sample of 100000 ***g*** supernatant fraction was placed on the column and 50 ml of the eluant was collected in 1 ml quantities.

The following solutions as 0·5 ml portions were run on the column and with water:

(*a*) 100000 ***g*** supernatant fraction before the Mg absorption measurement;

(*b*) 100000 ***g*** supernatant fraction after the Mg absorption measurement in the rumen;

(*c*) 100000 ***g*** supernatant fraction + 50 μg Mg as $MgCl_2 \cdot 6H_2O$;

(*d*) 100000 ***g*** supernatant fraction + ^{28}Mg (0·33 μCi/l);

(*e*) 100 μg Mg as $MgCl_2 \cdot 6H_2O$ in 0·5 ml 0·1 M-NaCl.

The organic material in the eluant was monitored by the absorbance at 280 nm; the Mg concentration was measured by atomic absorption spectrophotometry.

Analytical methods

Samples of the diets were dried and ground, as were the fractions from the rumen contents, before dry ashing at 480° for 12 h. The ash was dissolved in 6 M-hydrochloric acid with warming and diluted in 0·1 M-HCl for the Mg, Ca, Na and K analyses. Urine samples were diluted in 0·1 M-HCl while samples of 100000 ***g*** supernatant fractions and solutions from the Mg absorption experiments were diluted with trichloracetic acid (100 g/l) to give a 1:100 solution. Mg and Ca concentrations were determined by atomic absorption spectrophotometry on the acidified solutions containing 1 g lanthanum/l while Na and K concentrations were determined by flame emission photometry. ^{51}Cr was counted in a well-type scintillation counter (Gamma Guard; Tracer Laboratories, Weybridge, Surrey). The measurement of ^{14}C in the PEG in the presence of ^{28}Mg was delayed for 2 weeks to allow complete decay of the ^{28}Mg. The ^{14}C-labelled PEG was counted by liquid scintillation

Table 2. *Mean daily intakes, excretion and apparent absorption (mg/d) of magnesium determined from a 9 d balance study before and after continuous potassium infusion into the rumen of sheep fed on frozen grass and grass–maize pellets*

K intake	22 g/d (K_1)		50 g/d (K_2)		Difference ($K_2 - K_1$)	
			Frozen grass diet			
Sheep no.	5	6	5	6	5	6
Mg intake	1785	1796	1785	1796	0	0
Faecal output	1164	1291	1260	1330	96	39
Urinary excretion	518	112	286	31	−232	−81
Apparent absorption	621	505	525	466	−96	−39
Intake (%)	35	28	29	26		
			Grass–maize pellet diet			
Sheep no.	3	4	3	4	3	4
Mg intake	2330	2330	2330	2330	0	0
Faecal output	1496	1442	1645	1462	149	20
Urinary excretion	90	28	5	5	−85	−23
Apparent absorption	834	888	685	868	−149	−20
Intake (%)	36	38	29	37		

(LKB-Wallac Rackbeta, Stockholm, Sweden) after 1 ml sample had been dispensed in 10 ml scintillant (Bray, 1960).

The statistical significance of the difference between two treatment means for Mg absorption was determined from *t* tests using the residual mean square of a three-way analysis of variance with the main effects being sheep, time and treatment (SPSS, 1986).

RESULTS

The effects of diet on daily intakes, excretion and apparent absorption of Mg

The mean daily intakes, faecal output, urinary excretion and apparent absorption of Mg in sheep fed on frozen grass and grass–maize pellets are given in Table 2. The amounts of Mg apparently absorbed ranged from 505 to 621 mg/d for sheep fed on the frozen grass while the values for the sheep on the grass–maize diet were 788 and 834 mg/d. Sheep 5 had a significantly greater urinary excretion of Mg (518 mg/d).

The mean Ca, Na and K daily intakes were 4·87, 5·93 and 22·2 g/d and 6·02, 6·67 and 11·8 g/d for the sheep on the frozen grass and grass–maize pellets respectively.

Changes in the concentrations of Mg, Ca, Na and K in the rumen after feeding

The changes in the concentrations of Mg, Ca, Na and K in the rumen contents at 4, 8, 12 and 16 h after feeding are illustrated in Fig. 1. For both diets the changes in the concentrations of Ca and K were small over the 16 h period after feeding. However the concentrations of Mg significantly decreased and Na significantly increased between 4 and 16 h after feeding. The concentration ratio, Na:K increased from 2·2 to 3·6 and from 2·7 to 4·3 in rumens of sheep fed on the frozen grass and grass–maize pellet diet respectively.

The distribution of Mg in the rumen contents

The distribution of Mg in the rumen contents at 4 and 16 h after feeding, with the Mg in each fraction being expressed as a percentage of the total Mg in the rumen contents, is shown in Fig. 2. Approximately 50 and 60 % of the Mg in the rumen contents of both diets

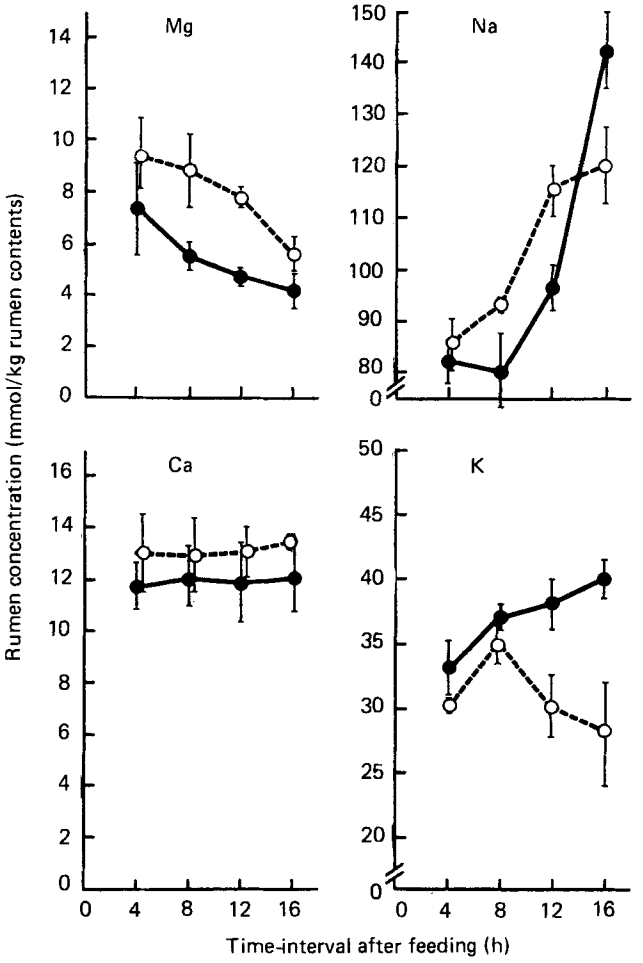

Fig. 1. Mean concentrations (mmol/kg contents) of magnesium, calcium, sodium and potassium in the ashed rumen contents at 4, 8, 12 and 16 h after sheep were fed on either frozen grass (●—●) or grass–maize pellets (○—○). Points are mean values with their standard errors represented by vertical bars.

was found to be associated with the strained rumen fluid at 4 and 16 h after feeding respectively. The Mg associated with the 100000 *g* supernatant fraction, regardless of diet, increased from approximately 31 to 38% as the time-interval after feeding increased (4 *v.* 16 h).

The mean amounts of Ca, Na and K associated with the 100000 *g* supernatant fraction, expressed as a percentage of the total in the rumen contents, were 10, 58 and 43% respectively, and were similar for both diets.

The gel filtration profiles of the 100000 g supernatant fractions

The elution profiles of (a) the 100000 *g* supernatant fraction, and (b) the 100000 *g* supernatant fraction plus MgCl on Sephadex G-15 column are shown in Fig. 3. As there were no significant dietary effects on the profiles only the values for the frozen grass are given.

The major organic peak had a V_e of 17 ml and a smaller peak with a V_e of 30 ml. As

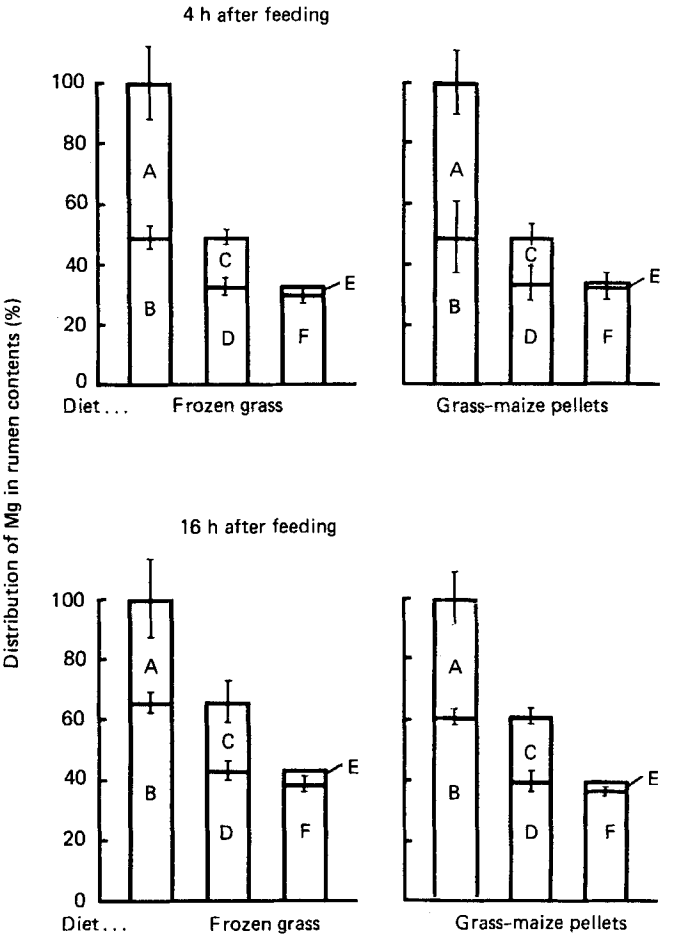

Fig. 2. The distribution of magnesium, expressed as a percentage of the total Mg, between various fractions from the rumen contents of sheep fed on frozen grass and on grass–maize pellets: (A), strained rumen particles; (B), strained rumen fluid; (C), 20000 *g* pellet; (D), 20000 *g* supernatant fraction; (E), 100000 *g* pellet; (F), 100000 *g* supernatant fraction. Values are means with their standard errors represented by vertical bars.

V_0 for the G-15 column was 17 ml the first and largest peak reflects the organic material with a molecular weight of greater than 1500 Da. The molecular weight of the organic material in the smaller peak was determined to be about 120 Da from the V_e for L-β-phenylalanine and L-tryptophan.

The Mg appeared between the two peaks (V_e 27 ml). About 90% of the total Mg in the 0·5 ml sample of 100000 *g* supernatant fraction placed on the column was recovered in tube nos. 22–32. A similar profile was obtained when ^{28}Mg labelled 100000 *g* supernatant fraction was run on the column. When 50 μg Mg as $MgCl_2 \cdot 6H_2O$ was added to the 100000 *g* supernatant fraction, left for several hours and run on the column, over 90% of the added Mg was recovered in tube nos. 22–32 (Fig. 3). A peak of Mg concentration similar to that eluted for the 100000 *g* supernatant fraction was observed when 100 μg Mg as $MgCl_2 \cdot 6H_2O$ in 0·1 M-NaCl was placed on the column to produce an ionic strength similar to that of the 100000 *g* supernatant fraction.

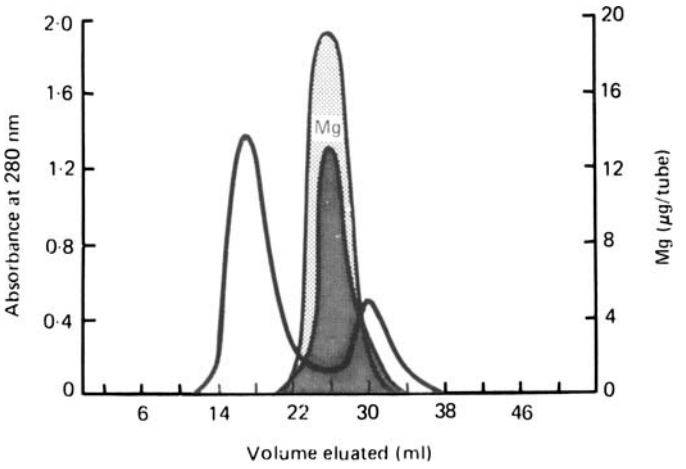

Fig. 3. The elution profile on a Sephadex G-15 column (240 × 15 mm; equilibrated with water) for the separation of organic substances and magnesium in the 100000 *g* supernatant fractions from sheep fed on frozen grass. (■) 100000 *g* supernatant fraction (▨) 100000 *g* supernatant fraction + 50 μg Mg as $MgCl_2 \cdot 6H_20$, (——) absorbance 280 nm.

Mg absorption from the temporarily-isolated, washed reticulo-rumen

In two sheep fed on either frozen grass or grass–maize pellets, the absorption rates of Mg from 2 litres of a high-Na low-K buffer placed in the emptied rumen were significantly higher ($P < 0{\cdot}001$) than those from a low-Na high-K buffer (10·34 *v.* 4·41 μmol/l per min) (Table 3). The Mg concentrations in the buffer were similar. The use of these buffers (Table 1) at the beginning of each series of experiments enabled the variation in Mg absorption by the rumen between study periods to be assessed. As there were no significant differences in the absorption rates of Mg from the same buffers or supernatants fractions used in the two sheep, the values obtained for each experimental solution have been pooled (Table 3).

The rates of absorption of Mg from 100000 *g* supernatant fractions prepared from rumen contents of sheep fed on either frozen grass or grass–maize pellets were not significantly different from corresponding inorganic buffers containing similar Mg, Na, K and Ca concentrations (Table 3). The concentrations of Mg in the 100000 *g* supernatant fractions varied between different preparations made over the experimental period, and those from sheep fed on the grass–maize pellets were consistently higher. The mean rate of net Mg absorption from the 100000 *g* supernatant fraction of rumen contents from sheep fed on grass–maize pellets was significantly higher ($P < 0{\cdot}05$) than that from the 100000 *g* supernatant fraction from sheep given frozen grass (11·33 *v.* 7·31 μmol Mg/l per min). The efficiency of the net absorption rate of Mg was also higher from the grass–maize-pellet supernatant fraction (24·6 *v.* 20·3 %/h).

The effects of an increase in K intake

(a) *Plasma Mg concentration*. The effect of increasing the K intake to 50 g/d on plasma Mg was complex as the Mg concentrations decreased within 12 h in both sheep fed on grass–maize pellets, but in only one of the sheep fed on frozen grass (Fig. 4). The decreases in plasma Mg ranged from 0·2 to 0·3 mmol Mg/l. The sheep did not become severely hypomagnesaemic or show signs of tetany, and the minimum plasma Mg concentration measured was 0·68 mmol/l.

Table 3. *Magnesium concentrations in experimental solutions, and the absorption of Mg from the temporarily-isolated and washed rumen of sheep*

(Mean values with their standard errors for five separate absorption studies on one sheep fed on frozen grass and three studies on one sheep fed on grass–maize pellets)

Solution	No. of observations	Mg concentration* (mmol/l)		Mg absorption (μmol/l per min)		Efficiency of net Mg absorption (%/h)	
		Mean	SE	Mean	SE	Mean	SE
E_1 Low-potassium high-sodium buffer	8	2·64	0·12	10·34	0·93	22·6	2·9
E_2 High-K low-Na buffer	8	2·75	0·16	4·41	0·23	9·1	0·7
E_3 100000 *g* supernatant fraction from frozen grass (FG)	7	2·07	0·23	7·31	1·01	20·3	2·3
E_4 Inorganic buffer with similar macro-element composition to FG 100000 *g* supernatant fraction	6	2·21	0·20	7·73	1·37	21·3	3·0
E_5 100000 *g* supernatant fraction from grass–maize pellet (GP)	7	2·34	0·42	11·33	2·18	24·6	6·1
E_6 Inorganic buffer with similar macroelement composition to GP 100000 *g* supernatant fraction	6	2·74	0·45	9·38	1·75	25·6	7·5

* Mean concentration of Mg in reticulo-rumen during an 80 min period; absorption was measured during the final 60 min of this period.

Statistical significance of difference between mean Mg absorption rates: E_1 *v.* E_2 $P < 0{\cdot}001$, E_5 *v.* E_3 $P < 0{\cdot}05$, E_3 *v.* E_4 and E_5 *v.* E_6 not significant.

(b) *The faecal and urinary excretion of Mg*. The increased K intake increased the faecal excretion of Mg and decreased urinary Mg output in all sheep studied regardless of diet (Table 2).

The Mg balance of sheep no. 5 was markedly different from the other sheep as it excreted large amounts of Mg in the urine. In this sheep, plasma Mg concentration did not decrease when K was infused into the rumen (Fig. 4).

(c) *The Mg, Ca, Na and K concentrations in the rumen contents*. The K infusion did not affect the concentrations of Ca and Mg in 100000 *g* supernatant fractions of rumen contents. For example, the Mg concentration in the 100000 *g* supernatant fraction from sheep fed on frozen grass and infused with KCl was 3·00 (SE 0·04) mmol/l relative to the control value of 2·60 (SE 0·27) mmol/l. With sheep fed on the grass–maize pellets the comparable values were 3·50 (SE 0·52) and 3·20 (SE 0·25) mmol/l respectively. All values are means of analyses of two collections made for each of two sheep. However, the K infusion caused the K concentration, regardless of the diet, to increase two- to three-fold while the Na concentration was decreased to about half the value of the control periods in the 100000 *g* supernatant fractions.

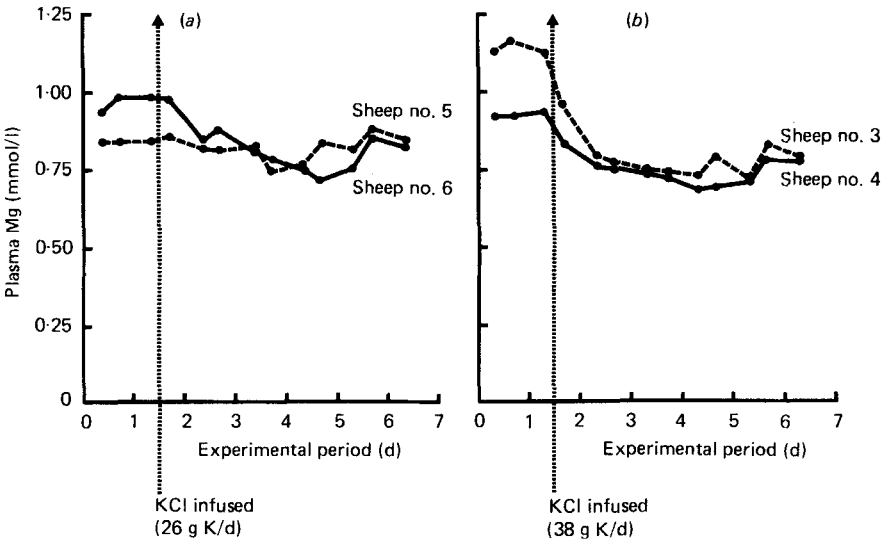

Fig. 4. Changes in plasma magnesium concentration following the start of an intrarumen infusion of potassium chloride in sheep fed on (*a*) frozen grass or (*b*) grass–maize pellets to increase potassium intake to 50 g K/d.

(d) *The distribution of Mg in rumen contents.* Intrarumen infusion of KCl had no effect on the distribution of Mg in the rumen contents of sheep fed on different diets. About 36–39% of the Mg in the rumen contents was found to be associated with the 100000 ***g*** supernatant fraction, and there were no apparent differences between supernatant fractions of rumen contents prepared from sheep fed on the different diets.

Effect of increasing K concentration on absorption of Mg from supernatant fractions and buffers in the rumen

The net rate of absorption (μmol/l per min) of Mg from 100000 ***g*** supernatant fractions and buffers in the isolated, washed rumens decreased in the three sheep as K concentrations increased (Fig. 5). Within a K concentration the differences between sheep in the net rates of Mg absorption were significant ($P < 0{\cdot}01$). Furthermore, within an individual sheep, increasing the K concentrations significantly decreased the net Mg absorption rates. Values were similar for 100000 ***g*** supernatant fractions and buffers. The magnitudes of the differences in the rate of Mg absorbed were similar when comparing one sheep with another for a given concentration of K or when the intraruminal concentration of K was changed in an individual sheep.

The absorption rates of ^{24}Mg and ^{28}Mg were expressed as a percentage of the ^{24}Mg and ^{28}Mg absorbed from the total initial amounts of each isotope placed in the rumen at the beginning of the absorption periods (Table 4). Although there was no significant differences between the mean percentage of ^{24}Mg and ^{28}Mg absorbed from the 100000 ***g*** supernatant fractions or the buffers containing similar concentrations of K, there were large differences between sheep as indicated by the SE values in Table 4.

When values for buffers and supernatant fractions containing similar concentrations were pooled, there were significant and similar decreases in the percentages of ^{24}Mg and ^{28}Mg absorbed as the K concentration was increased ($P < 0{\cdot}05$, Table 4). This indicated that there were no apparent differences between the rates of ^{24}Mg and ^{28}Mg absorption as intrarumen K concentration was gradually increased.

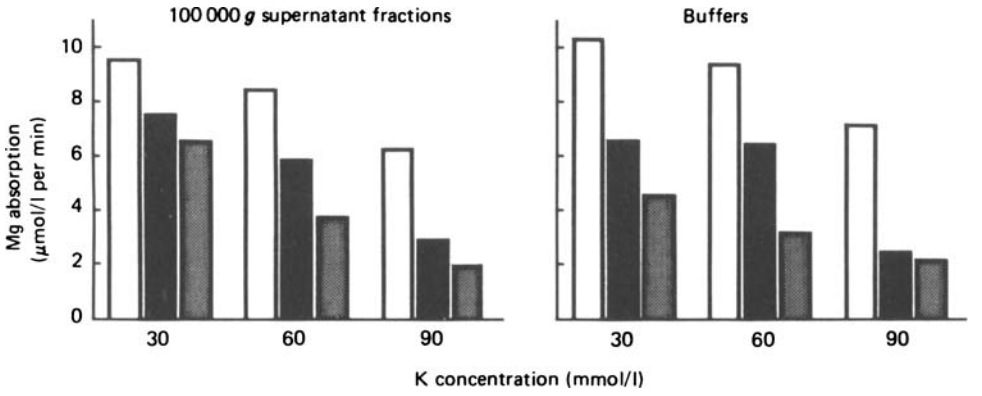

Fig. 5. Mean net magnesium absorption by the washed rumens of three sheep (□; ■; ▨) from 100000 *g* supernatant fractions and buffers containing different potassium and sodium concentrations (for details, see Table 1).

DISCUSSION

The concentration of Mg in the liquid phase of the rumen contents is an important factor which influences the rate of Mg absorption from the reticulorumen (Field, 1983). Mg is absorbed by an active, energy-dependent process which becomes saturated at a Mg concentration of 5 mmol/l (Brown *et al.* 1978; Martens, 1979). Therefore any factor which reduces the concentration of Mg ions at the absorptive site will adversely affect the rate of Mg absorption.

Rumen contents are a heterogeneous mixture of food particles, micro-organisms and organic and inorganic molecules. As Mg can bind to many organic molecules (Molloy & Richards, 1971), the Mg content of the rumen contents is unlikely to represent the concentration of Mg ions at the absorptive sites on the rumen wall. However the 100000 *g* supernatant fractions, which accounted for 30–38% of the total Mg in the rumen contents, contains no dietary particles or cellular debris but soluble inorganic or organic substances including Mg, presumably in a form suitable for absorption.

The gel-filtration studies showed that both the Mg incorporated in and the Mg added to the 100000 *g* supernatant fraction were associated with a fraction which had a V_e which corresponded to a molecular weight of about 200 Da. The Mg in the low-molecular-weight fraction must be associated with other molecules. These may consist merely of an hydration shell since Mg ions were eluted from an aqueous solution in the same position.

The temporarily-isolated and washed rumen preparation was used to quantify the efficiency of absorption of Mg, and the possible effect on it of an association of Mg with the organic material in the 100000 *g* supernatant fraction. The Mg was readily absorbed from the 100000 *g* supernatant fraction, regardless of the diet, and the presence of the organic substances in the supernatant fraction had no depressant effect on the efficiency of Mg absorption from the reticulo-rumen because a similar absorption rate was obtained using the electrolyte-buffer solutions which contained the same concentrations of Mg and other relevant macroelements as present in their matching 100000 *g* supernatant fractions.

The results of these gel filtration and rumen absorption studies suggest that any binding of Mg ions to organic moieties in the supernatant fraction obtained after 100000 *g* centrifugation of rumen contents is unlikely to play an important role in the restriction of Mg absorption from the recitulo-rumen.

It has been suggested that because Mg has a high binding affinity for various organic substances that this is an important factor, along with increasing dietary K levels, in

Table 4. *Absorption of ^{24}Mg and ^{28}Mg, expressed as a percentage of the initial amount of Mg added, by the temporarily-isolated and washed rumen from 100000 g supernatant fractions and buffers containing different potassium concentrations*

(Mean values with their standard errors)

K concentration* (mmol/l)		^{24}Mg absorption (%)				^{28}Mg absorption† (%)			
		100000 *g* supernatant fraction†		Buffer		100000 *g* supernatant fraction†		Buffer	
Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
30·5	1·4	18·0	3·5	16·4	3·4	17·8	6·9	20·5	2·5
60·5	1·9	13·3	7·6	13·4	5·6	12·6	3·9	12·2	4·5
87·8	1·7	7·9	3·8	9·9	4·9	10·1	4·1	9·0	4·5

* Mean K concentration during the 1 h absorption period.
† Values are means from 1 h absorption periods with three sheep.

predisposing ruminants to hypomagnesaemia. However, in the present study it has been observed that the Mg which was readily absorbed from the rumen contents is probably hydrated Mg ions, although the possibility that it is also associated with a low-molecular-weight organic fraction cannot be eliminated. Certainly such a fraction has no effect on the absorption of Mg from the reticulo-rumen.

The mean rates of net absorption of Mg from the 100000 *g* supernatant fractions were 7·3 and 11·3 μmol/l per min for sheep fed on frozen grass and grass–maize pellets respectively. The physiological effectiveness of the temporarily-isolated and washed rumen to absorb Mg was assessed by comparing the absorption rates, expressed as mg Mg/d, with the apparent Mg absorption values of sheep fed on the same diets, as the rumen is the major site of Mg absorption in sheep (Grace *et al.* 1974; Grace, 1983). Assuming that at least 2 litres of the 100000 *g* supernatant fraction bathes the absorptive areas of the rumen then the mean amounts of Mg absorbed from the temporarily-isolated washed rumen were estimated to be 505 and 781 mg/d for the sheep fed on the frozen grass and grass–maize diets respectively. These values compare favourably with the mean amounts of Mg apparently absorbed, namely 563 mg/d for sheep fed on frozen grass and 811 mg/d for sheep fed on the grass–maize diet. The amounts of Mg absorbed are also adequate in terms of meeting the Mg requirements of the sheep. A 50 kg lactating ewe producing 2 kg milk/d has a net Mg requirement of approximately 500 mg Mg/d (Agricultural Research Council, 1980).

The decrease in plasma Mg concentration, and reduced urinary and increased faecal Mg outputs observed following K infusion into the rumen are in agreement with earlier studies on sheep (Tomas & Potter, 1976). Increased K intake has not always been associated with a reduction in plasma Mg in ruminants, and it has been suggested that any effect of K on Mg homeostasis is dependent on the level of Mg intake (Suttle & Field, 1967, 1969; Field & Suttle, 1979). The diets given to the sheep in the present study provided from 1·7 to 2·3 g Mg/d, which was in excess of their requirements (Agricultural Research Council, 1980). Nevertheless, a depressant effect of K on Mg homeostasis was apparent to some degree in all our sheep.

The KCl infusions were associated with increased K and decreased Na concentrations in the 100000 *g* supernatant fractions from the rumen contents of sheep fed on both diets. The distribution and concentrations of Mg in the rumen contents, and proportion of Mg in the

100000 ***g*** supernatant fractions of rumen fluid did not change significantly as K concentrations increased. Although the Mg was absorbed from the 100000 ***g*** supernatant fractions at rates similar to those from inorganic buffers containing similar concentrations of Mg and other electrolytes, the net absorption of Mg from the rumen decreased as the K concentration was increased. It was therefore concluded that the effects of increased K intake on Mg metabolism were not due to decreased rates of release of Mg from the diet, altered distribution of Mg in rumen contents, or a decrease in the concentrations of Mg in rumen fluid, but were due to a decrease in the movement of Mg across the reticulo-rumen wall in response to the increased intrarumen K concentration.

Mg transport across the rumen epithelium has been associated with an active process linked to Na transport mediated by Na^+, K^+-ATPase activity on the serosal surface (Martens *et al.* 1978). The use of ^{28}Mg in 100000 ***g*** supernatant fractions and inorganic buffers in the isolated, washed rumen enabled the one-way efflux of ^{28}Mg to be compared with net ^{24}Mg absorption. In all three sheep studied, the net rates of efflux of ^{24}Mg and ^{28}Mg from supernatant fractions and buffers were decreased when the K concentration was increased, and the decreases were similar for both isotopes (Table 4). This finding is in agreement with that recently noted by Beardsworth *et al.* (1987), but contrasts with that of Care *et al.* (1984) who concluded from work with a rumen pouch that an increased influx of ^{24}Mg from the blood to the rumen pouch was mainly responsible for the reduced net efflux of ^{24}Mg. At the intrarumen Mg ion concentration of 2·4 mmol/l used by both groups, the electrochemical potential cannot support passive efflux of Mg ions from the rumen. Thus active transport must be responsible and one must conclude that the effect of the high rumen K concentration is probably by a direct inhibitory action on this active process. This conclusion has been confirmed by recent results obtained by Martens *et al.* (1987) from in vitro studies of Mg transport across rumen epithelium suspended in Ussing chambers. They showed that although the increase in potential difference across the epithelium, caused by high mucosal K concentration, does enhance the passive flux of Mg from the serosal to the mucosal side via a paracellular pathway, the magnitude of this response is far too small to account for the extent of the fall in net Mg flux from the mucosal to the serosal side.

One of the sheep used in the balance studies, and another used for the washed rumen experiments absorbed more Mg than the other sheep even when the K intake and concentrations in the rumen were increased. This has also been observed by others (P. M. Beardsworth and L. J. Beardsworth, personal communication). Field (1983) concluded that variation between ruminants in the efficiency of Mg absorption was greater than the inhibitory effects of K. The present study also showed that the differences between individual sheep in their ability to absorb Mg from the 100000 ***g*** supernatant fraction or inorganic buffer were greater than the effects of increasing K concentration (Fig. 4). The differences in efficiency of Mg absorption between individual sheep may reflect differences in the absorptive area of rumen papillae.

The authors thank Mr. P. M. Beardsworth, Mrs L. J. Beardsworth, S. Davis and the staff of the Department of Animal Physiology and Nutrition for assistance with the care of the sheep and the chemical analyses. Mr G. A. Anderson, University of Melbourne, assisted with the statistical analysis. The study was financed in part by the Australian Meat Research Committee.

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Printed in Great Britain