Magnesium absorption in mature ewes infused intrarumenally with magnesium chloride

BY A. F. MCLEAN*, W. BUCHAN AND D. SCOTT†

Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB

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1. The effects of magnesium supplementation on Mg absorption proximal and distal to the pylorus in ewes maintained on a grass diet were investigated using a combination of balance, digesta flow and electropotential measurements.

2. Three mature ewes each received by intrarumenal infusion a supplement of 0, 1, 2 and 3 g Mg/d in sequence over four 10-d periods.

3. Net Mg absorption distal to the pylorus took place down its electrochemical gradient, although the quantity absorbed remained small during the control and first infusion periods.

4. The bulk of Mg absorption occurred before the pylorus and, during the control and first infusion periods, took place against its electrochemical gradient. The net Mg absorption proximal to the pylorus rose with declining efficiency as Mg intake was increased. It is suggested that saturation of the absorption process at this site was occurring.

Recent studies have shown that the major site of magnesium absorption in the sheep is located at the rumen and that Mg uptake from this site is achieved by an active transport process (Martens *et al.* 1976, 1978; Field & Munro, 1977; Brown *et al.* 1978; Martens & Rayssiguier, 1980). In addition, a number of studies have shown that Mg absorption can also occur from sites distal to the pylorus (Grace & MacRae, 1972; Ben-Ghedalia *et al.* 1975; Field & Munro, 1977). However, it is not clear whether Mg absorption from both of these sites can vary in proportion to Mg supplementation or what transport mechanisms are involved. The present experiment was undertaken to obtain information concerning these points.

METHODS

Three adult (Suffolk × Greyface) ewes were used. Each was surgically prepared with a simple cannula (25 mm internal diameter) into the rumen and a T-shaped cannula (10 mm internal diameter) into the duodenum between 60 and 100 mm from the pylorus. The sheep were housed in individual metabolism cages which were equipped for the separate collection of urine and faeces. They were given a pelleted grass diet (717 g dry matter (DM)/d) which was continuously dispensed from a belt feeder over a 24 h period.

The experiment comprised an initial control period, during which the diet was given without supplementation, followed by three periods, during which the Mg supplementation was increased. The Mg supplement ($MgCl_2.6H_2O$) was infused intrarumenally and, together with the diet, provided intakes of 1·29, 2·29, 3·29 and 4·29 g Mg/d for the control, first, second and third supplementary periods respectively. Throughout the whole experiment, the sheep were also given 10 g/d of polyethylene glycol (PEG 4000; BDH Chemicals Ltd, Poole, Dorset) by continuous infusion into the rumen, as a marker for estimating digesta flow.

Each experimental period was of 10 d duration. Urine and faeces were collected daily at 09.00 hours over the last 6 d of each period. The volume of urine was recorded and 100-ml

* Present address: The West of Scotland Agricultural College, Auchincruive, Ayr.
† For reprints.

samples taken and stored separately at −20° prior to analysis. Faecal DM was determined by drying at 100° for 48 h. The faeces were then finely ground and 150 g samples taken for analysis. Blood and digesta samples were taken on the 5th and 9th days in each period. Blood was withdrawn from the jugular vein and the plasma was separated by centrifugation at 2000 *g* for 15 min and stored at −20°. Digesta samples were taken from the rumen at 12.00 and 15.00 hours by aspirating approximately 250 ml digesta via the rumen cannula. Duodenal samples were collected into polyethylene bags attached to the duodenal cannula at hourly intervals between 10.00 and 17.00 hours and pooled for each sheep. Both rumen and duodenal samples were homogenized and a 10 ml portion was immediately taken for DM and total Mg analyses. A further 30 ml portion was centifuged at 35000 *g* for 40 min and the resulting supernatant stored at −20° until being analysed for PEG. The electropotential difference between the blood in the jugular vein and the digesta contents in the rumen and duodenum were recorded during the control and second supplementary periods using the procedure described by Dobson (1959).

Concentrations of Mg in feed, digesta, faeces, urine and plasma were estimated using the automated colorimetric procedure described by Gitelman *et al.* (1966). The concentration of PEG in the digesta supernatant fraction was estimated turbimetrically using the procedure described by Malawar & Powell (1967).

The results were tested for significance using an *F* test.

RESULTS

The electropotential differences between blood and digesta contents at the rumen and duodenum measured during the second infusion period did not differ significantly from the values recorded during the control period. For this reason, the results from both periods have been combined. Mean (with SE) potential differences in the rumen and duodenum were 37·67 (1·108) and 13·30 (1·553) mV respectively (blood positive).

The values for urinary, faecal and total output, and total net absorption are shown in Table 1. Urinary and faecal excretion of Mg rose with increasing Mg intake, although Mg balance and apparent availability were unaffected.

The results given in Table 2 show the effect of $MgCl_2$ supplementation on the concentration of Mg in the supernatant fraction of rumen and duodenal digesta samples.

Table 1. *The effects of intraruminal infusion of magnesium chloride on Mg balance (g/d) in sheep given a pelleted grass diet*

(Mean values with pooled standard errors of the mean for the three sheep)

Treatment... Mg	Grass	Grass + 1 g Mg	Grass + 2 g Mg	Grass + 3 g Mg	SEM	Significance of linear trend†
Intake (g/d)	1·29	2·29	3·29	4·29	—	—
Excretion (g/d):						
Urine	0·32	0·52	0·70	0·93	0·026	$P < 0·001$
Faeces	0·94	1·68	2·55	3·27	0·065	$P < 0·001$
Total	1·26	2·20	3·25	4·21	0·063	$P < 0·001$
Net absorption (g/d)	0·36	0·61	0·74	1·02	0·065	$P < 0·001$
Retention (g/d)	0·03	0·09	0·04	0·08	0·063	NS
Apparent availability (%)	27·4	26·5	22·4	23·7	2·5	NS

NS, not significant.

† There was no significant quadratic component in any of the trends.

Table 2. *The effects of intraruminal infusion of magnesium chloride on the concentration of Mg in plasma, in rumen and duodenal digesta samples and on Mg absorption in sheep given a pelleted grass diet*

(Mean values with pooled standard errors of the mean for three sheep)

Treatment...	Grass	Grass + 1 g Mg	Grass + 2 g Mg	Grass + 3 g Mg	SEM	Significance of linear trend‡
Mg intake (g/d)	1·29	2·29	3·29	4·29	—	—
Mg concentration (mmol/l):						
Plasma	0·86	0·91	0·91	0·95	0·08	NS
Rumen supernatant fraction	4·03	7·72	10·83	15·70	1·29	$P < 0·01$
Minimum concentration needed for passive diffusion	7·76	8·21	8·21	8·57	—	—
Duodenal supernatant fraction	4·66	6·97	11·69	14·40	1·07	$P < 0·01$
Minimum concentration needed for passive diffusion	1·27	1·35	1·35	1·41	—	—
Mg absorption:						
Net absorption	0·36	0·61	0·74	1·02	0·065	$P < 0·001$
Proximal to pylorus	0·30	0·52	0·49	0·60	0·079	NS†
Distal to pylorus	0·06	0·09	0·25	0·42	0·096	$P < 0·05$

NS, not significant.
† $P < 0·1$.
‡ There was no significant quadratic component in any of the trends.

This table also shows the minimum concentrations of ionic Mg in the digesta that would be necessary for transport of Mg to occur by simple diffusion down its electrochemical gradient. These values were estimated from the electropotential difference and plasma concentration of Mg, assuming 55% of the total Mg in the plasma is in the ionic form (Walser, 1961) and using the relationship:

$$pd = \frac{RT}{2F} \ln \frac{C_1}{C_2} = 31 \log_{10} \frac{C_1}{C_2},$$

where pd is the potential difference, R is the gas constant, T is the absolute temperature, F is Faraday's constant, and C_1 and C_2 are the concentrations of ionic Mg in digesta and plasma respectively.

Total Mg concentration in the plasma was unaffected by the treatments whereas Mg concentration in the digesta supernatant fractions from the rumen and duodenum rose with increasing Mg intake. The observed concentrations of Mg in the rumen at the two lower levels of Mg intakes were less than that estimated for passive transport to occur whereas at the duodenum they were higher at all levels of Mg intake.

Nearly all (98 (SE 2·3)%) of the total Mg in the duodenal digesta was found to be in the liquid phase. Consequently, net Mg absorption from sites proximal (Mg in intake − Mg in duodenum) and distal (Mg in duodenum − Mg in faeces) to the pylorus have been calculated from the Mg concentration multiplied by the liquid flow rate at the duodenum. The liquid flow rates at the duodenum were unaffected by infusion; their means (with SE) being 9·90 (1·095), 9·72 (0·112), 10·95 (1·184) and 9·95 (0·275) litres/d for control, first, second and third supplementary periods respectively. Total net Mg absorption and net Mg absorption distal to the pylorus increased with Mg intake. Net Mg absorption proximal to the pylorus, however, increased with intake, but at a decreasing rate.

DISCUSSION

Since the increases in Mg supplementation were not random, the difference in the amounts of Mg absorbed may have been due to either the period or the quantity of $MgCl_2$ infused. However, as no biological mechanisms have been proposed which would account for the large differences in Mg absorption being due to a period sequence, it is more likely that these results are due to the increases in Mg intake.

The results presented in Table 2 show that the electrochemical gradients between the rumen contents and the blood at the two higher levels of Mg intake were conducive to transport of Mg across the rumen epithelium by passive diffusion. In contrast, Mg absorption at the two lower levels of Mg intake took place against the observed electrochemical gradients, suggesting that active transport across the rumen epithelium was occurring. Other studies in sheep (Field & Munro, 1977; Brown *et al.* 1978; Martens *et al.* 1978; Martens & Rayssiguier, 1980) and cattle (Martens, 1983) have provided evidence that absorption of Mg across the rumen epithelium is achieved by an active transport process which reaches saturation at high Mg concentrations. The results of this present study are consistent with these findings.

Absorption of Mg took place mainly proximal to the pylorus at the two lower levels of Mg intake. In other studies, similar observations were made when the Mg intakes of sheep infused with $MgCl_2$ were decreased below approximately 2·5 g/d (Strachan & Rook, 1975; Tomas & Potter, 1976; Field & Munro, 1977). The decline of the marginal Mg absorption rate proximal to the pylorus was complemented by an increase in absorption distal to the pylorus. This increase was particularly marked at the two higher levels of Mg supplementation. Similar results were obtained by Grace & MacRae (1972) who observed that only 2% of the total quantity of absorbed Mg was transported across sites distal to the pylorus when sheep were given grass continuously over a 24-h period. However, this proportion increased to 50% when the same daily quantity of grass was given in one meal. This was interpreted as being evidence for a 'pulse' of Mg having flowed through the forestomach, thus limiting the opportunity for absorption from this site (Grace & MacRae, 1972). It follows that such a 'pulse' would also increase the Mg concentration of digesta in the intestines and may have resulted in the increase in intestinal Mg absorption reported by these authors. The increase in Mg absorption distal to the pylorus seen in the present study may have arisen in a similar manner since the electrochemical gradients at the duodenum were conducive to the absorption of Mg by passive diffusion.

The results presented in Table 1 show that the absorption of Mg rose in relation to intake and that any Mg absorbed in excess of requirement was subsequently excreted in the urine. These findings highlight the dependence of ruminants on absorption of dietary Mg, since mature ruminants have no readily available store of Mg (Blaxter & McGill, 1956) and there appears to be no regulatory mechanism for Mg homeostasis (Martens & Rayssiguier, 1980). In the present study, a grass diet was given which, in Mg content, was typical of the diet of most grazing ruminants. It would appear, therefore, that giving Mg supplements to ruminants, when they are consuming diets which are low in Mg or when Mg absorption from the rumen is impaired, is advantageous.

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