

The site of magnesium absorption from the ruminant stomach

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1. A low-magnesium diet was fed to four sheep, each of which had been surgically prepared with a rumen fistula, a tube into the cranial one-third of the omasum, a tube to the cranial one-third of the abomasum and a re-entrant duodenal cannula. Mg, as gluconate or acetate, was continuously infused for 12–14 d in turn into (1) the caudal duodenal cannula, (2) the abomasum, (3) the omasum, (4) the rumen. A continuous infusion of the chromium–ethylenediaminetetraacetic acid complex (CrEDTA) was maintained to the rumen. The abomasal effluent which flowed through the cranial duodenal cannula was continually sampled and the flow of Mg calculated from the concentrations of Mg and CrEDTA. Blood and rumen fluid samples were taken and urine and faeces collected during each period of Mg infusion.

2. The Mg infused to either the abomasum or omasum was completely recovered at the duodenum, indicating a lack of net absorption of Mg from these stomach compartments. In contrast, 13.7–18.7 mmol (36–61 %) of the Mg infused to the rumen was not recovered at the duodenum which suggested that a substantial net absorption of the infused Mg occurred from the reticulo-rumen. Absorption of Mg caudal to the pylorus was not related to the site of Mg infusion and averaged 3.28 ± 0.56 (SEM) mmol/d.

3. Compared with the intraruminal infusion, the post-ruminal infusion of Mg was associated with decreased plasma and rumen fluid Mg concentrations, decreased urinary Mg excretion, decreased Mg balance and increased faecal Mg excretion.

4. It is concluded that no significant absorption of Mg occurs from either the omasum or abomasum in sheep and that the reticulo-rumen is the principal site of Mg absorption before the pylorus. Absorption of Mg post-ruminally is insufficient to maintain normal Mg status in the animal.

With the use of the re-entrant duodenal cannulation technique, it has been found in both cattle and sheep that most of the net absorption of magnesium occurs before the pylorus (Rogers & van't Klooster, 1969; Pfeffer, Thompson & Armstrong, 1970; Grace & MacRae, 1972; Kemp, van't Klooster, Rogers & Geurink, 1973). Although the rumen mucosa has been found to be permeable to Mg at very high concentrations in the rumen fluid, the average Mg concentrations normally found are considered to be less than those required to overcome the electrochemical gradient, and measured uptakes from the rumen contents have indicated Mg absorption from this site to be negligible (Care & van't Klooster, 1965; Phillipson & Storry, 1965). Care & van't Klooster (1965) have also reported that net Mg absorption from gastric pouches does not occur in the sheep, and this indicates a lack of Mg absorption from the abomasum. In addition, Pfeffer & Rahman (1974) have reported an apparent relative lack of absorption of Mg infused to the abomasum rather than to the rumen.

Based on these findings, the primary site for Mg absorption before the pylorus appears logically to be the omasum. Harrison (1971) found that the omasal epithelium is permeable to Mg *in vitro*, but the unfavourable electrochemical gradient and the

short period of time the digesta in the organ are factors which argue against the omasum being of major importance in Mg absorption.

The present study was undertaken to determine the site(s) of Mg absorption in the ruminant stomach, and results suggested that this was the reticulo-rumen rather than the omasum.

EXPERIMENTAL

Animals, housing and diet

Four Merino ewes, aged 2–5 years and weighing about 40 kg, were used for these studies. They were housed in an air-conditioned room in metabolism cages with facilities for separate collection of urine and faeces, and with rainwater freely available for drinking.

The dietary ration was divided into twelve equal portions which were dispensed at intervals of 2 h by an automatic feeder. Each daily ration contained 520 g dry matter made up of (g): tissue paper 290, starch 106, casein 60, molasses 20, polyethylene pellets 20, mineral mixture 24 (to provide (mg): $\text{Ca}_3(\text{PO}_4)_2$ 6480, CaCO_3 1880, K_2SO_4 5210, KCl 2860, K_2HPO_4 2160, NaCl 4560, ferric citrate 514, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 150, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 22.5, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 1.2, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 148, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 7.3, KI 0.6, $\text{Na}_2\text{SeO}_4 \cdot 0.2$), vitamin mix 0.1 (to provide (μg): retinol 375, cholecalciferol 6.25). The Mg content of the dietary components differed between mixed batches, and on analysis the rations were found to provide from 4.2 to 7.0 mmol Mg/d. In addition, two sheep (nos. 9081 and 2054) consistently ate only 400–450 g dry matter/d.

Surgical preparation of animals

Surgical procedures were used on each sheep to establish the following preparations: (1) a rumen fistula; (2) a silicone tube (2.6 mm i.d., 4.9 mm o.d.) into the omasum, projecting 30–40 mm into the lumen from a point on the greater curvature about 40 mm from the reticulo-omasal orifice, and secured in position by a method similar to that described by Driedger, Condon, Nimrick & Hatfield (1970) using a polyester-fibre skirt attached to the tube; (3) a silicone tube into the cranial one-third of the abomasum (Driedger *et al.* 1970); (4) a re-entrant duodenal cannula about 40–50 mm from the pylorus, with the cannula stems projecting through the body wall in the same dorsal plane about 60 mm apart.

Post-mortem examination of several preparations showed that the tube into the omasum projected beyond the tertiary lamellae to a position which enabled a solution infused through the tube to enter the cranial one-third of the omasum.

Sampling procedures

Results of preliminary studies indicated that the Mg in duodenal digesta collected adjacent to the pylorus (i.e. in the abomasal effluent) was present almost entirely in the fluid (> 98%) and that representative sampling of only the fluid was needed in order to measure the flow of Mg to the duodenum. A device was constructed from acrylic plastic such that digesta flowing up a tube (80 mm \times 16 mm i.d.) connected vertically to the cranial arm of the duodenal cannula would flow through an orifice

at a height of 60 mm into another tube (55 mm × 30 mm i.d.) connected to the caudal arm of the cannula. A sampling probe (5 mm o.d.) was inserted into the cranial tube, and the device sealed to the atmosphere after setting the level of digesta about 20 mm below the 'flow-over' orifice. This level was sufficient to cover the probe, but allowed reverse digesta flows to be fully accommodated by the large-bore caudal arm of the device. In this way, only abomasal effluent was sampled.

During the 7 d sampling period, a portion of duodenal digesta flow was withdrawn continuously at about 0.15 ml/min from the probe in the sampling device by means of a peristaltic pump. Daily samples were kept separate, and on occasions when sampling was incomplete due to a blockage, the sample was discarded. Urine and faeces were collected separately each day. On 2 d of the last 4 d of each sampling period, samples of jugular blood and rumen fluid were taken at 10.00 and 16.00 hours. Additional rumen fluid samples were taken at 10.00 hours on each of the other 5 d when Mg was infused to the abomasum or omasum.

Sampling and analytical procedures were the same as those described by Tomas, Jones, Potter & Langsford (1973) and Tomas & Potter (1975) except that faeces were dried at 105° for 24 h and urine was acidified with hydrochloric acid.

Experimental design

The experimental design involved the measurement of the flow of Mg from the cranial arm of the duodenal cannula when supplementary Mg was infused in turn into the caudal arm of the duodenal cannula, the abomasum, the omasum and the rumen.

The sheep were initially given the experimental diet for an adaptation period of 7–10 d, during which time they received a daily drench of 1.5 g magnesium oxide/d. After adaptation the Mg supplementation was given as a continuous infusion at about 380 ml magnesium gluconate (magnesium acetate to sheep no. 2054)/d into the appropriate infusion site, in the order duodenum, abomasum, omasum and rumen. The quantities of Mg infused are given in Table 1. An inadvertent error in the preparation of the infusion solutions for sheep no. 2010 resulted in only half the planned quantity of Mg being infused to this animal.

A continuous intraruminal infusion of a complex of chromium with ethylenediaminetetraacetic acid (CrEDTA) was begun at the same time as the Mg infusion and maintained throughout the experiment. A period of 5 d was allowed for digesta Mg flows to stabilize on each treatment before sampling began. Polyethylene glycol (PEG) was included in the Mg infusate at a concentration of 50 g/l in order to estimate both contamination of the sampled digesta by infusate during the duodenal infusion, and any backflow to the rumen of infusate directed into the abomasum or omasum. There was a 7 d sampling period for each infusion site. On the basis of the results from two sheep, the abomasal infusion of Mg and PEG to the two remaining sheep was withheld subject to the omasal infusion results, and was finally omitted.

Statistical analysis

The results were analysed as a two-way classification of sheep and treatments in which the two missing values relating to the abomasal infusion were estimated and

the tests of significance were based on a pooled error with seven degrees of freedom. To determine whether the estimated rates of absorption from infusion into the omasum and rumen were different from zero, the tests were based on the between-sheep variability with three degrees of freedom.

Since the allocation of the treatment order in each sheep was non-random the results were confounded by the sequence of treatments, and the differences in absorption may have been due either to the time or to the site of infusion. On biological grounds no mechanism can be proposed for large differences in absorption due to time and, therefore, it is more probable that the results reflect differences due to the site of infusion.

RESULTS

Infusate location

On no occasion was PEG detected in the duodenal sample during Mg infusion to the caudal arm of the duodenal cannula. This suggested that no significant backflow of infusate to the sampling probe occurred, thus proving the validity of the measured baseline Mg flow. A backflow of infusate to the rumen was detected in only one sheep during Mg infusion post-ruminally. Rumen fluid from sheep no. 9081 contained from 0.21 to 0.61 mg PEG/ml during omasal infusion of Mg. The amounts of PEG required to maintain these concentrations were calculated from the extent of dilution of the CrEDTA infused intraruminally, and on this basis, entry of PEG to the rumen from the 18.8 g/d infused to the omasum was 1.95, 3.25, 3.84, 0, 0 and 1.66 g for each of the six consecutive days of the collection period. Hence for the 6 d period, about 23 of 245 mmol Mg infused to the omasum of this sheep entered the rumen.

Absorption of Mg

Results for the absorption of Mg from the different stomach compartments are given in Table 1. Significant absorption of infused Mg occurred only from the rumen ($P < 0.001$). Absorption from either the omasum or abomasum was not significantly different from zero ($P > 0.05$). Though not significant, the small apparent absorption obtained for sheep no. 9081 may have been due to backflow of infusate from the rumen.

The total net absorption of Mg before and after the pylorus as estimated from Mg input, duodenal Mg flows and faecal Mg excretion are given in Table 2. Except when Mg was infused to the rumen, there was a net secretion of Mg before the pylorus (1.35 ± 0.30 mmol/d) whereas a net absorption from the remainder of the gut was consistently obtained for all infusions (3.28 ± 0.56 mmol/d). Total absorption from the whole gastrointestinal tract was 1.76 ± 0.70 and 18.65 ± 2.27 mmol/d (3.75 ± 1.75 and $44.4 \pm 0.44\%$ of intake) for Mg infusion post-ruminally and intraruminally respectively.

Excretion and balance of Mg

Values for urinary and faecal excretion of Mg, and Mg balances are given in Table 2. Intraruminal infusion of Mg was associated with substantially higher Mg balances, higher urinary Mg excretion and lower faecal Mg excretion than was post-ruminal

Table 1. *The mean amount of magnesium entering the sheep's stomach from the diet and infusate, the amount of Mg leaving the stomach, and the apparent absorption of Mg infused into different compartments (mmol/d)*

Sheep no ...	(Mean values; no. of determinations/treatment given in parentheses)													
	2010			2086			9081			2054				
Site of Mg infusion* ...	Duo	Ab	Om	Rum	Duo	Ab	Om	Rum	Duo	Om	Rum	Duo	Om	Rum
Dietary Mg	7.0	7.0	7.0	5.2	5.2	5.2	5.1	5.5	2.9	3.8	3.7	3.3	3.0	3.9
Infused Mg	23.0	23.0	23.1	22.4	40.4	45.8	46.4	39.0	39.4	40.8	40.3	50.5	49.0	48.1
Flow of Mg to Duo: Mean	8.0	30.9	31.2	16.7	6.7	52.4	51.4	28.4	5.4	44.5	27.0	5.4	54.9	36.3
SEM	0.98	1.70	1.50	0.92	0.31	0.77	1.27	1.57	0.12	1.94	1.21	0.41	1.24	1.45
Infused Mg flowing to Duo†	(4)	(4)	(5)	(6)	(7)	(7)	(7)	(7)	(7)	(6)	(6)	(7)	(7)	(7)
Apparent absorption of infused Mg before pylorus	—	22.9	23.2	8.7	—	45.7	44.6	21.6	—	39.0	21.6	—	49.5	30.9
Absorption of infused Mg (%)	—	0.1	-0.1	13.7	—	0.1	1.8	17.4	—	1.8	18.7	—	-0.5	17.2
	—	0.5	-0.5	61.1	—	0.3	3.8	44.6	—	4.3	46.4	—	-1.0	35.8

Duo, duodenum; Ab, abomasum; Om, omasum; Rum, rumen.

* For details of procedures, see pp. 38-9.

† Duodenal Mg flow minus the baseline duodenal flow due to non-infusate sources.

Table 2. *The mean excretion of magnesium in the urine and faeces, Mg balance, and the net absorption of Mg from the whole digestive tract and the sections before and after the pylorus (mmol/d)*

(Mean values for four sheep, except for abomasal infusion where two sheep were used)

	Site of Mg infusion†				SE‡
	Duodenum	Abomasum	Omasum	Rumen	
Mg in faeces	42.8	38.1	41.4	23.4***	1.60
Mg in urine	2.0	0.3	1.3	13.7***	1.02
Mg balance	-2.0	2.1	1.7*	4.9**	0.99
Net Mg absorption before pylorus	-1.8	-1.2	-0.8	14.9***	0.93
Net Mg absorption after pylorus	1.9	3.6	4.1*	3.7	0.77
Net Mg absorption from whole digestive tract	0.1	2.4	3.2	18.6***	1.16

Mean values were statistically significantly different from those for duodenal infusion: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details of procedures, see pp. 38-9.

‡ SE does not apply to abomasal infusion results.

Table 3. *Mean concentrations (mmol/l) of magnesium and calcium in plasma, and of Mg in rumen fluid*

(Mean values for four sheep, except for abomasal infusion where only two sheep were used)

	Site of Mg infusion†				SE‡
	Duodenum	Abomasum	Omasum	Rumen	
Rumen fluid Mg	0.49	0.47	0.47	2.54*	0.68
Plasma Mg	0.72	0.52	0.77	1.15**	0.08
Plasma Ca	2.38	2.54	2.44	2.34	0.03

Mean values were statistically significantly different from those for duodenal infusion: * $P < 0.05$, ** $P < 0.01$.

† For details of procedures, see pp. 38-9.

‡ SE does not apply to abomasal infusion results.

infusion of Mg. During intraruminal infusion of Mg, urinary Mg excretion was $32.3 \pm 1.3\%$ of Mg intake and $72.8 \pm 2.7\%$ of total net Mg absorption.

Plasma and rumen fluid samples

The effect of the different sites of Mg infusion on the plasma calcium and Mg levels is indicated in Table 3. Infusion of Mg post-ruminally was associated with reduced plasma and rumen fluid Mg concentrations in comparison with the intraruminal infusion. Plasma Ca concentrations were unaffected by treatment.

DISCUSSION

It is clear from the results presented here that practically all the net Mg absorption which occurs before the pylorus in sheep is from the reticulo-rumen. Indeed, in these experiments absorption from this stomach compartment accounted for 70–91% of the total Mg absorption from the gastrointestinal tract. Only very small amounts, if any, are absorbed from either the omasum or abomasum. Rogers & van't Klooster (1969) concluded from the results of reported studies (Stewart & Moodie, 1956; Care & van't Klooster, 1965; Phillipson & Storry, 1965) that absorption of Mg from the reticulo-rumen is negligible, except at very high concentrations of Mg in the rumen fluid. However, Care & van't Klooster (1965) had reported an absorption rate of 0.165 mmol Mg/h from the reticulo-rumen contents of an anaesthetized sheep in which the rumen fluid level of Mg was 1.75 mmol/l. This rate is equivalent to 3.96 mmol/d, which is indeed a significant quantity. In normal physiological conditions and with a more usual Mg concentration in rumen fluid of 2.5 mmol/l (Storry, 1961; Rogers & van't Klooster, 1969; Tomas & Potter, 1975), a substantial absorption of Mg from this organ could be envisaged. In the present work, the Mg levels in the rumen fluid samples taken during the infusion of 40–50 mmol Mg/d to the rumen were 2.2–3.6 mmol/l and were associated with a net absorption of 15.6–17.7 mmol Mg/d from the reticulo-rumen. In comparison with the results of Care & van't Klooster (1965) this represents a fourfold increase in Mg absorption at twice the rumen fluid Mg concentration, but in this instance the measurements were extended over several days on conscious, relatively normal animals.

Experiments based on the concentration ratios, digesta Mg:inert marker in the gut of slaughtered animals, in order to identify the sites of Mg absorption, have not produced consistent results. Perry, Cragle & Miller (1967) found no Mg absorption from the rumen of calves, but the results reported by Marongiu (1971) indicate a considerable absorption of Mg from the rumen of sheep. However, although Marongiu (1971) found no absorption of Mg from the omasum, Perry *et al.* (1967) reported evidence to the contrary, although they dismissed this evidence as an artifact of their marker technique.

The suggestion that the omasum may be a significant site of Mg absorption in the ruminant stomach has been made by several authors (e.g. Rogers & van't Klooster, 1969; Ben-Ghedalia, Tagari, Zamwel & Bondi, 1975). This view is not supported by the evidence reported in this paper. It is possible that the method of introducing the Mg to the omasum may have confined the infused Mg to only a small area of the total omasal absorptive surface, particularly when the nature of the propulsive movements of the omasum is considered (Buéno & Ruckebusch, 1974). If this were the situation the concentration gradient of Mg from this area of the omasal mucosa to blood would be very high. However, it seems highly likely that some of the infusate would have entered the omasal groove and then been distributed into most of the omasal lumen (Sellers & Stevens, 1966), especially in view of the backflow of infusate to the rumen which occurred in one animal. Further, it is probable that much of the Mg leaving the rumen in normal circumstances moves rapidly to the abomasum through the omasal

groove, and only a portion is moved through the interlaminal spaces of the omasum. Hence, if the omasum were the principal site of absorption of intraruminally infused Mg, it seems likely that significant absorption would also be detected during the omasal infusion in this experiment.

The results of a number of studies have suggested that the omasum is a site for substantial absorption of volatile fatty acids, sodium, bicarbonate and water (Engelhardt & Hauffe, 1975). Harrison (1971) found a small transfer of ^{28}Mg from the mucosal to the blood side of a portion of omasal lamella prepared *in vitro*. Also, absorption of Ca by omasal tissue *in vitro* has been reported (Timet, Herak, Emanovic & Kraljevic, 1974), but concentrations higher than normal were used. Further, in view of the similarities in the epithelial lining and transmembrane potential difference between the rumen and omasum (Sellers & Stevens, 1966; Rogers & van't Klooster, 1969), the anatomical adaptation of the omasum for absorption (Lauwers, 1973), and the increased concentration of digesta minerals due to absorption of water, the possibility that some Mg is absorbed from the omasum should not be discarded. In particular, extrapolation of these results to cattle requires caution because of the relatively larger omasum in this species compared with sheep (Phillipson, 1970).

The lack of absorption of Mg from the abomasum in this study agrees with the results of Care & van't Klooster (1965) from their experiments with gastric pouches in the sheep's abomasum, and also explains the apparent lack of absorption of Mg infused to the abomasum in the work of Pfeffer & Rahman (1974).

Net Mg absorption from the intestines alone was insufficient to maintain both the plasma Mg levels and Mg balances which were obtained when absorption occurred from the whole gastrointestinal tract. These findings (Tables 2 and 3), for the relative apparent absorptive capacity of the stomach *v.* the intestines are consistent with those of other workers (Rogers & van't Klooster, 1969; Pfeffer *et al.* 1970; Grace & MacRae, 1972; Kemp *et al.* 1973; Pfeffer & Rahman, 1974). Of course certain sections of the intestine, such as the mid-ileum and colon, are probably important areas of net Mg absorption, but these are largely nullified by the net secretion of Mg into other sections (Marongiu, 1971; Ben-Ghedalia *et al.* 1975). The over-all net absorption of Mg from the intestines is a balance between the absorptive and secretory processes, and may change under circumstances different from those imposed in the experiment here. There is no reason to suggest, however, that the Mg salts which were infused to the gut should have any specific perturbing effect on the Mg absorption processes.

Behar (1974) reported that in the rat intestine Mg appears to be transported principally by solvent drag, and this is directly related to the bulk water flow across the membrane, although a difference in the permeability of ileum and colon to Mg transfer by this mechanism was noted. However, the likely net transepithelial flux of water from the rumen (Engelhardt, 1970) is sufficient for only a small proportion of the estimated Mg absorption to occur in this manner since rumen fluid Mg levels averaged only 2.54 mmol/l during the intraruminal infusion. Similarly, for the omasum an average absorption rate of 700 ml water/d (Engelhardt, 1970) could transport no more than about 1.5 mmol Mg/d by solvent drag. It appears that other transport mechanisms are responsible for Mg absorption from the rumen and, furthermore,

the omasal infusion results suggest that the mucosa of the ruminant forestomach may not be freely permeable to the bulk flow transfer of Mg.

The relatively unphysiological conditions imposed in many of the studies of Mg absorption from the rumen of sheep may have limited any transport processes involved and, coupled with the short-term nature of the experiments, led to the conclusion that absorption did not normally occur from this organ. Certainly the results presented here for sheep indicate a definite absorption of Mg from the reticulo-rumen, and that absorption from either the omasum or abomasum is not significant.

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