The effect of ruminal phosphate concentration on the absorption of calcium, phosphorus and magnesium from the reticulo-rumen of the sheep

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1. The absorption rates of calcium, inorganic phosphate (P_i) and magnesium were determined from buffered solutions placed in the temporarily isolated and washed reticulo-rumen of conscious sheep. The basic composition of these solutions was similar to that found in supernatant fractions of ultracentrifuged rumen contents.

2. The P_i concentrations studied in these solutions were 2, 8·7, 14, 17·3 and 38 mmol/l. The initial concentration of Ca was 2·0 mmol/l and that of Mg was 2·5 mmol/l in all experiments.

3. Increasing the P_i concentration in the rumen solution from 2 to 38 mmol/l resulted in increases in the net absorption rates of both Ca and P_i, and a decrease in the potential difference across the wall of the rumen.

4. Similarly, increasing the P_i concentration from 2 to 17·3 mmol/l resulted in an increase in the net absorption rate of Mg from the rumen.

5. Mineral analysis of strained rumen fluid or a 30000 *g* centrifugate of strained rumen fluid revealed a reduced P_i concentration in sheep fed on frozen spring grass as opposed to the pellet + hay diet. The values obtained were within the range studied.

There is recent in vitro evidence to suggest that inorganic phosphate (P_i) ions are absorbed across the reticulo-rumen (Breves *et al.* 1986, 1988). The extent of this P_i absorption appears to be dependent on the P_i concentration in the rumen, within the range 0–15 mmol/l. Similarly, net P_i absorption from the omasum has also recently been described (Edrise & Smith, 1986).

Grazing ruminants may experience suboptimal phosphorus intakes on spring grass (Ritchie & Fishwick, 1977) and hypophosphataemia has been reported to develop during hypomagnesaemic tetany (Simesen, 1970). Another feature common to the condition of clinical hypomagnesaemia is hypocalcaemia (Hemingway & Ritchie, 1965).

The current study was undertaken to investigate the relation between the absorption rates of calcium, P_i and magnesium from the isolated reticulo-rumen at different rumen P_i concentrations. The P_i concentrations used were within the physiological ranges for P-replete and P-depleted sheep (G. Breves, personal communication).

MATERIALS AND METHODS

Anaesthesia and surgery. Seven large crossbred ewes and wethers were prepared with large rumen fistulas. The sheep used were 1·5–5 years of age. The rumen fistulas were established under halothane–oxygen anaesthesia, following induction of anaesthesia by an intravenous injection of thiopentone (Pentothal; May & Baker).

Experimental protocol. The sheep were trained for use in the temporarily isolated, washed rumen technique (Martens & Rayssiguier, 1980; Care *et al.* 1984). Briefly, this technique involved restraining the conscious sheep by a loose neck yoke and removing the rumen cannula and rumen contents. The contents were stored at 40° until the end of the experiment when they were returned to the sheep. Washing buffer (2 litres), at 40°, was poured into the rumen and agitated manually in order to dislodge material from the rumen

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epithelium. The buffer was removed by suction and the procedure repeated until the effluent was free from rumen contents. The washed rumen was isolated according to Martens & Rayssiguier (1980). The base of the oesophagus was temporarily occluded by an inflated cuff, and the saliva collecting cranial to the obstruction was withdrawn by continuous suction. This was pumped into the abomasum via a Foley catheter (Eschmann; 75–100 ml, 30 catheter, 10·0 mm) passing through the reticulo-omasal orifice, the cuff being inflated in the abomasal entrance to complete the seal. The oesophageal cuff was connected to a naso-gastric tube, which was anchored at the nasal end once the cuff was situated in the appropriate place (80–100 mm from the distal end of the oesophagus). Air was allowed to bleed into the lower oesophagus in order to prevent the oesophagus from collapsing and occluding the outflow of saliva. The experimental solutions were introduced into the isolated, washed reticulo-rumen, and continually gassed with carbon dioxide via a tube fitted with a diffusing nozzle.

During an experiment, the fistula in the rumen was sealed by inner and outer foam-rubber flanges held together by plastic tapes tied externally. Tubes for saliva collection and re-circulation, CO_2, and the air bleed to the saliva collector, ran through both flanges.

Between experimental observations, sheep were usually maintained on 1 kg commercial pelleted diet (500 g dried grass and 500 g beet pulp/kg, with added trace elements) plus chopped hay.

Sheep were not fed in the morning before an experiment which began at 07.00 hours. Sheep were always used in pairs, to minimize stress due to isolation, and stood in crates for the whole day. The sheep were fed after completion of the experiment (21.00 hours), and food and water were consumed readily. These sheep were left for at least 7 d before re-use. The experimental environmental conditions were kept as constant as possible.

Measurement of potential difference (PD) across the rumen wall. The method used was that of Dobson (1959), except that blood was not allowed to clot in the venous catheter but was mixed with heparinized saline (9 g sodium chloride/l) to facilitate repeated observations. The sheep were prepared with jugular venous catheters on the day before an experiment, and the PD was recorded between the rumen contents and the venous blood by means of electrodes (40 g agar/l in saturated potassium chloride) connected to calomel half cells and a high impedance millivolt-meter.

Solutions used to measure mineral absorption rates. The compositions of solutions used for mineral absorption studies are displayed in Table 1, and are based on mineral analyses of supernatant fractions of centrifuged rumen contents.

Before each mineral absorption period was begun, 2 litres of the experimental solution to be used was placed in the rumen to equilibrate for up to 20 min. The solutions were heated to 40° and contained CrEDTA as a fluid marker. This was prepared according to Downes & McDonald (1964). The pH of the solution was adjusted, using CO_2, to 6·6–6·8 before use. In the case of the higher phosphate solutions the mean pH was reduced to 6·2.

Measurement of mineral absorption rates. The equilibrium solution was removed under suction and a 10 ml portion of the test solution was taken as the original sample. Of this solution, 2 litres were immediately placed into the rumen and another 10 ml sample taken 5 min later – the time-zero sample. Sampling was repeated at 30 and 60 min. The remaining solution was then removed and its volume noted.

The absorption rate of the mineral (mmol/2 litres per h) was calculated according to Grace *et al.* (1988) and assumes that CrEDTA is a non-absorbable substance at normal osmotic pressure (Dobson *et al.* 1976).

Mineral analyses. Mg, Ca and CrEDTA concentrations were determined by atomic absorption spectrophotometry (Instrumentation Laboratory 151). The ionized Ca concentration was determined by use of a Ca-sensitive electrode (Nova 2; Clandon

Table 1. *Composition of intraruminal solutions of differing phosphate concentration (mmol/l)*

Phosphate	2	8·7	14	17·3	38	2	20
Sodium	108	108	108	110	108	60	60
Potassium	30	30	30	30	30	90	90
Calcium	2	2	2	2	2	2	2
Magnesium	2·5	2·5	2·5	2·5	2·5	2·5	2·5
Ammonium	10	10	10	10	10	10	10
Chloride	80	80	80	80	80	80	84
Bicarbonate	45	39	33	20	20	45	45
Acetate	30	30	30	30	30	30	30
Propionate	10	10	10	10	10	10	10
Butyrate	5	5	5	5	5	5	5
Total cations	152·5	152·5	152·5	154·5	152·5	164·5	164·5
Total anions	172·0	172·7	172·0	162·3	183·0	172·0	194·0
Total ions	324·5	325·2	324·5	316·8	335·5	336·5	358·5
Glucose	5	5	5	5	5	5	5

Scientific Ltd). Sodium and potassium concentrations were determined by flame photometry (Corning 400) and P_i concentrations were determined by spectrophotometry, with absorbance monitored at 340–380 nm (Cobas Mira; Roche).

Expt 1. Mineral absorption from the isolated reticulo-rumen

Seven sheep were used for this experiment, which consisted of a series of subsections in which mineral absorption rates were compared using solutions of two differing P_i concentrations. In each subsection the effect of the solution with the lowest P_i concentration (2 mmol/l) was compared to that with the higher P_i concentration. This was 8·7, 14·0 or 17·3 mmol P_i/l, depending on the experimental subsection. Thus, with any one sheep in one experimental day, two solutions of 2 mmol P_i/l were studied, followed by two solutions of either 8·7 or 14·0 or 17·3 mmol P_i/l.

Further subsections of this experiment included the use of three sheep in which two measurements of mineral absorption rates from a solution containing 2 mmol P_i/l were made, followed by two sets of measurements using 38 mmol P_i/l solutions. The latter concentration might be expected during phosphate supplementation.

The reasons for duplicating each solution were twofold. First, it allowed the experimenter to detect any residual effects of a previous solution of differing composition, and second it provided a safeguard against total loss of results from a treatment caused by a burst Foley catheter cuff.

Expt 2. Rumen P_i concentrations

Rumen contents were withdrawn via a large rumen fistula from five sheep fed on either a commercial pelleted diet plus hay or a frozen spring grass diet. The rumen contents were strained through muslin cheesecloth. Some of the strained rumen fluid (SRF) was analysed and some was centrifuged at 30000 g and the supernatant fraction analysed for P_i.

RESULTS

Expt 1

Comparison of the net P_i absorption rates from the rumen buffered solutions showed that the P_i absorption rate increased as the intraruminal P_i concentration was increased from 2 to 8·7, 14 and 17·3 mmol/l (Fig. 1).
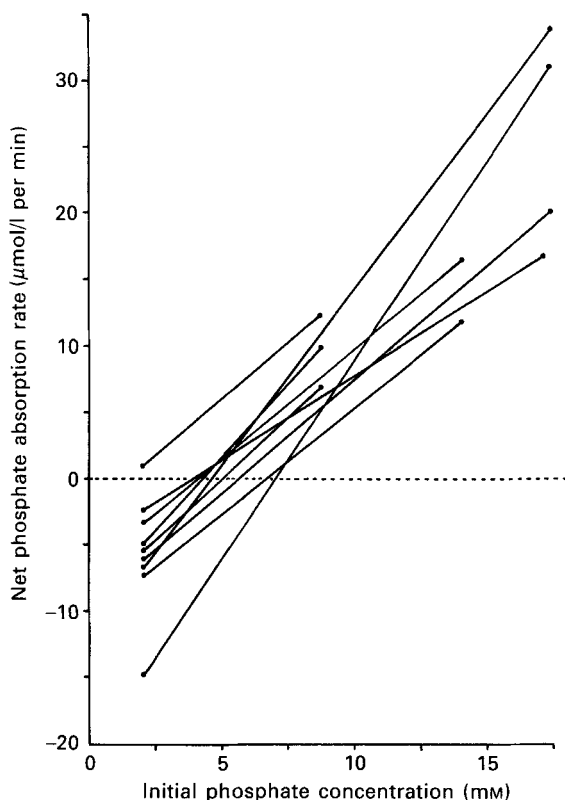
Fig. 1. Expt 1. The average net phosphate absorption rates (μmol/l per min) for individual sheep over a range of initial phosphate concentrations in the rumen solution (for details of procedures, see pp. 715–717).

Similarly, the net Ca absorption rate from the rumen solutions increased as the P_i concentration in the solution was increased from 2 to 8·7, 14 and 17·3 mmol/l (Fig. 2).

Comparison of net Mg absorption rates from the rumen buffers showed that the Mg absorption rate was greater when the P_i concentration in the buffer was increased from 2 to 8·7 and 17·3 mmol/l (Fig. 3). The variation between animals in this Mg response to increasing P_i concentration was greater than that noted for Ca and P_i.

Conversely, the transmural PD associated with these rumen solutions tended to be consistently lower when the P_i concentration in the solution was increased from 2 to 8·7, 14 and 17·3 mmol/l (Fig. 4).

Preliminary studies with two sheep showed that qualitatively similar changes in P_i, Ca and Mg absorption rates occurred when the rumen P_i concentration was increased in the presence of a high K concentration, 90 mmol/l.

The P_i absorption rates from the rumen increased in all three sheep studied as the rumen P_i concentration was increased from 2 to 38 mmol/l, i.e. from −2·8 to 19·9, −2·9 to 57·7 and −2·5 to 3·0 μmol/l per min. Similarly, the Ca absorption rates were increased, i.e. from 0·4 to 18·4, 2·7 to 15·7 and −4·5 to 2·5 μmol/l per min. In contrast, the Mg absorption rates decreased in all three sheep, i.e. from 10·5 to 9·8, 11·1 to 8·3 and 4·8 to 1·7 μmol/l per min. In the two sheep monitored, the rumen transmural PD decreased, i.e. from 29·6 to 25·6 and 42·8 to 35·9 mV.
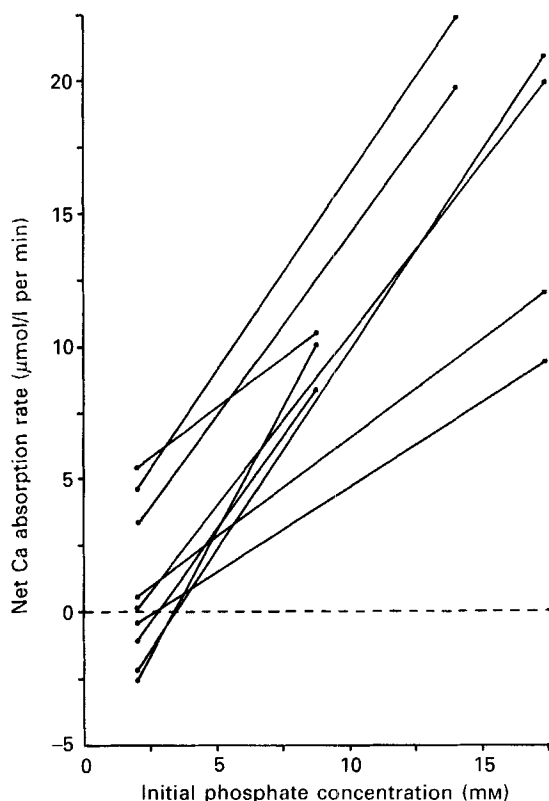
Fig. 2. Expt 1. The net calcium absorption rates (μmol/l per min) for individual sheep over a range of initial phosphate concentrations in the rumen solution (for details of procedures, see pp. 715–717).

Table 2. *Expt 2. The inorganic phosphate (P_i) concentration in fractions of rumen contents from sheep fed on different diets*

Diet*	Rumen fraction	P_i (mmol/l) Mean	SEM
Hay + pellets	Strained rumen fluid	26·03	2·51
Frozen grass	Strained rumen fluid	13·00	2·83
Hay + pellets	Pooled sample of 30000 g supernatant fraction	19·76	—
Frozen grass	Pooled sample of 30000 g supernatant fraction	15·52	—

* For details, see p. 716.

Expt 2. Rumen P_i concentrations

The P_i concentrations in SRF and in a 30000 g centrifugate of SRF were determined from rumen contents of sheep fed on either a commercial pellet and chopped hay diet or frozen spring grass, each sampled 6 h post feeding.

The P_i concentrations in the SRF from sheep fed on different diets were markedly different, the frozen spring grass being lower (Table 2). Similarly, the P_i concentration in
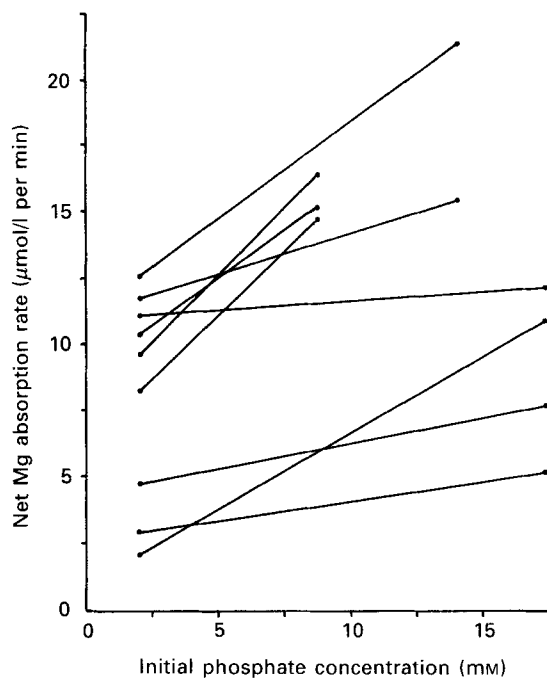
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Fig. 3. Expt 1. The average net magnesium absorption rates (μmol/l per min) for individual sheep over a range of initial phosphate concentrations in the rumen solution (for details of procedures, see pp. 715–717).
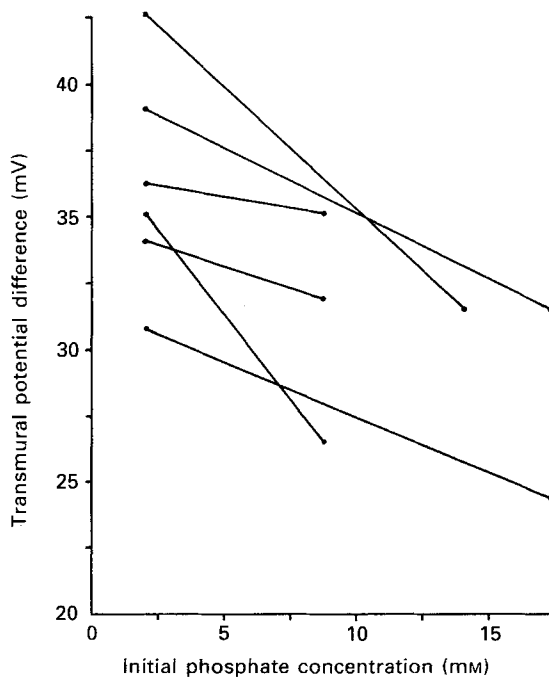

Fig. 4. The transmural potential difference (mV) blood positive for individual sheep over a range of initial phosphate concentrations in the rumen solution (for details of procedures, see pp. 715–717).

the supernatant fraction obtained by centrifuging the grass SRF at 30000 g was lower than that in the other supernatant fraction. Both values were found to be within the range of P_i concentrations used (Table 1).

DISCUSSION

Recent evidence has highlighted the role of the rumen as an organ for P_i absorption (Breves & Holler, 1986; Breves *et al.* 1986, 1988). Although earlier workers demonstrated the ability of the rumen epithelium to transport P_i in both directions (Scarisbrick & Ewer, 1951; Parthasarathy *et al.* 1952; Sperber & Hydén, 1952; Wright, 1955), the results varied considerably, and the conclusion drawn was that substantial net P_i transfer does not occur across the rumen wall, although this epithelium is capable of transporting both ^{32}P or unlabelled P_i down a concentration gradient under a variety of conditions. Similarly, Scott & Buchan (1987) were unable to support the theory that $^{32}P_i$ is transported across any part of the forestomach epithelium of sheep, although $^{32}P_i$ does appear in the plasma when the digesta is allowed to pass into the small intestine. The results of Scott & Buchan (1987) are not compatible with the results presented in the present paper or with those of Breves & Holler (1986) or Breves *et al.* (1988). The reason for this is unclear, but it may be attributable to the differences in experimental technique. The most obvious difference is the use of inorganic buffers by Breves *et al.* (1986, 1988) and ourselves, whereas Scott & Buchan (1987) studied $^{32}P_i$ absorption from digesta. There is a possibility that the $^{32}P_i$ may have been bound by digesta. However, we did not find this to be so (L. Beardsworth, P. Beardsworth and A. Care, unpublished results) and Scarisbrick & Ewer (1951) have observed $^{32}P_i$ transfer from rumen digesta to the rumen vein. Without further information on the pH, P_i, Ca and ammonia concentrations in the digesta, the possibility of the $^{32}P_i$ becoming unavailable for absorption because of binding to digesta cannot be decided.

It has been demonstrated in vitro and in vivo that P_i absorption across the rumen wall increases as the rumen P_i concentration is increased (Breves *et al.* 1986, 1988). We have confirmed this, and our results indicate that net P_i absorption occurs only at P_i concentrations above a mean of 4·3 mmol/l (Fig. 1). This agrees well with the value of 4·1 mmol/l from Breves *et al.* (1988). The rumen P_i concentration has been shown to vary with the diet (Table 2). On a diet containing only 2·2 g P/kg dry matter, Grings & Males (1987) reported that the rumen supernatant-fraction P_i concentration was still within the range 16–20 mmol/l, despite the suboptimal P intake. Conversely, Breves *et al.* (1987) showed that a decrease of 85% in the P_i concentration within the rumen was associated with a 60% decrease in total daily salivary P output (from 6·25 to 2·4 g) caused by the ingestion of a P-deficient diet (0·96 g P/d compared with 4·2 g P/d in control sheep).

The results of the present study indicate that at normal physiological rumen P_i concentrations, i.e. 16–20 mmol/l (Table 2), the capacity of the rumen to absorb Ca is greatly enhanced relative to that at P_i concentrations compatible with induced P depletion, i.e. 2 mmol/l (G. Breves, personal communication). The effect on Mg absorption rate was similar but occurred to a lesser extent. Preliminary studies showed that the effect on Ca absorption was maintained up to P_i concentrations of 38 mmol/l, but Mg absorption was reduced at that P_i concentration. It should be noted that as the P_i concentration of the intraruminal solution was increased from 2 to 17·3 mmol/l, the ionized Ca concentration decreased from 1·66 (SE 0·04) to 1·20 (SE 0·04) mmol/l, despite a fall in pH from 6·60 to 6·19, and without a change in total Ca concentration. This observation indicated that the increased Ca absorption rate observed under conditions of increased P_i concentration could not be attributed to an increase in the ionized Ca concentration within the 17·3 mmol P_i/l solution. The mechanism of Ca transport across the rumen wall is unknown, but it may

be similar to the coupled $Ca–P_i$ transport which may be present in rat small intestine (Walling, 1977). The observation that the Ca absorption rate is not decreased as the transmural PD is increased by a high intraruminal K concentration (Beardsworth *et al.* 1987) would suggest that the process of Ca absorption across the rumen wall is mainly active.

The results from these experiments suggest that the maintenance of P repletion in grazing animals is likely to facilitate the absorption of P_i and Ca from the rumen. This is also true to a lesser extent for Mg. Acute clinical hypomagnesaemia (grass tetany) is often accompanied by hypocalcaemia, and it has been reported that many hypomagnesaemic animals may appear normal until clinical symptoms are triggered by a further factor, commonly hypocalcaemia (Hemingway & Ritchie, 1965). Hypophosphataemia has also been reported to be associated with clinical hypomagnesaemia. That is, animals turned out to spring pasture may be exposed not only to an increased intake of K but to a decreased P intake, which may result in decreased absorption of Ca, Mg and P_i from the rumen. On the other hand, it appears that if P supplementation were able to produce a very high rumen P_i concentration (38 mmol/l) there might be interference with Mg absorption from the rumen. At such high levels, even at the acidic pH of the rumen, precipitation of guanite $(MgNH_4PO_4.6H_2O)$ may begin to occur (Axford *et al.* 1982).

The present study highlights the potential importance of preventing P deficiency in the grazing ruminant which may be susceptible to hypocalcaemia and hypomagnesaemia. It indicates that an intraruminal P_i concentration of 15–20 mmol/l is desirable to improve the absorption of P_i, Ca and Mg across the rumen wall.

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