Solubility and net exchange of calcium, magnesium and phosphorus in digesta flowing along the gut of the sheep

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1. The changes in the solubility of calcium, magnesium and phosphorus in digesta flowing along the intestinal tract and the net movement across the intestinal wall of these elements were determined in six rams, each equipped with three T-shaped cannulas; cannulas were placed in a total of six different sites of the small intestine. Cr_2O_3 was used as a marker substance to measure the rate of flow of the digesta.

2. The concentrations of soluble Ca, Mg and P decreased as digesta moved along the intestine. The greatest fall in soluble Ca occurred after the first 3 m of the intestine, while a significant decrease in soluble Mg was found only at 15 and 25 m from the pylorus. The concentration of soluble P in digesta decreased until the 7 m site and then remained stable. In the faeces, the level of soluble Mg was approximately 4 times higher than, and that of Ca equal to, the levels of Mg and Ca found in digesta flowing through the upper intestine. Unlike Ca and Mg, a very low concentration of soluble P was found in the faeces.

3. In the duodenum, 84, 78 and 62 % of the total Ca, Mg and P respectively were soluble, whereas in the digesta flowing through the terminal ileum the corresponding values were 3·2, 7·2 and 19 % for Ca, Mg and P respectively.

4. The forestomachs and the colon were found to be the main sites of Mg net absorption; 1·12 mmol/h was apparently absorbed from the stomach and 1·05 mmol/h from the colon. The upper small intestine (1–3 m from the pylorus) appeared to be the major site of Ca and P absorption.

5. In the last 10 m of the small intestine, considerable amounts of minerals were secreted; 4·70, 0·96 and 1·85 mmol Ca, Mg and P/h respectively were added to the digesta flowing between 15 and 25 m from the pylorus. The effect of the increase in the pH of digesta along the small intestine on the solubility of these minerals is discussed.

Absorption of calcium, magnesium and phosphorus in the gut of the sheep has been estimated using animals equipped with duodenal and ileal re-entrant cannulas (Bruce, Goodall, Kay, Phillipson & Vowles, 1966; Pfeffer, Thompson & Armstrong, 1970), and by radioisotope techniques (Wright, 1955; Jones & Mackie, 1959; Field, 1961). There is little information, however, concerning the movement of these minerals at sites along the small intestine.

In contrast to the situation in man and other single-stomached animals, the pH of material flowing through the upper section of the sheep's small intestine remains acidic (Kay, 1969; Ben-Ghedalia, Tagari, Bondi & Tadmor, 1974) and this may affect the absorption of Ca, Mg and P. Simple diffusion has been suggested as the main mechanism of Ca and Mg absorption in the sheep (Phillipson & Storry, 1965) and he rat (Aldor & Moore, 1970). Information on changes in the concentrations and proportions of soluble Ca, Mg and P at sites along the small intestine may help in understanding the mechanisms of absorption.

There are conflicting reports on the effect of dietary Ca:P ratio on P absorption in

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Table 1. *Calcium, magnesium and phosphorus contents of the foodstuffs given to sheep (mmol/kg air-dried material)*

Foodstuff	Ca	Mg	P
Concentrate*	450	87	145
Vetch (*Vicia sativa* L.) hay	443	154	115

* Contained (per kg): 250 g barley, 250 g maize, 250 g wheat, 150 g cottonseed hulls, 50 g cottonseed meal, 50 g mineral mixture, 1200 μg retinol and 10 μg ergocalciferol.

ruminants (Young, Lofgreen & Luick, 1966; Ricketts, Weinman, Campbell & Thumbleson, 1970). The subject has considerable practical as well as scientific importance as diets based upon Ca-rich forages are widely used. By following the fate of Ca and P in digesta passing along the intestine of the sheep, a better understanding of the interactions between Ca and P in the gut might be achieved.

The studies in this paper relate to the net exchange of Ca, Mg and P in different sections of the gastrointestinal tract of the sheep and to the changes in the solubility of these elements along the gut. The experiment was done with sheep that were cannulated at different sites along the small intestine and given a diet with a high Ca:P ratio.

EXPERIMENTAL

Animals, feeding and sampling

Generally, the sheep, diets and the cannulation procedures were the same as those described previously (Ben-Ghedalia *et al.* 1974). Six Awassi rams, each cannulated with three T-shaped Perspex cannulas, which involved six different sites along the small intestine, were used. The sites of cannulation were: 0·05, 1, 3, 7, 15 and 25 m from the pylorus, the most posterior site of cannulation being located approximately 0·2 m from the ileo-caecal junction. Unfortunately, two of the rams which were equipped with cannulas at the 7 m site rejected the cannulas at the beginning of the experiment. The rejection was accompanied by a slight inflammation, after which the site of rejection healed up within 2 weeks and the experiment, which had been interrupted, began again.

Cr_2O_3-impregnated paper (6 g/d) was used as a reference material to estimate the flow of digesta and excretion of faeces. The marker was given twice daily before half the daily ration (600 g concentrate mixture and 400 g vetch (*Vicia sativa* L.) hay/d) at 07.30 and 19.30 hours. The content of Ca, Mg and P in the feeds is given in Table 1. Tap-water was offered *ad lib.* and intake was not recorded, but our previous observations on their drinking behaviour suggested that for sheep kept on similar diets during the same season (spring), the water intake would be in the range 1–3 l/d. The content of Ca, Mg and P in the drinking-water was 2·83, 0·53 and 2·10 mmol/l respectively. The influence of the limited amounts of these elements in drinking-water should be considered with reference to the net exchange between the mouth and the duodenal cannula.

Samples, each of about 25 ml digesta, were taken from each cannula six times daily

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for 4 successive days 1, 3, 5, 7, 9 and 11 h after the morning feed. The samples obtained on the 4 successive days from each cannula were pooled for each sheep. The pooled samples were kept frozen at $-20°$ until used for total and soluble Ca, Mg and P analysis.

Analytical procedures

For total Ca, Mg and P determination, foodstuffs, freeze-dried digesta and faeces were ashed at 500° for 2 h. The ash was dissolved in 6 M-HCl, evaporated to dryness on a water bath, re-dissolved in concentrated HCl and again evaporated to dryness. The dry residue was dissolved in warm 6 M-HCl, filtered, washed and made up to volume. The solution obtained was analysed for total Ca, Mg and P. Analysis of soluble Ca, P and Mg in digesta was done on the supernatant fraction obtained after centrifugation at 70000 ***g***. The samples were then diluted with twice-distilled water and analysed. To determine the soluble fraction of these elements in faeces, 0·5 g of freeze-dried faeces were shaken with 9·5 ml twice-distilled water for 1 h and the supernatant fraction, after centrifugation at 70000 ***g***, used for analysis.

Ca and Mg were determined using an atomic absorption spectrometer (Model no. 303, Perkin Elmer). P was determined colorimetrically using the *p*-aminophenol-sulphate method. The method used to calculate the flows of digesta at the different sites along the intestine was described by Ben-Ghedalia *et al.* (1974).

Statistical analysis

An incomplete block analysis was used to compare the results obtained at six sites on the basis of samples taken from two or three cannulas in each of six rams. Because the design was not balanced, the standard error of the difference between the means is not the same for each pair of sites. The least and greatest values are given in Tables 2 and 3. The residual standard deviation is also given as a measure of the variability of the measured values.

RESULTS

Samples taken 7 m from the pylorus were available from only one animal, and this should be borne in mind when changes occurring immediately before and after this site are considered.

Ca, Mg and P fractions in digesta

The concentrations of the total and soluble Ca, Mg and P in digesta sampled at different sites of the small intestine are shown in Table 2. As in the previous study (Ben-Ghedalia *et al.* 1974) the effects of rams were found to be non-significant for all measurements. The mean values, calculated using an incomplete block analysis, showed that the cannulation site had a significant effect on the soluble Ca concentration and on the total and soluble concentrations of Mg and P.

As the flow rate of digesta along the intestine decreased (Ben-Ghedalia *et al.* 1974) the concentrations of total Ca and Mg increased, suggesting that Ca and Mg on the one hand and water on the other are not absorbed in a parallel pattern. The changes in the levels of soluble Ca and Mg in digesta passing along the intestine (Table 2) are quite independent. A considerable fall in the concentration of soluble Ca occurs after the

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Table 2. *The pH and the mean concentrations of total and soluble† calcium, magnesium and phosphorus (mmol/kg) in digesta sampled at different sites of the intestine and in the faeces of three sheep fitted with* T-*shaped cannulas*

	Ca		Mg		P		
	Total	Soluble	Total	Soluble	Total	Soluble	pH (range)
Sample							
Intestine							
Distance of site from pylorus (m)							
0·05	39·1	33·1	7·35	5·75	34·2	21·3	2·60–3·00
1	37·1	27·9	6·85	5·10	29·3	15·4	3·54–4·65
3	39·7	26·8	7·60	6·05	17·6	5·46	4·11–5·15
7‡	56·5	15·3	11·2	6·35	20·7	3·20	5·95–7·02
15	73·0	4·53	18·6	1·62	16·6	5·66	7·80–8·15
25 (terminal ileum)	98·0	3·08	24·3	1·72	25·4	5·06	7·70–8·22
Faeces	57·8	34·2	125·5	22·5	167	1·73	
Residual SD	96·5	3·72	13·5	1·67	16·3	2·35	
SE of difference between means:							
Least value	86·5	3·34	12·1	1·50	14·6	2·11	
Greatest value	151	5·82	21·2	2·34	25·5	3·68	
	Significance of the factors						
Effects of rams	NS	NS	NS	NS	NS	NS	
Effects of cannulas	NS	***	***	***	**	**	

NS, not significant.
** $P < 0.01$; *** $P < 0.001$.
† Concentration in the supernatant fraction from digesta and twice-distilled-water soluble material from faeces after centrifugation at 70000 *g*.
‡ One sheep only.

3 m site and continues until the terminal ileum, while a significant decrease in soluble Mg occurs probably only after the 7 m site.

Very high concentrations of soluble Ca and Mg were obtained in the faeces. The level of soluble Mg was approximately four times higher, and that of Ca the same as the concentrations of these elements found in the digesta flowing through the upper intestine.

The soluble P content of digesta decreased apparently until the 7 m site and then remained stable until the terminal ileum. Unlike that of Ca and Mg, the concentration of soluble P in the faeces was very low. The solubility of the three elements, expressed as a percentage of total, decreased as the digesta flowed down the small intestine. In the duodenum (0·05 m site), 84, 78 and 62% of the total Ca, Mg and P respectively were soluble, whereas in the digesta flowing through the terminal ileum (25 m site), the values were 3·2, 7·2 and 19% respectively.

Up to the 7 m site there appeared to be a concurrent decrease in the solubility of the Ca and P. Between the 7 m site and the 15 m site there was a continuous fall in the soluble Ca fraction but an increase in the soluble P fraction. In this work no distinction

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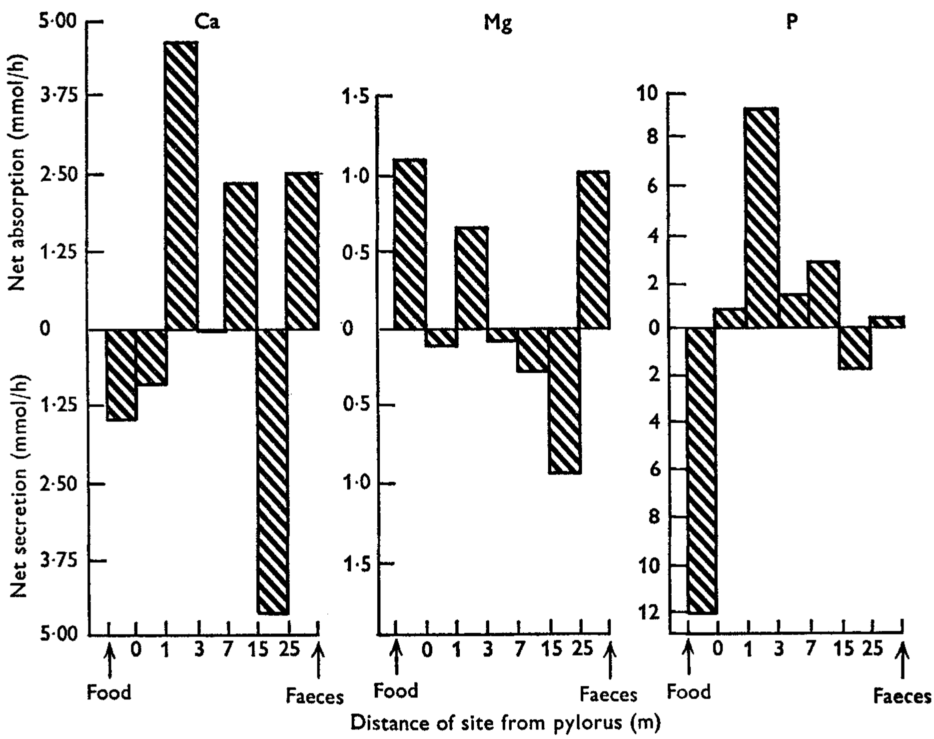

Fig. 1. Net absorption and net secretion (mmol/h) of calcium, magnesium and phosphorus between sites along the gastrointestinal tract posterior to the pylorus in the sheep.

was made between organic and inorganic P, therefore the contribution of each form to the P-soluble fraction could not be evaluated.

Net exchange of Ca, Mg and P along the intestine

Table 3 shows the flow rates for total and soluble Ca, Mg and P along the intestine. The net exchange of these elements is illustrated in Fig. 1. The statistical analysis showed a significant effect of the cannulation sites on the total and soluble Ca flow rates, the soluble Mg flow rate and the total and soluble P flow rate. A substantial net absorption of Mg and a large net secretion of P occurred between the mouth and the duodenum. Following the net movements of Ca, Mg and P passing along the intestine, it is clear that the sites and directions of net exchange differ for each of these three elements. While the upper small intestine (1–3 m from the pylorus) seems to be the major site of Ca net absorption, the forestomachs and colon are the main sites for Mg absorption. Net absorption of P occurs along the section of the intestine between 0·05 and 15 m from the pylorus, although the most active site for P absorption is the upper small intestine.

Along the 1st m of the intestine there is a certain quantitative increase of Ca and Mg, but it is likely that the absolute addition of Ca and Mg into this section by the

Table 3. *Mean flow rates (mmol/h) for total and soluble† calcium, magnesium and phosphorus at different sites of the intestine of three sheep fitted with T-shaped cannulas*

Sample	Ca Total	Ca Soluble	Mg Total	Mg Soluble	P Total	P Soluble
Food	18·6	—	4·69	—	5·56	—
Intestine						
Distance of site from pylorus (m)						
0·05	20·1	17·0	3·57	2·79	17·5	10·9
1	21·0	15·7	3·69	2·73	16·7	8·79
3	16·4	11·5	3·30	2·40	7·46	2·42
7‡	16·5	4·48	3·08	1·75	6·07	0·93
15	14·0	0·88	3·37	0·29	3·19	1·08
25 (terminal ileum)	18·7	0·60	4·33	0·31	5·04	0·96
Faeces	16·2	0·97	3·28	0·59	4·65	0·01
Residual SD	2·16	1·61	0·40	0·17	2·45	1·19
SE of difference between means:						
Least value	1·94	1·45	0·36	0·15	2·20	1·08
Greatest value	3·38	2·53	0·63	0·26	3·83	1·86
Significance of the factors						
Effects of rams	NS	NS	NS	NS	NS	NS
Effects of cannulas	*	***	NS	***	***	***

NS, not significant.

* $P < 0·07$; *** $P < 0·0001$.

† Concentration in supernatant fraction from digesta and twice-distilled-water soluble material from faeces after centrifugation at 70000 *g*.

‡ One sheep only.

common bile duct was masked by concomitant absorption. Substantial net secretion of Ca, Mg and P was observed along the last 10 m of the small intestine.

DISCUSSION

Studies on sheep cannulated at the duodenum and ileum have contributed significantly to the knowledge of mineral absorption (Bruce *et al.* 1966; Rogers & Van't Klooster, 1969; Pfeffer *et al.* 1970). However, it is impossible from the results of these studies to assess the net changes which occur in the different parts of the small intestine. As the small intestine appears to be the major site for Ca and P absorption in cows (Rogers & Van't Klooster, 1969) and sheep (Phillipson & Storry, 1965; Pfeffer *et al.* 1970) the net absorption and net secretion of Ca, Mg and P at sites along the intestine were studied in this work.

The solubility of minerals in the gut is one of the main factors affecting their absorption. Van't Klooster (1967) has shown that 90% of the total Ca and Mg and 73% of the total P passing to the duodenum of cows is in a soluble form, in comparison to 12, 34 and 7% of the total Ca, Mg and P respectively flowing through the ileum. The values obtained in this work are comparable.

The rise of pH as the digesta advances along the intestine is accompanied by a

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concomitant decrease in the solubility. The pancreatic juice flowing into the section of the intestine 0·05–1 m from the pylorus affects to some extent the solubility of these minerals (Kay, 1969), but it seems that the intestinal secretion acts as the major factor in decreasing the soluble amounts of Ca, Mg and P in digesta flowing through the lower parts of the intestine. The effect of pH on the amount of soluble Mg in digesta, however, is not as marked.

This study has shown that the upper small intestine is the main site for Ca absorption, although this process continues to some extent up to the 15 m site. These results are compatible with those of Phillipson & Storry (1965), who studied the absorption of Ca and Mg from solutions by isolated sections of the small intestine of the sheep. In addition, they found that no absorption occurred in the duodenum or in the terminal ileum, which agrees with the results presented here. Scott (1965) has shown that both Ca and Mg were absorbed from inorganic solutions placed in ileal loops of the sheep. However, in those experiments in which the concentrations of Ca and Mg were close to those found in digesta passing along the ileum, very little net absorption occurred, and in some instances a net secretion of Mg and Ca was found. The localization of the upper small intestine as the major site for Ca absorption in different species such as the horse (Schryver, Craig, Hintz, Hogue & Lowe, 1970) and the rat (Sernka & Borle, 1969) is undoubtedly connected with the solubility of Ca in this area.

Jones & Mackie (1959), studying Ca secretion into the gut of the sheep, showed that after intravenous injection of ^{45}Ca, a considerable amount of the tracer was recovered in the lower half of the small intestine. In the present study, approximately 4·75 mmol Ca/h was found to be secreted in the lower 10 m of the small intestine. It would appear that the upper part of the small intestine is mainly a site of absorption whereas the lower part is apparently a site of secretion. In an experimental system of duodenal–ileal cannulated sheep, the Ca absorbed in the upper small intestine may be masked quantitatively by the endogenous secretions in the lower part. This may explain the very slight quantitative changes in Ca flowing through the small intestine found by Pfeffer *et al.* (1970).

The present results demonstrate that a substantial amount of the ingested Mg was absorbed before the duodenum. As the rumen epithelium appears to be impermeable to Mg^{2+} (Phillipson & Storry, 1965) and no net absorption of Mg occurs in the abomasum and duodenum (Care & Van't Klooster, 1965), it is possible that the omasum is the site of Mg absorption. Appreciable net absorption of Mg before the duodenum has been reported by Rogers & Van't Klooster (1969), Pfeffer *et al.* (1970) and Kemp, Van't Klooster, Rogers & Guerink (1973). The net movement of Mg in the upper small intestine found in this work, although not statistically significant, agrees with the findings of Care & Van't Klooster (1965) which showed that Mg was absorbed from Thiry Vella loops located 3 and 10 m from the pylorus. However, high dietary levels of Mg would increase the relative importance of the forestomachs as the main site of Mg absorption (Kemp *et al.* 1973). The colon was shown to be another important site of Mg absorption. The proportion of soluble Mg in faeces was very low, but the results in Table 2 showed that the concentration of soluble Mg in the faeces was

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about four times higher than in digesta passing through the upper small intestine. As the formation of the faecal pellets is connected with an intense absorption of water as the digesta passes along the colon, it is clear that this process also concentrates solutes and this would facilitate the absorption (passive) of Mg and possibly other elements from the colon. Net absorption of Mg from the large intestine was also reported by Pfeffer *et al.* (1970) and Rook (1972). By comparison, in the rat the upper small intestine seems to be the major site of Mg absorption (Urban & Schedl, 1969; Aldor & Moore, 1970).

Large volumes of saliva are secreted continuously by the sheep, up to 16 l/d. Assuming that the P content of saliva is about 16·2 mmol/l (Kay, 1960), the addition of about 200 % of P between the mouth and duodenum would be expected. In view of the fact that the reticulo-rumen and the omasum appear to be impermeable to P (Wright, 1955), it seems that this increase in P could be an absolute gain. Substantial gains of P between mouth and duodenum were reported also by Bruce *et al.* (1966) and Pfeffer *et al.* (1970). It is generally accepted that P absorption occurs mainly in the intestine but very little information concerning P absorption from sections of the intestine of the sheep is available. The results presented in this paper suggest that an appreciable amount of P is absorbed from the upper small intestine. About 339 mmol P/d were apparently absorbed from the section of the intestine 0·05–15 m from the pylorus, which is 2·5 times the quantity that was ingested.

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