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## Studies on the amount and composition of digesta flowing through the duodenum of dairy cows. 2. Sites of net absorption of magnesium and calcium from the alimentary tract

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### Summary

1. The amounts of magnesium and calcium in the digesta flowing through the duodenum were measured direct in two cows fitted with re-entrant duodenal cannulas. A winter ration and a ration consisting of freshly mown herbage were given to both cows. Balance trials were carried out in the usual manner. Duodenal digesta were collected for 120 h in the middle of each balance period. In four other cows fitted with a T-piece cannula, the flow of duodenal digesta, Mg and Ca were estimated indirect by reference to poly(ethylene glycol) (PEG) and Cr<sub>2</sub>O<sub>3</sub>. Balance trials were carried out on these cows as above, and duodenal digesta were sampled every 2 h for 120 h in the middle of each balance period.

2. At normal Mg intakes there were no significant differences in net absorption of Mg proximal and distal to the duodenal cannula. At high Mg intakes net Mg absorption occurred mainly proximal to the duodenal cannula. Net Ca absorption was restricted to the intestines and a considerable net Ca secretion occurred proximal to the duodenal cannula. The amounts of chromium sesquioxide (Cr<sub>2</sub>O<sub>3</sub>), Mg and Ca which passed through the duodenum during the first 24 h of digesta collection were measured and were compared with the amounts which passed during each 120-h collection period. Recoveries of Cr<sub>2</sub>O<sub>3</sub> averaged 85 and 98% at the end of 24 and 120 h respectively. The amounts of Mg and Ca which passed in the first 24 h were 99 and 96% of the mean amounts that passed during the 120-h collection. Experiments which combine the total collection of digesta for short periods (up to 24 h) with a correction factor to adjust first day's flow of digesta to give 100% recovery of the indicator may involve a considerable error.

3. Reliable conclusions on the sites of net Mg absorption could not be drawn, as the error attached to the indirect estimates in these four cows was too great, with respect to the small total net absorption of Mg. The results suggested, however, that Ca absorption was restricted to the intestines, with some secretion of Ca proximal to the duodenum.

### Introduction

It is now generally agreed that hypomagnesaemia in the bovine arises by an inadequate absorption of magnesium from the elementary tract (Rook et al., 1958; Rook and Balch,

1958; Kemp et al., 1960, 1961). The site of net absorption of magnesium from the alimentary tract of ruminants has been called in question recently. Studies with fistulated cows (Rogers and van 't Klooster, 1969) and with sheep (Grace, 1972) have indicated that the main site of magnesium absorption is proximal to the duodenum. This conclusion conflicts with that of other workers, who reported that magnesium was absorbed almost entirely from the small intestine in cattle (Perry et al., 1967) and sheep (Field, 1961). Smith (1962), working with fistulated calves, reported similar results for calves more than four months old, although he found that the large intestine was a very important absorption site in the very young non-ruminating calf. The reticulo-rumen of sheep was found to be unimportant, except at high magnesium concentrations in the rumen fluid (Stewart and Moodie, 1956; Care and van 't Klooster, 1965; Phillipson and Storry, 1965). Because of these conflicting conclusions, and because the validity of the sampling technique used by Rogers and van 't Klooster (1969) could not be proven in those experiments, the sites of absorption of Mg and Ca have been re-examined.

The present paper describes the results obtained by direct measurements in two cows fitted with re-entrant cannulas in the duodenum and by indirect estimates in four cows fitted with simple T-piece duodenal cannulas. This study was part of the experiment to assess the accuracy of indirect versus direct measurements of the flow of digesta and nutrients through the duodenum of cows (see van 't Klooster et al., 1972).

### Experimental

Cows 3 and 4 were fitted with re-entrant duodenal cannulas, and cows 1, 2, 5 and 6 with T-piece duodenal cannulas. All cows had previously been fitted with rumen cannulas. During two experimental periods (I and III), two different rations were fed to each cow and full balance trials with separate collection of faeces and urine were carried out. Full details of the balance procedure, dosing with indicator (all cows), sampling procedure, analytical methods for PEG and  $\text{Cr}_2\text{O}_3$  etc. are given in Part 1 (van 't Klooster et al., 1972). Details of the experimental cows, the rations given to the cows and the Mg supplementation (cows 1, 2 and 3 only) are given in Tables 1 and 2.

Duodenal collections were made over 5 continuous days (120 h) in the middle of each balance period. At 30 min past each hour about 700 ml digesta from cows 1, 2, 5 and 6 were allowed to flow into a plastic 1-litre bottle. The digesta were mixed and sampled (200 ml) and the remainder was returned to the duodenum. The samples were stored at  $-25^\circ\text{C}$  until required.

The concentration of Mg and Ca was determined in the acidified duodenal contents

Table 1. Age, body-weight, date of calving and type and situation of the duodenal cannula of the experimental cows. All animals were fitted with a rumen cannula.

Cow No	Age (years)	Body-weight (kg)	Date of calving	Type and situation of the cannulas used
1	5	537	6.1.70	Proximal duodenal cannula
2	5	503	28.2.70	Distal duodenal cannula
3	6	508	14.2.70	Re-entrant duodenal cannula in the distal duodenum
4	4	480	27.2.70	Re-entrant duodenal cannula in the distal duodenum
5	5	498	12.2.70	Proximal duodenal cannula
6	5	605	22.2.70	Distal duodenal cannula

Table 2. Details of the experimental rations given to the cows.

Exp. No	Cow No	Milk yield (kg/day)	Rations (kg/day)			MgO added (g/day)
			hay	concentrates	dry matter intake	
I	1	21.68	8.0	10.0	14.71	50
	2	19.16	8.0	8.0	13.40	50
	3	15.46	8.0	8.0	13.19	50
	4	16.00	7.0	8.0	12.27	0
	5	19.63	8.0	8.0	13.41	0
	6	26.21	8.0	11.0	15.85	0
II	1	18.63	All animals fed		13.03	50
	2	13.87	on freshly		10.22	50
	3	10.40	mown herbage		9.61	50
	4		(early stage			
	5	16.77	of growth)		11.68	0
	6	20.41			13.08	0
III	1	16.54	All animals fed		11.70	50
	2	14.84	on freshly		10.91	50
	3	11.78	mown herbage		11.48	50
	4	11.61	(older stage		9.37	0
	5	15.11	of growth)		12.42	0
	6	18.04			11.35	0

and in the ashed feeding stuffs and faeces by atomic absorption flame spectrophotometry. The standards used and the samples were diluted with  $\text{SrCl}_2$  solution (10 g/litre) after precipitation of sulphate.

The flow of digesta through the duodenum was measured directly (120 continuous hours in each experiment) in cows 3 and 4. The concentrations of Mg and Ca in proportional samples (2%) were estimated for all samples from the even hours and in bulk samples for each day (day samples) which were composed from twelve samples from the odd hours of each day. The calculation of the amounts of Mg and Ca passing through the duodenum was based on these analyses.

The flow of digesta, Mg and Ca through the duodenum of cows 1, 2, 5 and 6 was estimated indirectly by reference to PEG and  $\text{Cr}_2\text{O}_3$  as follows. Fixed amounts of each sample for the even hours were combined to form one bulk sample for each cow for each collection period of five days. Each bulk sample, therefore, was composed of sixty samples (van 't Klooster and Rogers, 1969). Samples from each odd hour were bulked to form five-day samples per cow. Analytical results of these day samples were used to estimate the day-to-day variation between the bulked samples. The quantities of Mg and Ca that passed through the duodenum of cow 1, 2, 5 and 6 were calculated for the even and odd hours separately as:

$$\text{total Mg or Ca/12 h} = \frac{\text{dose of indicator/12 h}}{\text{content of indicator/kg digesta}} \times \text{g Mg or Ca/kg digesta}$$

where the digesta samples used for analysis were the bulk samples of the even hours and odd hours, respectively. Agreement between estimates for the even and odd hours was good. The sum of both estimates represents the total amount of magnesium or calcium that passed through the duodenum per day. By comparing the amounts of Mg and Ca

ingested and the amounts calculated to pass through the duodenum with those excreted in the faeces, the net absorption proximal and distal to the duodenal cannulae could be estimated.

## Results

### *The availability and balance of Ca and Mg*

Table 3 shows the mean daily intakes and excretions of Ca and Mg and the concentrations of Mg in plasma of the six cows on a winter ration and on a ration of freshly mown herbage. Blood samples were taken at the end of each experimental period. The availability of Ca averaged 20 and 21% and the availability of Mg averaged 15 and 21%, respectively. (Availability = % of intake not excreted with the faeces and corrected for removal of digesta samples.)

The secretion of Ca and Mg in milk was determined mainly by the milk yield, as one might expect. The Ca excretion in urine was low in both experiments. The Ca balances appeared to be normal for the stages of lactation.

Excretion of Mg in urine was lowest in cows 4 and 5 on the grass ration and coincided with the lowest plasma Mg levels measured in these experiments. Plasma Mg levels remained normal in the other cows and in all 6 cows on the winter ration. This suggests that the amount of available Mg was adequate to meet the requirements (Kemp et al., 1960), except in cows 4 and 5, that were given the herbage ration. The variation in the Mg balances was somewhat greater than in other trials at this laboratory (Kemp et al., 1960) but, taking into account the greater fluctuation in daily faeces production in the present experiments, these balances are acceptable.

### *Sites of Mg absorption as determined by direct and indirect measurements in the same cows.*

Table 4 shows the mean intake of Mg, the rate of passage of digesta, the amount of Mg calculated to pass through the duodenum, the net amounts absorbed proximal to the re-entrant duodenal cannulas, the amount excreted in the faeces and the net amounts absorbed distal to the duodenal cannulas. Results are given for the net absorption measured direct and estimated indirect.

A significant absorption of magnesium occurred proximal to the duodenal cannulas in both cows in Expts I and III. Distal to the duodenal cannula there was also a significant absorption in cow 4 given the winter ration and in cows 3 and 4 given the herbage ration. The net absorption proximal to the duodenal cannulas in both experiments was greater in cow 3 with extra magnesium than in cow 4 with a normal magnesium intake. The differences in net absorption between the cows on the two levels of magnesium intake appeared to be less distal to the duodenal cannulas. The higher net absorption in the cow with the high magnesium intake took place mainly proximal to the duodenal cannula. On the normal magnesium intake there were no significant differences in net absorption proximal and distal to the duodenal cannulas.

It has been shown (van 't Klooster et al., 1972) that indirect estimates of the flow of digesta through the duodenum gave good agreement with direct measurements. Moreover, there were no significant differences between the Mg concentration of the proportional and the non-proportional samples of digesta. However, a relatively small difference in total flow of digesta, whether estimated direct or by reference to PEG or Cr<sub>2</sub>O<sub>3</sub> is associated with a large error in the estimates of the net amounts of Mg and Ca absorbed proximal or distal to the cannula. This is most noticeable for Mg. For instance, in cow 3, Exp. I, a

48 Table 3. Intake of calcium and magnesium, output in faeces, urine and milk and plasma Mg concentrations in lactating cows fed on winter rations (Exp. I) and on freshly cut herbage (Exp. III).

Experiment No	Cow	Intake (g/day)		In faeces (g/day)		In milk (g/day)		In urine (g/day)		Availability <sup>1</sup> (%)		Balance (g/day)		Plasma Mg (mg/100 ml)
		Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg	
I winter ration	1	129.4	70.6	97.0	57.4	28.0	2.4	1.3	7.5	24	18	1.8	1.8	2.54
	2	110.4	65.3	89.4	58.4	22.5	1.9	0.2	6.6	18	10	-2.8	-3.2	2.39
	3	109.9	64.9	90.4	55.0	18.3	1.4	0.1	4.0	16	14	-1.1	2.4	1.92
	4	105.7	33.7	87.0	28.9	18.5	1.6	0.2	2.3	16	13	-2.2	-0.5	2.24
	5	110.8	35.8	89.2	30.7	23.3	2.0	0.4	3.0	19	13	-3.1	-0.9	2.22
	6	140.9	44.6	103.3	35.2	31.3	3.0	2.4 <sup>2</sup>	5.7 <sup>2</sup>	26	20	2.5	-0.1	2.43
III herbage ration	1	81.8	47.5	59.4	34.9	18.7	1.7	0.6	6.9	27	26	2.3	3.8	2.19
	2	76.4	46.2	64.6	37.8	16.2	1.3	0.5	4.0	15	17	-5.6	2.9	2.19
	3	81.7	47.2	68.6	35.3	13.0	1.0	0.9	6.0	14	24	-2.4	4.2	2.06
	4	65.0	14.3	50.5	11.1	12.6	1.1	1.0	1.0	21	21	-0.3	1.1	1.63
	5	88.1	19.0	67.7	15.7	17.6	1.4	1.1	1.1	22	16	0.9	1.0	1.71
	6	77.5	17.3	55.0	13.4	20.6	1.8	2.0	1.8	28	22	-0.8	0.7	2.21

<sup>1</sup> Percentages of the intake not excreted in the faeces, but corrected for the removal of duodenal samples.

<sup>2</sup> Contaminated with faeces.

Table 4. Net absorption of magnesium proximal and distal to the duodenal cannula (DC) measured direct and estimated by the indicator dilution technique with poly(ethylene glycol) (PEG) and chromium sesquioxide (Cr<sub>2</sub>O<sub>3</sub>) in two cows fed on two rations (mean values, with their standard errors where shown).

Experi- ment No	Cow No	Method	Mg intake (g/day)	Rate of flow through DC		Net Mg absorption proximal to DC (g/day)	Mg excreted in faeces (g/day)	Net Mg absorption distal to DC <sup>1</sup> (g/day)
				total digesta (kg/day)	Mg (g/day)			
I winter ration	3	direct PEG Cr <sub>2</sub> O <sub>3</sub>	64.9 ± 0.07	289 ± 4.3	57.4 ± 1.4	7.5 ± 1.4	55.0 ± 0.8	1.3 ± 1.6
				280	55.6	9.3		-0.5
				298	59.2	5.7		3.1
4	4	direct PEG Cr <sub>2</sub> O <sub>3</sub>	33.7 ± 0.07	202 ± 2.9	31.6 ± 0.8	2.1 ± 0.8	28.9 ± 0.2	2.0 ± 0.8
				199	30.5	3.2		0.9
				199	30.5	3.2		0.9
III herbage ration	3 <sup>2</sup>	direct PEG Cr <sub>2</sub> O <sub>3</sub>	47.2 ± 0.07	353 ± 3.8	40.0 ± 0.5	7.2 ± 0.5	35.3 ± 0.1	4.1 ± 0.5
				346	39.0	8.2		3.1
				413	46.6	0.6		10.7
4	4	direct PEG Cr <sub>2</sub> O <sub>3</sub>	14.3 ± 0.07	225 ± 2.6	13.2 ± 0.2	1.1 ± 0.2	11.1 ± 0.1	1.8 ± 0.2
				216	12.8	1.5		1.4
				224	13.3	1.0		1.9

<sup>1</sup> Corrected values for sampling duodenal digesta.

<sup>2</sup> Blockages occurred in the cannula.

Table 5. Net absorption of calcium and magnesium proximal and distal to the duodenal cannula (DC) estimated by the indicator dilution technique with poly(chylene glycol) (PEG) and chromium sesquioxide (Cr<sub>2</sub>O<sub>3</sub>) in cows fitted with simple T-piece duodenal cannulas and fed on two rations.

Experiment No	Cow No	Method	Intake			Rate of flow past DC			Net absorption proximal to DC			Excreted in faeces			Net absorption distal to DC <sup>1</sup>		
			Ca (g/day)	Mg (g/day)	digesta (kg/day)	Ca (g/day)	Mg (g/day)	Ca (g/day)	Mg (g/day)	Ca (g/day)	Mg (g/day)	Ca (g/day)	Mg (g/day)	Ca (g/day)	Mg (g/day)		
I winter ration	1	PEG	129.4	70.6	272	149.8	68.4	-20.4	2.2	97.0	57.4	51.0	10.2				
		Cr <sub>2</sub> O <sub>3</sub>	129.4	70.6	239	131.5	60.0	-2.1	10.6	97.0	57.4	32.7	1.8				
	2	PEG	110.4	65.3	270	124.6	64.8	-14.2	0.5	89.4	58.4	33.7	5.6				
		Cr <sub>2</sub> O <sub>3</sub>	110.4	65.3	272	125.6	65.4	-15.2	-0.1	89.4	58.4	34.7	6.2				
	5	PEG	110.8	35.8	316	125.8	36.6	-15.0	-0.8	89.2	30.7	34.9	5.4				
		Cr <sub>2</sub> O <sub>3</sub>	110.8	35.8	288	123.8	33.4	-13.0	2.4	89.2	30.7	32.9	2.2				
6	PEG	140.9	44.6	340	160.1	40.6	-19.2	4.0	103.3	35.2	55.3	5.0					
	Cr <sub>2</sub> O <sub>3</sub>	140.9	44.6	316	150.0	37.8	-9.1	6.8	103.3	35.2	45.2	2.2					
III herbage ration	1	PEG	81.8	47.5	291	95.7	43.0	-13.9	4.5	59.4	34.9	35.3	7.7				
		Cr <sub>2</sub> O <sub>3</sub>	81.8	47.5	266	87.4	37.5	-5.6	10.0	59.4	34.9	27.0	2.2				
	2	PEG	76.4	46.2	305	91.3	45.2	-14.9	1.0	64.6	37.8	25.8	6.9				
		Cr <sub>2</sub> O <sub>3</sub>	76.4	46.2	298	89.2	44.2	-12.8	2.0	64.6	37.8	23.7	5.9				
	5	PEG	88.1	19.0	362	104.7	20.9	-16.6	-1.9	67.7	15.7	35.8	5.0				
		Cr <sub>2</sub> O <sub>3</sub>	88.1	19.0	343	99.0	17.8	-10.9	1.2	67.7	15.7	30.1	1.9				
6	PEG	77.5	17.3	359	89.8	16.1	-12.3	1.2	55.0	13.4	34.0	2.6					
	Cr <sub>2</sub> O <sub>3</sub>	77.5	17.3	339	85.1	15.2	-7.6	2.1	55.0	13.4	29.3	1.7					

<sup>1</sup> Corrected values for sampling duodenal digesta.

Table 6. Net absorption of calcium proximal and distal to the duodenal cannula (DC) measured direct and estimated with the indicator dilution technique with poly(ethyleneglycol) (PEG) and chromium sesquioxide (Cr<sub>2</sub>O<sub>3</sub>) in two cows fed on two rations (mean values with their standard errors where shown).

Experi- ment No	Cow No	Method	Ca intake (g/day)	Rate of flow of Ca through DC (g/day)	Net Ca absorption proximal to DC (g/day)	Ca excreted in faeces (g/day)	Net Ca absorption distal to DC (g/day)
I winter ration	3	Direct	109.9 ± 0.26	122.3 ± 3.4	-12.4 ± 3.4	90.4 ± 1.7	29.6 ± 3.8
		PEG		117.6	-7.7		24.9
		Cr <sub>2</sub> O <sub>3</sub>		125.2	-15.3		32.5
III herbage ration	4	Direct	105.7 ± 0.26	120.9 ± 2.7	-15.2 ± 2.7	87.0 ± 0.8	31.1 ± 2.8
		PEG		120.3	-14.6		30.5
		Cr <sub>2</sub> O <sub>3</sub>		120.3	-14.6		30.5
III herbage ration	3 <sup>1</sup>	Direct	81.7 ± 0.26	95.3 ± 1.2	-13.6 ± 1.2	68.6 ± 0.3	25.1 ± 1.2
		PEG		94.2	-12.5		24.0
		Cr <sub>2</sub> O <sub>3</sub>		112.4	-30.7		42.2
III herbage ration	4	Direct	65.0 ± 0.26	72.7 ± 1.1	-7.7 ± 1.1	50.5 ± 0.7	20.7 ± 1.3
		PEG		71.8	-6.8		19.8
		Cr <sub>2</sub> O <sub>3</sub>		74.4	-9.4		22.4

<sup>1</sup> Blockages in the cannula.



small difference (3%) between the estimates of digesta flow measured direct and by reference to PEG resulted in a difference of 24% in the estimate of Mg absorbed proximal to and of more than 100% distal to the duodenal cannula.

The peculiar values for the amounts of Mg calculated to pass the duodenum by reference to  $\text{Cr}_2\text{O}_3$  in cow 3, given the herbage ration, were caused by extremely low  $\text{Cr}_2\text{O}_3$  concentrations in the digesta on one of the five days of the duodenal collection. The low passage of  $\text{Cr}_2\text{O}_3$  in this experiment may have been due to abomasal distension resulting from the blockages of the cannula occurring in this instance (van 't Klooster et al., 1972).

The mean recoveries of  $\text{Cr}_2\text{O}_3$  and PEG during the first 24 h of each 120-h collection of duodenal digesta were 85 and 95%, respectively. The mean amounts of Mg which were calculated by direct measurement to pass in the same 24 h equaled the mean daily amounts for the 120-h periods. The duodenal recovery of  $\text{Cr}_2\text{O}_3$  and PEG over the 120-h periods averaged 100.3 and 98.3%, respectively (van 't Klooster et al., 1972). It follows that a correction of the amount of Mg which was measured direct over the first 24 h to give a theoretical recovery of 100% of  $\text{Cr}_2\text{O}_3$  would result in much higher estimates of the amount of Mg passing through the duodenum than was found by direct measurements over 120-h periods.

#### *Sites of Mg absorption as examined by indirect measurements in cows with T-piece duodenal cannulas*

Table 5 gives for cows 1, 2, 5 and 6 the results as shown in Table 4 for the cows with re-entrant cannulas. For some reason unknown, the estimates of flow based on the PEG content of the duodenal digesta were higher in most instances than the estimates of flow based on the  $\text{Cr}_2\text{O}_3$  content of the digesta. Consequently, the estimates of the amounts of Mg passing in the digesta showed the same differences. The average net absorption of Mg from the entire tract in these cows was 7.4 g/day. By reference to PEG, the mean net absorptions proximal and distal to the duodenum were 1.3 and 6.1 g/day, respectively; by reference to  $\text{Cr}_2\text{O}_3$ , the values were 4.4 and 3.0 g/day, respectively. It was not possible to decide which of the two indicators was the more likely to yield the more accurate result in these cows. When the two indicator techniques were considered as duplicates the net absorptions of Mg proximal and distal to the cannulas were about 2.9 and 4.5 g/day, respectively. Because of the considerable variation between the two indicators in respect of the small amounts of Mg absorbed, reliable conclusions on the sites of Mg absorption could not be drawn from these results.

#### *Sites of Ca absorption*

Table 5 shows the net amounts of Ca absorbed by reference to PEG and  $\text{Cr}_2\text{O}_3$  in cows 1, 2, 5 and 6. Table 6 shows the amounts absorbed by cows 3 and 4, estimated direct and also by reference to PEG and  $\text{Cr}_2\text{O}_3$ . The general results in cows 1, 2, 5 and 6 suggested that about 13 g Ca/day was secreted proximal to the duodenal cannulas and about 35 g/day was absorbed from the intestines. These amounts agree with the amounts measured directly in cows 3 and 4. Absorption of calcium proximal to the duodenal cannulas can not be excluded, but net absorption is restricted to the intestines.

## **Discussion**

The present results showed no significant difference between the availability of Mg for cows given a supplement of MgO compared with cows not given the supplement. This

agrees with earlier conclusions (Kemp et al., 1961). The results also show that the availability of Mg may be less in a winter ration than in spring herbage.

The results of the direct measurements in cows 3 and 4, fitted with re-entrant cannulas in the duodenum, showed that significant net absorption of Mg occurred from the stomachs as well as from the intestines. With normal Mg intakes there were no significant differences between the two sites, whereas with high Mg intakes the stomachs were much more important than the intestines. This agrees with earlier experiments of Rogers and van 't Klooster (1969) and also with the findings of Kemp (1959) and Kemp et al. (1963), that plasma Mg concentrations in hypomagnesaemic cows rise within 2 h after dosing with 40 g MgO, or more, in Mg-containing cakes.

The volume of saliva, gastric juice, bile and pancreatic juice secreted in these cows would amount to about 300 litres and would add 1.5 - 2.0 g Mg to the digesta, proximal to the duodenal cannula. Therefore the total absorption of Mg proximal to the sampling point would be increased by this amount.

The recovery of PEG and Cr<sub>2</sub>O<sub>3</sub> from duodenal digesta of sheep (van 't Klooster et al., 1969) and cows (van 't Klooster et al., 1972) was almost complete, and indirect estimates of digesta flow based on PEG agreed very well with those based on Cr<sub>2</sub>O<sub>3</sub>. Furthermore, the estimate of duodenal digesta flow in the earlier work with cows (van 't Klooster and Rogers, 1969) showed only a 3% difference between the values based on PEG and those based on Cr<sub>2</sub>O<sub>3</sub>. In those experiments, the means of the values based on both indicators were used and the results indicated that net absorption of Mg occurred mainly from the stomachs. In the present experiments, with the indicator dilution technique in the four cows fitted with simple T-piece cannulas, the estimates of the duodenal flow of digesta, Mg and Ca, based on PEG, were about 6% higher than those based on Cr<sub>2</sub>O<sub>3</sub>. This difference between the two indicators resulted in a large error attached to the small amounts of Mg absorbed and does not allow definite conclusions on the sites of Mg absorption to be drawn from these results.

The errors attached to direct measurement of the flow of digesta and nutrients through the duodenum are such that the errors attached to estimates of net absorption proximal or distal to the cannula are relatively large, particularly when the amounts absorbed are small. As the net absorption increases, for instance with Mg in cows receiving extra MgO, the reliability of the estimates of segmental absorption increases. The same comments apply with even more force to indirect estimate made by the indicator dilution technique. The cause of the discrepancy between the PEG and Cr<sub>2</sub>O<sub>3</sub> results in these four cows could not be determined.

The present findings that net absorption of Ca occurs mainly or exclusively from the intestines is in agreement with earlier conclusions (Smith, 1962; Cragle et al., 1964; Phillipson and Storry, 1965; Yan and Thomas, 1965; Perry et al., 1967; van 't Klooster, 1967; Rogers and van 't Klooster, 1969). The results suggested that the net secretion proximal to the duodenal cannulas was about 13 g Ca/day. Secretion products would add about 6 g/day if their Ca content is similar to that of sheep (Storry, 1960), but it is possible that the content is higher in cows. However, the daily endogenous faecal loss of Ca was estimated to be about 20 mg/kg body weight in cows (Visek et al., 1953; Hansard et al., 1954; Conrad et al., 1956). In our experiments, the endogenous faecal loss of Ca would have been about 10 g/day, on this basis. If one assumes that the availability of endogenous Ca in the digesta is the same as that of dietary Ca, and that most of the endogenous Ca is secreted proximal to the duodenal cannula, this would result in a secretion of over 12 g Ca/day proximal to the cannula.

A negative relationship between the calcium intake and the availability of Ca can be

deduced from several experiments (Duncan, 1958). The intake of Ca and the daily milk yields in cows given the herbage ration were considerably lower than in those giving the winter ration. The combination of lower Ca intake with lower milk yield may explain the lack of difference in Ca availability between Exp. I and III because a lower milk yield goes with a lower availability.

Several workers have taken the low recovery of the indicators over the first 24-h period of collection as an indication of a depressed flow rate. Therefore, they adjusted the measured flow rate to a value which would give a theoretical 100% recovery of the indicator on the assumption that the rate of passage of nutrient and indicator are depressed in the same way. In cows 3 and 4 the flow of Ca and Mg during the first 24 h was adjusted to 100% recovery of  $\text{Cr}_2\text{O}_3$  and considerable differences were noted between the adjusted values and the mean flow of Ca and Mg, which were measured directly over 5-day periods. The technique combining short periods of direct measurement with adjustments to 100% recovery of indicator saves much of the labour and tedium involved in total collection of digesta over long periods. However, the errors involved are often large and the basic assumption that indicator and nutrient flow are correlated has been shown to be invalid, both in these and in previous experiments (van 't Klooster et al., 1969).

In the present experiments the potential difference between blood and lumen of the reticulo-rumen was found to be about 25 mV in cows given the winter ration and about 40 mV in cows given the herbage ration for all six cows. If a Mg ion concentration in blood is assumed to be 1.2 meq/litre it can be calculated from the formula of Nernst that for passive absorption of Mg from the reticulo-rumen the concentration of Mg ions in the rumen fluid would have to exceed 8 meq/litre in the rumen fluid in Exp. I and 16 meq/litre in Exp. III. The concentrations of Mg in rumen fluid on the day following the end of the duodenal collection period (rumen fluid samples taken at four different times between the morning and evening feed) were found to be lower than those necessary for a passive transport of Mg, except in the cows receiving extra Mg in Exp. I. On a few occasions the levels reached 16 meq/litre in these cows.

For a passive transport of Mg from the omasum to blood, even higher concentrations of Mg ions would be necessary, since the potential difference between blood and omasal contents was found to be about 20% greater than the difference between blood and rumen contents. This raises the question of the mechanisms underlying the absorption process of Mg from the forestomachs.

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