Studies on the flow of zinc, cobalt, copper and manganese along the digestive tract of sheep given fresh perennial ryegrass, or white or red clover

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1. Sheep fitted with a rumen fistula and either a re-entrant cannula at the proximal duodenum or a re-entrant cannula at the terminal ileum were given twice daily 480–520 g dry matter as fresh perennial ryegrass, or white or red clover. Flows of digesta were corrected to 100 % recovery of chromic oxide.

2. The quantities (g/24 h) of zinc and cobalt leaving the stomach were significantly greater than those in the food. No significant change was found in the quantities of copper and manganese. Significantly smaller quantities of Co (all three diets) and Zn (all diets except red clover) left the small intestine than those which entered this region. No significant differences in the quantities of Cu and Mn entering and leaving the small intestine were found. Significantly smaller quantities of Zn, Co, Cu and Mn were excreted in the faeces than entered the large intestine.

3. From the flow results it was determined that there was a significant net secretion of Zn and Co in the stomach, and a significant net absorption of Zn (except with the red-clover diet) and Co from the small intestine, and of Zn, Co, Cu and Mn from the large intestine.

The importance of trace elements, and their characteristic signs of deficiency in the ruminant, are well documented but more information is needed about their metabolism (Underwood, 1971). Although it is known that the apparent availability of many micro-elements is low and that dietary factors can influence the absorption of some, there is not sufficient information about their passage along the digestive tract and their sites of absorption or secretion.

In this study the flows of zinc, cobalt, copper and manganese along the digestive tract were determined using sheep equipped with re-entrant cannulas. Values obtained for flows were corrected to mean 24 h flows (MacRae & Armstrong, 1969) to determine the quantities of the micro-elements entering and leaving the stomach, small and large intestine.

EXPERIMENTAL

Sheep

Eight castrated male Romney Marsh sheep (1·5–2·5 years old) weighing 37–42 kg were equipped with rumen fistulas; four of these animals were fitted with a re-entrant cannula at the proximal duodenum (50 mm beyond the pylorus) and the other four were fitted with a re-entrant cannula at the terminal ileum (400–500 mm from the ileo-caecal valve) (Brown, Armstrong & MacRae, 1968). All sheep were housed indoors in wooden metabolism crates fitted with stainless-steel grate floors, wooden

food boxes and plastic water buckets. Distilled water was freely available for drinking. Care was taken during the experiments to avoid contamination of samples with micro-elements from the surroundings, and distilled water was used for rinsing the food and water containers.

Diet and feeding procedure

The three pasture species studied were: *Lolium perenne* L., 'Grasslands Ruanui' perennial ryegrass; *Trifolium repens* L., 'Grasslands 4700' white clover; *Trifolium pratense* L., 'Grasslands Hamua' red clover. Pure stands had been established for several years and were maintained by annual dressings of 200 kg potassium super-phosphate/ha. The perennial ryegrass received, in addition, 200 kg ammonium sulphate/ha. Weeds in the clover were controlled using 'Tropotox' MCPB (May and Baker Ltd, Dagenham, Essex) at the rate of 3–4 l/ha and the clover in the perennial ryegrass was controlled using 'Gramoxone' 24-D (ICI (New Zealand) Ltd, Auckland, New Zealand), 1·5 l/ha. During the experiment the perennial ryegrass and white clover were cut at a height of 80–140 mm and the red clover was cut at a height of 200–300 mm. Harvesting was at 08.00 hours daily and after a thorough mixing, a sample was taken for immediate determination of dry matter (Ulyatt & MacRae, 1974) to estimate the amount of fresh pasture required to give dry-matter intakes ranging from 480 to 520 g/d. The calculated amount of fresh food was weighed, divided into two equal portions and each portion was given at 09.00 and 16.30 hours. A second sample was taken and dried at 105° for 24 h to determine accurately the dry-matter content, and the dry-matter intake of the sheep.

The experiment lasted 9 months: the perennial ryegrass was harvested during April and May, the white clover during June and July and the red clover during October and November.

Experimental design and sampling procedures

A summary of the experimental design in terms of diet fed, numbers of sheep, collection dates and number of samples collected is shown in Table 1.

After a preliminary feeding period of 3–4 weeks there was a 9 d balance period in which collections of urine and faeces were made. The daily output of urine was recorded and portions (10 ml/l) were bulked to give one sample/sheep per collection period. The daily faecal output was measured and samples were taken for dry-matter determinations and for chemical analysis. For 3 d periods all daily faecal samples were bulked to give three faecal samples/sheep per collection period. The fresh pasture was sampled throughout the experiment; samples for each species were bulked every 3 d, to give three samples for the 9 d balance period and six samples for the subsequent 2–3 weeks of 24 h collection periods, that is, a total of nine samples/diet.

In the 2 or 3 weeks after the 9 d balance period two, three or four 24 h collections/sheep (i.e. twelve–sixteen collections/diet) of duodenal and ileal digesta were made using the techniques of MacRae & Armstrong (1969) and Ulyatt & MacRae (1974). The re-entrant cannulas were disconnected and all the digesta leaving the proximal cannula were collected, weighed and sampled about every 2 h (Beever, Thomson,

Table 1. *Design of the experiment showing diet fed, number of sheep, collection dates and number of samples collected*

No. of samples collected

Diet		Perennial ryegrass				White clover				Red clover			
Collection period		3–11 May		15 May–7 June		1–9 July		10–30 July		24 Oct.–1 Nov.		3–27 Nov.	
Sample		Urine	Faeces	Duodenal	Ileal	Urine	Faeces	Duodenal	Ileal	Urine	Faeces	Duodenal	Ileal
Sheep no.	Position of cannula												
441	Duodenal re-entrant (50 mm beyond the pylorus)	1	3	4	—	1	3	4	—	1	3	3	—
647		1	3	4	—	1	3	2	—	1	3	4	—
731		1	3	4	—	1	3	2	—	1	3	3	—
786		1	3	4	—	1	3	4	—	1	3	4	—
1102	Ileal re-entrant (400–500 mm from the ileo-caecal valve)	1	3	—	4	1	3	—	2	1	3	—	3
1054		1	3	—	3	1	3	—	2	1	3	—	3
1035		1	3	—	3	1	3	—	4	1	3	—	3
1038		1	3	—	3	1	3	—	4	1	3	—	3

Pfeffer & Armstrong, 1971). The amount of sample taken for analysis was 14 and 21 % of the duodenal and ileal digesta respectively. After sampling, material collected in the preliminary feeding period was added to the remaining digesta to make them up to the original collected weight. The digesta were then warmed to 40° and re-introduced into the distal cannula at a rate approximating to the flow of digesta from the proximal cannula. All samples of food, digesta, faeces and urine were stored at $-10°$. Chromic oxide, which was used as an inert marker to correct the values obtained for 24 h flow of digesta to mean 24 h flows (MacRae & Armstrong, 1969), was placed in the rumen, through the rumen fistula, as Cr_2O_3-impregnated paper pellets (3 g/sheep) twice daily, prior to feeding.

Analytical methods

Samples of food, duodenal and ileal digesta and faeces were freeze-dried and ground, using a porcelain mortar and pestle, before analysis. Sub-samples (1 g for Zn, Cu and Mn determinations; 5 g for Co determination) were ashed at 500° for 6–8 h, treated with 15 M-HNO_3 and re-ashed if necessary to destroy all organic matter, before extracting the ash using 10 ml 2 M-HCl. The urine samples (25 ml for Zn, Cu and Mn determinations; 60 ml for Co determination) were dried and digested using an acid mixture (16 M-HNO_3–18 M-H_2SO_4–9 M-$HClO_4$ (10:2:4, by vol.)). Analytical grade chemicals were used to prepare all reagents and all glassware was acid-washed and thoroughly rinsed in glass-distilled water. All samples were diluted, and Zn, Cu and Co contents were determined by atomic absorption spectrophotometry. In the instance of Co, the method of additions (Perkin-Elmer, 1971) was used to eliminate matrix effects resulting from the high concentration of solids in the samples. As the urine samples contained high levels of K, KCl was added to the standards used in the determination of micro-element contents for the urine. The chromium content of samples was determined by atomic absorption spectrophotometry using the method of Williams, David & Iismaa (1962). The titanium content of the pasture samples, which indicated the amount of soil contamination (Field & Purves, 1964), was determined by X-ray fluorescence using the method described by Healy (1968).

Statistical analysis

All results from each sheep, for each collection period, were used to calculate the mean flow rates of micro-elements with their standard errors (Table 3) for each diet, at each sampling site. The significance of the difference between the flow of a micro-element entering and leaving a region of the digestive tract was determined using a t test.

RESULTS

The average micro-element composition for the pasture species used in this study are shown in Table 2.

The levels of Zn, Co and Cu were similar for all plants but the Mn content of the grass was about twice that for the clovers. The Cr_2O_3-impregnated paper contained (μg/g) 8 Zn, 0·072 Co, 1·9 Cu, 18 Mn. From Ti determinations the extent of the soil

Table 2. *The micro-element composition (mg/kg) for fresh perennial ryegrass,*
white clover and red clover given to sheep

(Mean values for nine samples/diet, three samples taken during the 9 d balance period and
six samples taken during the 2–3 week period of 24 h digesta collection)

	Zinc	Cobalt	Copper	Manganese
Perennial ryegrass	25	0·41	8·8	75
White clover	28	0·53	9·1	34
Red clover	31	0·48	11·0	39

contamination for the perennial ryegrass, white clover and red clover was estimated to be 0·8, 1·4 and 0·25 % respectively.

For perennial ryegrass, white clover and red clover respectively the mean ($\pm$ SE) daily dry-matter intakes (g/24 h) were 480 (11), 489 (15), 520 (13); the corrected mean ($\pm$ SE) 24 h dry-matter flows (g/24 h) were: at the duodenal cannulas, 293 (20), 276 (12), 356 (19); at the ileal cannulas, 181 (7), 167 (7), 259 (12); the mean ($\pm$ SE) quantities of dry matter (g/24 h) excreted in the faeces of the sheep given these diets were: 118 (13·2), 92 (6·5), 127 (11·2). The recoveries of Cr_2O_3 in the faeces, for all sheep, irrespective of diet, ranged from 97 to 102 %.

From the dry-matter flows along the digestive tract and the micro-element concentration in the digesta and faeces, the flows of these elements along the digestive tract were determined. The Zn, Co, Cu and Mn contents for the diets, for digesta entering and leaving the small intestine, and the amounts excreted in the faeces are shown in Table 3.

For all sheep, irrespective of diet, the quantities of Zn and Co leaving the stomach region were significantly greater ($P < 0·05$) than those in the food. There were no significant differences between the quantities of Cu and of Mn entering and leaving the stomach region.

For all diets the quantities of Co entering the small intestine were significantly greater ($P < 0·05$) than those leaving the small intestine, but there were no significant differences between the quantities of Cu or Mn entering and leaving the small intestine. For sheep fed on red clover there was also no difference between the quantities of Zn entering and leaving the small intestine. However, for sheep fed on perennial ryegrass and white clover the quantities of Zn entering the small intestine were significantly greater ($P < 0·05$) than those leaving the small intestine. The amounts of Zn, Co, Cu and Mn excreted in the faeces were significantly less ($P < 0·05$) than those leaving the small intestine.

The sites of net absorption and net secretion of Zn, Co, Cu and Mn, as determined from their flows along the digestive tract, are shown in Fig. 1.

There was a net secretion of Zn and Co into the stomach region and a net absorption of Zn, Co, Cu and Mn from the large intestine for all sheep, irrespective of diet. A small net absorption of Zn from the small intestine was found for sheep given perennial ryegrass, and for those given white clover; there was a greater net absorption of Co from this region for all sheep.

Table 3. *The quantities (mg/24 h) of zinc, cobalt, copper and manganese in the diet, entering and leaving the small intestine, excreted in the faeces and urine, and apparently retained, for sheep†* given fresh perennial ryegrass (PR), white clover (WC) or red clover (RC)

(Mean values with their standard errors for no. of samples given in parentheses)

	Zn						Co					
	PR		WC		RC		PR		WC		RC	
Sample‡	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Diet	11·8	0·12 (9) **	12·7	0·38 (9) *	16·3	0·37 (9) **	0·20	0·005 **	0·24	0·006 *	0·25	0·006 *
Digesta												
Entering duodenum	18·8	1·38 (16) *	14·0	0·62 (12) *	21·5	1·24 (14) NS	0·32	0·032 **	0·30	0·026 *	0·31	0·021 *
Leaving ileum	14·7	0·57 (13) ***	12·4	0·57 (12) *	20·6	1·14 (12) **	0·14	0·019 *	0·19	0·042 *	0·24	0·019 **
Faeces	10·2	0·75 (24)	10·8	0·43 (24)	15·7	0·19 (24)	0·07	0·011	0·07	0·017	0·09	0·025
Urine	0·9	0·16 (8)	0·7	0·09 (8)	1·0	0·19 (8)	0·05	0·017	0·08	0·024	0·08	0·032
Apparent retention	0·7	0·30	1·2	0·43	−0·4	0·32	0·08	0·007	0·04	0·014	0·08	0·033
Apparent availability (%)	13		15		4		65		70		66	

	Cu						Mn					
	PR		WC		RC		PR		WC		RC	
Sample	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Diet	4·0	0·07 NS	4·0	0·09 NS	5·8	0·15 NS	34·6	0·71 NS	14·9	0·32 NS	18·8	1·30 NS
Digesta												
Entering duodenum	4·1	0·13 NS	4·2	0·28 NS	6·3	0·42 NS	37·5	4·71 NS	16·1	1·15 NS	21·4	1·47 NS
Leaving ileum	4·2	0·14 **	4·1	0·26 **	6·5	0·34 *	40·2	1·99 **	18·2	1·06 **	24·7	1·67 **
Faeces	2·9	0·15	2·7	0·11	5·3	0·18	32·8	1·40	12·6	0·55	18·4	0·41
Urine	0·1	0·05	0·2	0·05	0·1	0·04	0·3	0·19	0·1	0·04	0·1	0·05
Apparent retention	1·0	0·32	1·1	0·13	0·4	0·07	1·5	0·56	2·2	0·67	0·3	0·12
Apparent availability (%)	30		34		9		5		15		2	

NS, not significant.

For each micro-element, the differences between mean values for the amounts in the diet and those entering the duodenum and those leaving the ileum, the amounts leaving the ileum and those in the faeces, were significant: * $P < 0.05$, ** $P < 0.01$.

† Eight sheep received each of the diets during the experimental period (for details, see Table 1); four sheep were fitted with duodenal re-entrant cannulas (50 mm beyond the pylorus) and four sheep were fitted with ileal re-entrant cannulas (400–500 mm from the ileo-caecal valve).
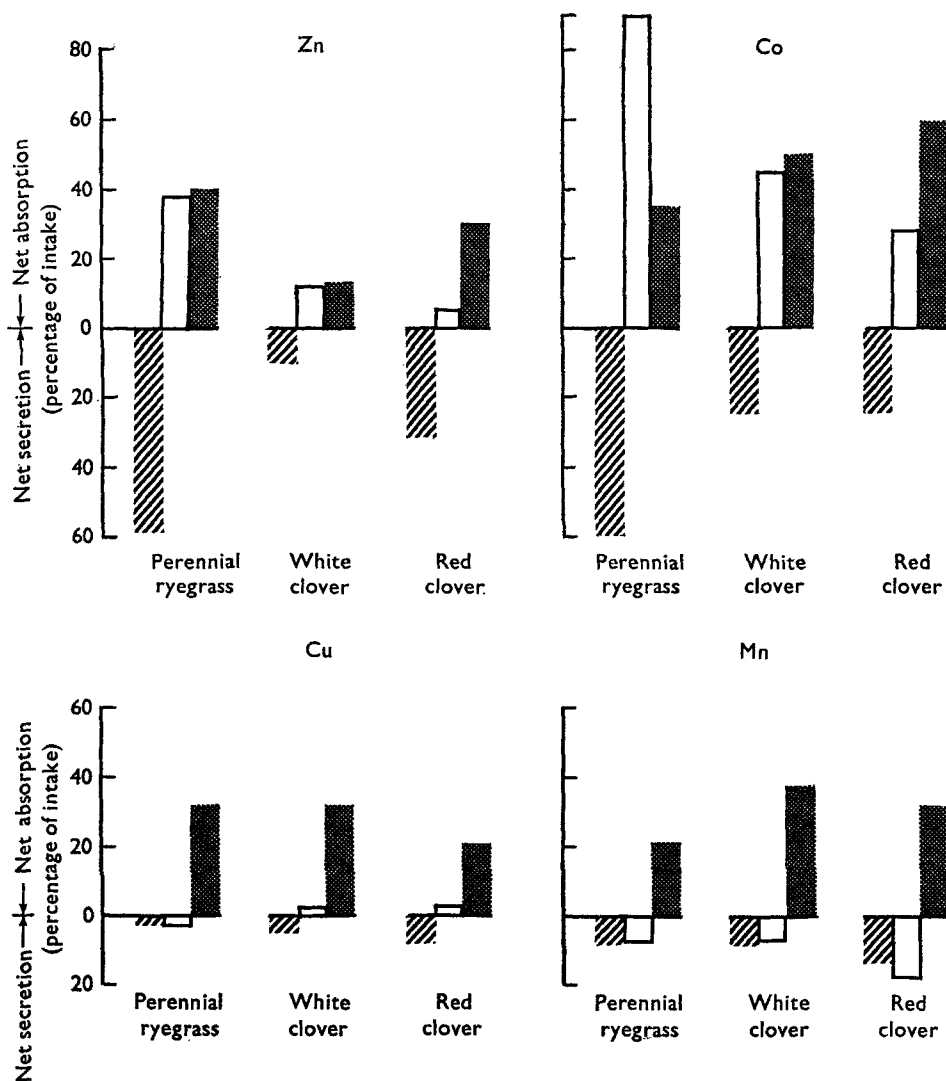
‡ For details, see p. 74 and Table 1.

Fig. 1. The sites of net secretion and net absorption of zinc, cobalt, copper and manganese, as determined from their flows along the digestive tract (amounts for each micro-element expressed as a percentage of intake), for sheep given fresh perennial ryegrass, white clover or red clover. Eight sheep received each of the diets during the 9-month experimental period (for details of experimental design, see Table 1); four sheep were fitted with duodenal re-entrant cannulas (50 mm beyond the pylorus) and four sheep were fitted with ileal re-entrant cannulas (400–500 mm from the ileo-caecal valve). ▨, Stomach; □, small intestine; ▦, large intestine.

The results of the balance experiment (Table 3) indicated that there was a positive apparent retention of Co, Cu and Mn for all sheep, and of Zn for sheep given perennial ryegrass or white clover. The apparent availability of Co was high (average value for the three diets 67 %) compared with that for Zn and for Mn (less than 15 %). The apparent availability of Cu was 30 and 36 % for perennial ryegrass and white clover respectively and 9 % for red clover. Except for Co, the faeces were the main pathway

for the excretion of the micro-elements, as the faecal losses relative to intake ranged from 66 to 97 %, and urinary losses were less than 8 %. The losses of Co in the faeces and urine were similar (26–35 % of intake).

<div align="center">DISCUSSION</div>

The amounts of Zn, Cu and Mn found in perennial ryegrass, white clover and red clover were similar to those previously reported (Fleming, 1965). The high values obtained for Co reflect some soil contamination (less than $1\cdot5$ %) of the plant material during harvesting. However, in this study the Co contribution from the soil cannot be regarded as a contaminant. Under New Zealand farming conditions the grazing sheep can ingest more than 25 kg soil annually (Healy, 1972), and Rigg & Askew (1934) have reported that soil Co is available to the sheep; the oral administration of soil to animals given Co-deficient diets was found to prevent bush sickness. Although the availability of the Co in the soil ingested during this study was unknown, about 80 % of the total Co intake was of plant origin. The Cr_2O_3-impregnated paper did contain trace amounts of Zn, Co, Cu and Mn but when these were compared with the dietary intakes the contamination from this source was less than $0\cdot5$ % for Zn, Co and Cu, and less than 1 % for Mn.

The assumptions made, and the validity of using a marker to correct the values obtained for flow rates of digesta to mean 24 h flows, has been discussed fully elsewhere (MacRae & Armstrong, 1969; Grace & MacRae, 1972; Ulyatt & MacRae, 1974). Also, for most micro-elements, the net absorption or net secretion is represented by a small difference between the quantities entering and leaving the region under study, but in the instance of the dietary organic constituents which are readily digested, a net or apparent absorption is represented by a large difference between the quantities entering and leaving the absorptive region. The approach in this study was therefore to make a large number of measurements (three diets, twelve–sixteen collections/diet) for the flow of Zn, Co, Cu and Mn along the digestive tract, and, therefore, obtain reasonable estimates of the net movements of these elements across the wall of the digestive tract.

Diet was not found to influence the pattern for the net movements of the micro-elements across the walls of the digestive tract. A net secretion of Zn and Co into the stomach region was found. Although for Co there have been no previous reports of secretion, for Zn there have been reported secretion into the rumen, the transfer taking place through the rumen epithelium (Watson & Kastelic, 1967) as well as in the saliva (Hiers, Miller & Blackmon, 1968). Net absorption of Zn and Co took place from the small intestine. This is in agreement with the results of earlier studies of Hiers *et al.* (1968) for Zn, and of Hedrich, Elliot & Lowe (1973) for cyanocobalamin or Co, as considerable quantities of dietary Co are used in the synthesis of cyanocobalamin (Hedrich *et al.* 1973). As considerable quantities of Cu (Van Ravesteyn, 1944) and Mn enter the small intestine in the bile, as well as through the intestinal wall in the instance of Mn (Bertinchamps, Miller & Cotzias, 1966), it is suggested that in this study the quantities of Cu and Mn entering the small intestine in various endo-

genous secretions may have been similar to the amounts being absorbed and therefore a significant net absorption of Cu and Mn would not have been found. In view of this it is interesting that in man it has been reported that of the 2–5 mg Cu ingested, 0·6–1·6 mg is absorbed while 0·5–1·3 mg is excreted in the bile (Cartwright & Wintrobe, 1964). Further, since Zn is secreted into the small intestine in the pancreatic juice, bile and other secretions (Sheline, Chaikoff, Jones & Montomery, 1943; Pekas, 1966), the actual absorption of Zn from the small intestine would be greater than the net absorption found in this study.

Although it could be concluded from the results of earlier studies, mainly with simple-stomached animals, that the small intestine was the main site of absorption and endogenous secretion of the micro-elements (O'Dell & Campbell, 1970), in the present study a considerable net absorption of Zn, Co, Cu and Mn was found to take place in the large intestine. While it is well documented that the absorption of water and minerals, particularly sodium (Kay & Pfeffer, 1970), takes place in the large intestine, further studies will be required to substantiate the above findings.

As previously reported (Underwood, 1971), it was also found that most (66–97 %) of the ingested Zn, Cu and Mn was excreted in the faeces with small amounts (less than 8 %) being excreted in the urine of sheep. However, in the instance of Co about equal quantities were excreted in the faeces and urine. This is in contrast to the results of Comar, Davis & Taylor (1946) with cattle, where most of the Co was found to be excreted in the faeces. In humans the main pathway for Co excretion is not clear: Harp & Scoular (1952) found that for women most of the Co was excreted in the urine but for girls, Engel, Price & Miller (1967) found that the faeces were more important than the urine as a pathway for Co excretion. The apparent availability, which is the net absorption of the element from the digestive tract expressed as a percentage of the intake, may give only limited information about the extent of the net absorption or net secretion of some micro-elements within various regions. For example, in this study, a considerable net secretion of Zn and Co into the stomach region was followed by a greater net absorption from the intestine region and therefore the quantities of the above micro-elements moving across the wall of the digestive tract were far greater than was reflected by the apparent availability or total net absorption from the digestive tract.

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