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Absorption and secretion of ^{65}Zn in the stomach and intestinal tract of sheep exchanging digesta via duodenal re-entrant cannulas*

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1. The absorption and secretion of ^{65}Zn in the stomach and intestinal tract regions was studied in sets of two and three sheep which were exchanging digesta via re-entrant cannulas in the proximal duodenum. One sheep from each of the three three-sheep sets was dosed intraruminally with the radioisotope. One sheep from the three two-sheep sets received an intravenous dose.

2. Measurements of ^{65}Zn in blood plasma from intraruminally dosed sheep showed that there was no apparent absorption from the stomach region. Measurements from sheep receiving radioactive digesta intraduodenally showed that mean apparent absorption of ^{65}Zn was 0·07 and mean true absorption was 0·103. There was a large variation in endogenous recycling of ^{65}Zn into the stomach region.

3. Secretion of ^{65}Zn into the stomach and intestinal regions in the intravenously dosed sheep of the two-sheep sets was calculated on the basis of total recovery over 10 d in the digesta and faeces. The present study showed that for every 1 molecule Zn secreted into the stomach region, 2·1 molecules were secreted into the intestinal tract region.

Several studies of the movement of zinc in the gastrointestinal tract have been carried out using ruminant animals equipped with intestinal cannulas (Grace, 1975; Bertoni *et al.*, 1976; Stevenson & Unsworth, 1978; Ivan *et al.* 1979, 1983). Many of these studies demonstrated a secretion of Zn into the stomach. Several other studies of endogenous secretion of Zn in ruminants were also carried out (Miller *et al.* 1966; Weston & Kastelic, 1967; Stake *et al.* 1974; Grace & Gooden, 1980) but the proportions of endogenous Zn secreted into the stomach and the intestinal tract regions were not determined. Thus the contribution made by the stomach in ruminants to the absorption of Zn is not clear.

Innovative techniques of digesta transfer at the proximal duodenum between two or three sheep in conjunction with automatic digesta samplers were used in the present study. The results showed no active absorption of ^{65}Zn from the stomach region and a ratio of 1:2·1 for endogenous secretion of ^{65}Zn into the stomach and the intestinal tract regions.

EXPERIMENTAL

Animals and diet

Fifteen castrated crossbred sheep weighing 42–49 kg were used. Surgery was performed under general anaesthesia at least 3 months before measurements were started. A re-entrant cannula (Ivan & Johnston, 1981) was placed in the proximal duodenum. At 14 d before the initiation of measurements, the sheep were transferred into plastic metabolism cages (Ivan & Hidiroglou, 1980) and were given 125 g hay and 25 g pelleted concentrate every 3 h. The concentrate consisted of (g/kg): ground barley 970, limestone 10, dicalcium phosphate 10, sodium chloride–micromineral–vitamin mixture 1·0. The mixed diet contained 83 μg Zn/g dry matter. The sheep had continuous access to drinking water.

Experimental procedures

Expt 1. The proportions of endogenous ^{65}Zn that were secreted into the stomach and intestinal tract regions were measured in this experiment. Two sheep (A, B) of similar body-weight were interconnected via re-entrant cannulas and two (1, 2) automatic digesta

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samplers (Fig. 1). The samplers have been described previously (Ivan *et al.* 1985). The digesta flowing from the stomach of sheep A was collected and sampled in sampler 1 and returned to the proximal duodenum of sheep B. Correspondingly, the digesta flowing from the stomach of sheep B was sampled in sampler 2 and returned to the duodenum of sheep A. All samplers were programmed to accumulate a subsample of 13–16% of the total digesta for each 4 h period. Digesta from a third (donor) sheep was automatically added into the returning digesta to compensate for the volume sampled. Digesta was warmed to 40° before being transferred by the sampler into a digesta return system positioned above the digesta-receiving sheep, and finally allowed to enter the duodenum under gravity.

At 1 d after the interconnection of the sheep and samplers, sheep A was dosed intravenously with a sterile solution of 1 mCi carrier-free ^{65}Zn as zinc chloride (New England Nuclear, Lachine, Quebec) in 10 ml saline (9 g sodium chloride/l). The timers on both samplers were adjusted to zero time and collections of faeces and urine were initiated in both A and B sheep. Faeces and urine were sampled every 24 h. Blood samples (10 ml) were obtained from the jugular vein of each sheep at 0·5, 1, 2, 4, 6, 12 and 24 h on the 1st day after dosing, every 12 h on the 2nd day and once daily thereafter. The plasma was separated from the cellular constituents by centrifugation at 600 *g* for 20 min. The experiment lasted for 10 d. It was later repeated twice, each time with two new sheep of similar body-weight (three replicates).

Expt 2. The absorption of ^{65}Zn from the stomach and intestinal tract regions was measured in this experiment. The experimental procedure was the same as for Expt 1 except three sheep (A, B, C) and three digesta samplers (1, 2, 3) were used (Fig. 1). Thus, the digesta flowing from the stomach of sheep A was sampled in sampler 1 and returned to the duodenum of sheep B. Correspondingly, the digesta from sheep B was sampled in sampler 2 and returned to sheep C, and that of sheep C was sampled in sampler 3 and returned to sheep A. Sheep A was dosed intraruminally via a stomach tube with a solution of 1 mCi ^{65}Zn (as zinc chloride) in 50 ml saline. The tube was rinsed with 50 ml saline. The experiment was repeated twice (three replicates) as per Expt 1.

Analytical procedures

Triplicate samples of faeces, urine, digesta (flowing from the stomach of one sheep and entering the proximal duodeum of other sheep) and blood plasma were counted for ^{65}Zn in a Beckman 300 gamma counter together with a ^{65}Zn standard, background vials, and three working standards containing a known quantity of the ^{65}Zn solution which was used for dosing the sheep. The counts were converted into disintegrations/min. The efficiency of counting was between 18·34 and 18·46%.

Analysis of the Zn in the diet and in samples of freeze-dried digesta was performed using a Perkin-Elmer atomic absorption spectrophotometer (model 460) following nitric acid–perchloric acid digestion.

Calculations

$$\text{Apparent absorption} = \frac{{}^{65}\text{Zn in digesta entering the duodenum} - {}^{65}\text{Zn in faeces}}{{}^{65}\text{Zn in digesta entering the duodenum}}.$$

Secretion into the intestinal tract (S) = ^{65}Zn recovery in digesta flowing from the stomach × 2·1. (For every 1 molecule Zn secreted into the stomach region over 10 d, 2·1 molecules were secreted into the intestinal tract.)

$$\text{True absorption} = \frac{{}^{65}\text{Zn in digesta entering the duodenum} - ({}^{65}\text{Zn in faeces} + \text{S})}{{}^{65}\text{Zn in digesta entering the duodenum}}.$$

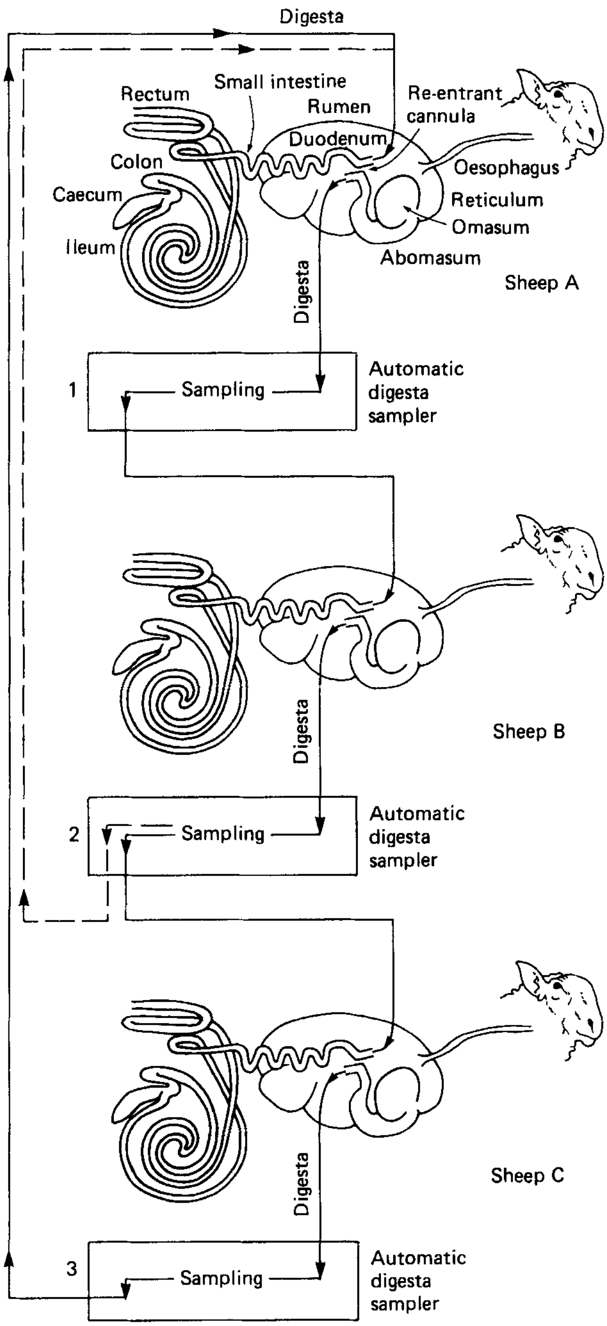

Fig. 1. Diagram of exchange of digesta betwen two (– – –) or three (——) sheep interconnected via duodenal re-entrant cannulas and automatic digesta samplers. Sheep A was dosed with ^{65}Zn intravenously (two-sheep system; Expt 1) or intraruminally (three-sheep system; Expt 2).

Table 1. *Expt 1. Cumulative percentage recovery of radioactivity in the digesta flowing from the stomach, and in faeces and urine of two sheep interconnected via duodenal re-entrant cannulas*

(Mean values with their standard errors for three sheep; A sheep were dosed intravenously with 1 mCi ^{65}Zn)

Time interval after dosing (h)	Digesta		Faeces				Urine			
	A sheep		A sheep		B sheep		A sheep		B sheep*	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
24	0·3	0·04	1·7	0·68	0·6	0·29	0·06	0·019	0·01	0·007
48	1·0	0·18	5·6	1·33	4·1	1·27	0·09	0·030	0·03	0·021
72	2·2	0·37	8·2	1·48	11·9	1·70	0·11	0·035	0·05	0·032
96	3·3	0·66	9·6	1·85	26·7	0·70	0·13	0·040	0·06	0·045
120	4·3	0·74	11·1	1·79	40·7	1·69	0·14	0·041	0·07	0·051
144	5·1	0·92	12·7	1·91	51·1	2·01	0·16	0·043	0·09	0·063
168	5·8	1·02	13·8	1·06	59·5	2·74	0·17	0·044	0·12	0·079
192	6·7	1·05	14·8	2·35	66·3	1·41	0·18	0·044	0·13	0·087
216	7·3	1·07	15·8	2·43	73·5	2·40	0·20	0·040	0·14	0·091
240	7·7	1·11	16·5	2·62	88·4	4·18	0·21	0·040	0·16	0·101

* Percentage of the radioactivity received in the digesta from A sheep.

RESULTS

Expt 1. The cumulative percentage recovery of the radioactivity in the digesta flowing from the stomach and in the faeces and urine of the A and B sheep is summarized in Table 1. In the A sheep, a total of 24·5% of injected radioactivity was recovered over the 10 d period, comprising 7·7% in the digesta, 16·5% in faeces and 0·21% in urine. Of the radioactivity entering the intestinal tract of the B sheep, 88·4% was excreted in faeces and 0·16% in urine. The amount of radioactivity detected in the digesta flowing from the stomach of the B sheep was negligible.

In the A sheep, the percentage of radioactivity recovered in the digesta flowing from the stomach over the 10 d period comprised 32% of that accumulated in both digesta and faeces (Fig. 2). The ratio of cumulative ^{65}Zn excretion in faeces:digesta declined from 6·1 on the 1st day to 2·1 on day 8. After day 8 the ratio remained constant at 2·1. Therefore, over the 10 d period, for every 1 molecule Zn secreted into the region, 2·1 molecules were secreted into the intestinal tract region. The 2·1 ratio = ^{65}Zn excretion in faeces: ^{65}Zn in digesta leaving the stomach, and is based on cumulative ^{65}Zn recovery over the 10 d period.

Expt 2. The mean percentage recovery of the radioactivity in the digesta from the A sheep for the 10 d period was 98·2 (SE 3·47). With the 0·6% recovery of radioactivity in the stomach of these sheep (Table 2) the total mean recovery was 98·8%.

The highest average flow of ^{65}Zn from the stomach of the A sheep was found during the 1st day following dosing (Fig. 3), with 50% of the radioactivity leaving the stomach within 48 h. There was a rapid increase in the specific activity which indicated proportional mixing of the radioisotope with the stable Zn in the digesta immediately after dosing (measured in the first set of sheep only), reaching peak values between 8 and 10 h and rapidly declining thereafter (Fig. 4). Both the quantity of the radioactivity (Fig. 3) and the specific activity in the digesta (Fig. 4) were very small after 7 d. No significant amounts of radioactivity were detected in the plasma, urine or faecal samples of the A sheep.

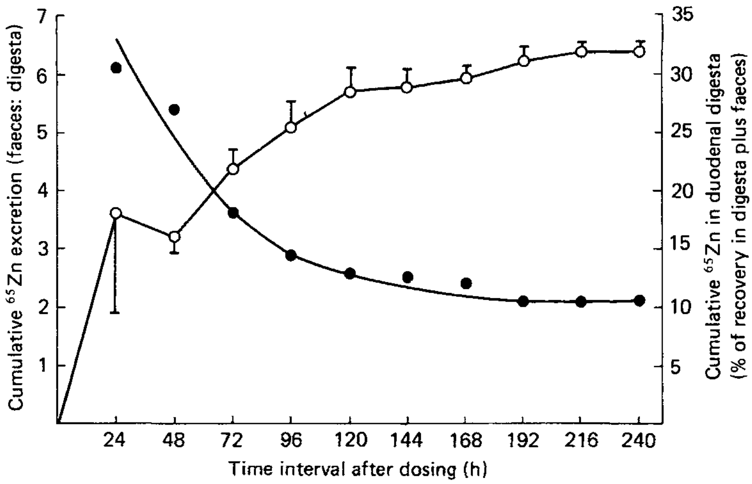

Fig. 2. Expt 1. Ratio of faeces: digesta (●) of cumulative ^{65}Zn excretion and cumulative ^{65}Zn recovery (○) in duodenal digesta flowing from the stomach of A sheep. Each A sheep was dosed intravenously with 1 mCi ^{65}Zn. Points are mean values of three sheep with their standard errors represented by vertical bars. For illustration of exchange of digesta between two sheep, see Fig. 1.

Table 2. *Expt 2. Recovery of ^{65}Zn (% of dose) in the stomach contents and the wall of the reticulo-rumen, omasum and abomasum of A sheep killed 10 d after intraruminal dosing with ^{65}Zn*

(Mean values with their standard errors for three sheep)

	Mean	SE
Stomach contents	0·528	0·1141
Reticulo-rumen wall	0·017	0·0075
Omasal wall	0·003	0·0012
Abomasal wall	0·003	0·0010
Total stomach recovery	0·551	0·1045

^{65}Zn excretion in faeces of the B sheep (Fig. 5) showed a similar pattern to that for ^{65}Zn in the digesta flowing into these sheep (Fig. 3), except the peak excretion appeared 1 d later and 50% of the radioactivity was excreted within 72 h. The peak concentration of ^{65}Zn in the plasma (Fig. 6) of the B sheep appeared at 36 h after dosing, indicating that the highest absorption of the radiozinc in the B sheep occurred during the 2nd day after dosing of the A sheep.

The absorption and secretion of ^{65}Zn in the B and C sheep are summarized in Table 3. The intestinal absorption obtained in these sheep represents the absorption from the entire intestinal tract, starting from the re-entrant cannulas placed in the proximal duodenum. The 1·6% of radioactivity recovered in the digesta flowing from the stomach of the B sheep and subsequently entering the duodenum of the C sheep, originated from the absorbed ^{65}Zn which was subsequently recycled within the B sheep. Therefore, the apparent absorption of 0·129 in the C sheep represents an intestinal reabsorption of endogenous ^{65}Zn that was recycled into the stomach of the B sheep. The mean apparent reabsorption was approximately double the original apparent absorption of 0·07 measured in the B sheep.

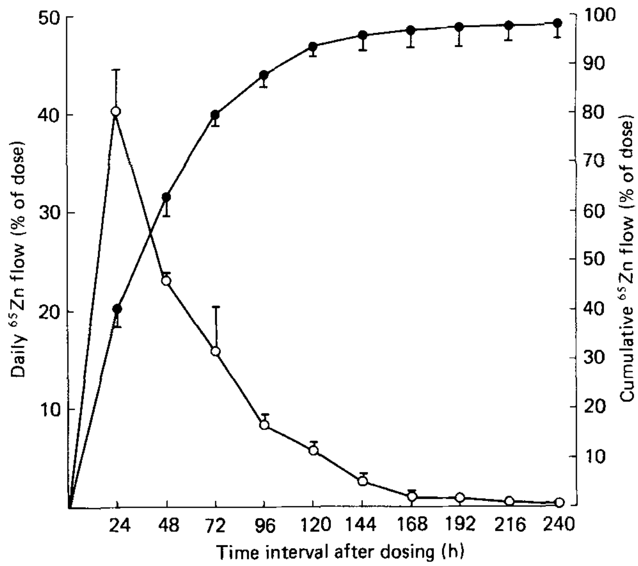

Fig. 3. Expt. 2. Daily (○) and cumulative (●) flow of ^{65}Zn in the digesta flowing from the stomach of A sheep which were each dosed intraruminally with 1 mCi ^{65}Zn. Points are mean values of three sheep with their standard errors represented by vertical bars. For illustration of digesta transfer among set of three sheep, see Fig. 1.

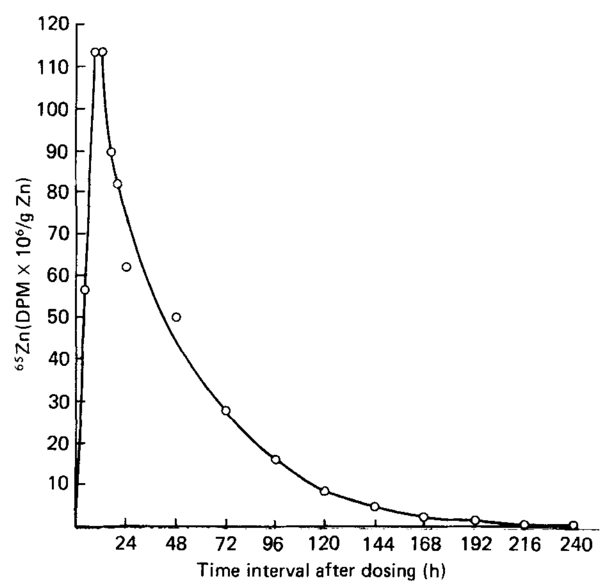

Fig. 4. Expt 2. Specific activity of ^{65}Zn (disintegrations/min (DPM) $\times 10^6$/g Zn) in the digesta flowing from the stomach of A sheep in the first replicate. The sheep was dosed intraruminally with 1 mCi ^{65}Zn. For illustration of digesta transfer among set of three sheep, see Fig. 1.

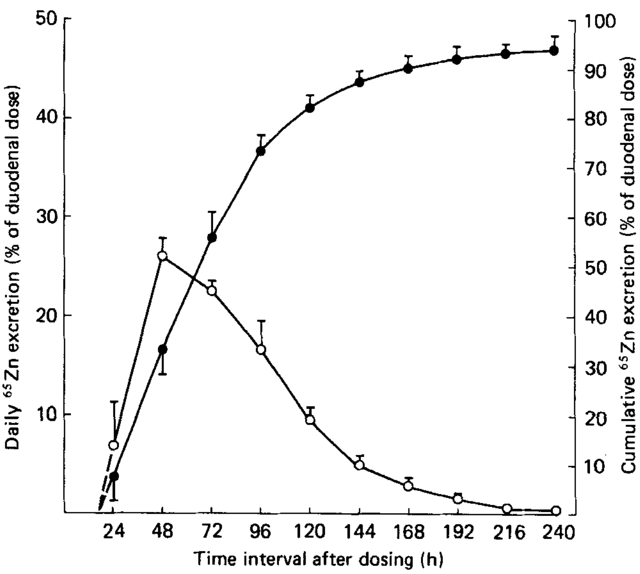

Fig. 5. Expt 2. Daily (○) and cumulative (●) ^{65}Zn excretion in faeces of B sheep. Points are mean values of three sheep with their standard errors represented by vertical bars. The duodenal dose is the amount of radioactivity entering the duodenum of B sheep in the digesta flowing from the stomach of A sheep during 10 d after intraruminal dosing of A sheep with 1 mCi ^{65}Zn. For illustration of digesta transfer among set of three sheep, see Fig. 1.

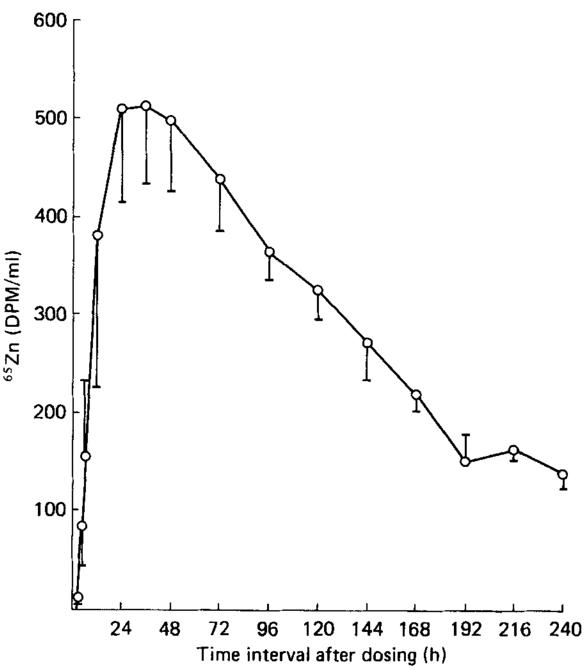

Fig. 6. Expt 2. Concentration of radioactivity in the plasma of B sheep (disintegrations/min (DPM) per ml per 10^6 DPM dosed) following the intraruminal dosing of each A sheep with 1 mCi ^{65}Zn. Points are mean values of three sheep with their standard errors represented by vertical bars. For illustration of digesta transfer among set of three sheep, see Fig. 1.

Table 3. *Expt 2. Absorption and secretion (proportion of duodenal dose)* of radiozinc in B and C sheep of three sets, each consisting of three sheep (A, B, C), exchanging digesta via the duodenal re-entrant cannulas (see Fig. 1)*

(Mean values with their standard errors for three sheep. Each B sheep received radioactive digesta flowing from the stomach of A sheep, and each C sheep from the stomach of B sheep. Each A sheep received digesta from C sheep and was dosed intraruminally with 1 mCi ^{65}Zn)

	B sheep		C sheep	
	Mean	SE	Mean	SE
Secretion into the stomach	0·016	0·006	—	—
(% of absorbed)	(27·3)	(9·63)	—	—
Secretion into the intestinal tract†	0·034	0·0126	—	—
Apparent absorption	0·070	0·0208	0·129	0·0470
True absorption‡	0·103	0·0284	—	—
Urinary secretion	0·0007	0·00012	—	—

* The duodenal dose of radioactivity is defined as the amount in the digesta flowing from the stomach of the previous sheep in the set, minus the radioactivity in the sample portion removed by the automatic sampler.

† Secretion into the stomach × 2·1 (S)

‡ $$\frac{^{65}\text{Zn in digesta entering the duodenum} - (^{65}\text{Zn in faeces} + \text{S})}{^{65}\text{Zn in digesta entering the duodenum}},$$

assuming the same ratio, endogenous secretion into the stomach region: that into the intestinal tract region over a 10 d period, in Expt 1 (A sheep) and Expt 2 (B sheep).

However the variation among the C sheep was also much higher than that among the B sheep.

DISCUSSION

Experimental method

The method described here for exchange of digesta at the point of the proximal duodenum between two or three sheep was similar to that published previously (Ivan *et al.* 1981; Kelleher *et al.* 1983). It was, however, greatly improved in the present study by the replacement of simple digesta pumps by the automatic digesta samplers. The samplers facilitated time-related measurements of recovery of the radioactivity flowing from the stomach of each sheep and of amounts of radioactivity entering the proximal duodenum of the second sheep in the system. They also maintained the digesta entering the duodenum at constant temperature.

There were no visible signs of stress on the animals during the 11 d period in which sheep were interconnected via the duodenal re-entrant cannulas and digesta samplers, and daily feed intake remained constant. The technique was successful in preventing recycling of the radioactivity into the stomach of the A sheep and facilitated the separation of the stomach and intestinal tract regions for the purpose of measurement of absorption and endogenous secretion within these gastrointestinal regions of all the sheep.

Absorption and secretion of ^{65}Zn

The results of the present study show that in the sheep there is no apparent absorption of ^{65}Zn before the duodenum. This was illustrated by a high percentage recovery of the intraruminally administered radioactivity in the digesta flowing from the stomach region of the dosed sheep and, more importantly, by the lack of detectable radioactivity in the blood of these sheep. Previous studies of Zn metabolism in sheep and cattle, employing cannulation of the gastrointestinal tract or slaughter techniques, have indicated a net secretion of Zn into the stomach (Grace, 1975; Bertoni *et al.* 1976; Ivan & Grieve, 1976; Stevenson & Answorth, 1978; Ivan *et al.* 1979). The majority of these studies concluded that the net secretion into the stomach exceeded the net absorption from the stomach. However, as Grace (1975) pointed out the values for net absorption or net secretion obtained in these studies are based on measurements of small differences between the quantities of Zn entering and leaving the region under study. Therefore, the previously used techniques are prone to large experimental errors and so cannot be used for accurate determination of secretion or absorption of Zn within the stomach of ruminants. The virtue of the technique used in the present study was that it utilized easily quantified labelled Zn and prevented a significant recycling of the absorbed label into the stomach of intraruminally dosed sheep.

Considerable quantities of ^{65}Zn are adsorbed onto the rumen wall of sheep (Arora *et al.* 1969; Ivan, 1979) with values (percentage dose/kg fresh tissue) being approximately 5·6 at 48 h and 1·3 at 96 h after dosing. In the present study only 0·02% of the dose was found in the entire reticulo-rumen wall 10 d after dosing, and only approximately 0·5% of the dose was recovered in the tissues and contents of the stomach of the dosed sheep. It therefore appears that although the rumen wall could adsorb a considerable proportion of dietary Zn, there is probably no active transport of the adsorbed Zn across the wall. Using rumen sacs, Arora *et al.* (1969) found that only negligible amounts of Zn were transferred from the inside to the outside. Zn is therefore recycled back into the rumen and flows with the digesta out of the stomach region and into the intestinal tract where the active absorption process begins.

It has been shown (Grace, 1975) that net absorption of Zn can occur in both the small and large intestine of sheep in spite of the possibility of a considerable Zn secretion into the small intestine (Stake *et al.* 1974). In the present study, the B sheep in Expt 2 represented the absorption from the entire intestinal tract or, more specifically, from the entire gastrointestinal tract, as there was no active absorption from the rumen of the A sheep. The mean apparent absorption in the B sheep (0·07) was within the range reported by Grace (1975). There was, however, no contribution from the endogenous secretion into the stomach of these sheep. Results of Expt 1 showed that the ratio of endogenous secretion into the stomach region: that into the intestinal tract region over the 10 d period was 1:2·1. Assuming the same ratio for the B sheep in Expt 2, and taking into consideration the values for the secretion into the stomach of these sheep, it was calculated that the mean true absorption in the B sheep was 0·103. This is in good agreement with Suttle *et al.* (1982), despite the contrasting methods used by the present and former authors. However, there was an appreciable variation among individual sheep in the present experiment which was probably due to differences in the quantity of Zn recycling into the gastrointestinal tract. Such a concept is supported by the results of Weston & Kastelic (1967) which show appreciable differences between sheep in the quantity of intravenously dosed ^{65}Zn present in the rumen contents. It is, however, noteworthy that in the present Expt 1 the ratio of ^{65}Zn secretion into the stomach: that into the intestinal tract was similar for all three A sheep (1:2·0, 1:2·3, 1:2·1).

It should be noted that the radiozinc entering the C sheep in the present Expt 2 was entirely of endogenous origin which was recycled into the stomach of the B sheep. The radioactivity in the C sheep was therefore very low, with only negligible amounts being detected in the digesta flowing from the stomach and in the plasma and urine of these sheep. The main purpose of incorporating sheep C into the system was to prevent a significant contamination of sheep A with recycled radioactivity. However, a relatively high radioactivity in faeces permitted calculation of apparent absorption of ^{65}Zn in the C sheep. A higher efficiency of absorption was found for recycled ^{65}Zn than for ^{65}Zn which entered via the rumen.

Transfer of endogenous Zn into the rumen has been reported to take place through the rumen epithelium and in saliva (Weston & Kastelic, 1967; Hiers *et al.* 1968) and into the small intestine in the bile, pancreatic juice and other secretions (Stake *et al.* 1974). Suttle *et al.* (1982) did not find appreciable amounts of Zn being recycled via the saliva. In the present Expt 1, recycling via saliva was not measured but the total secretion into the stomach region of the A sheep was higher than previously reported for the rumen (Weston & Kastelic, 1967). The sum of the ^{65}Zn recoveries in the digesta and faeces of the A sheep over 10 d, and the sum of the urinary recoveries in the A and B sheep over 10 d, were similar to previous faecal and urinary recoveries from intravenously dosed sheep (Ivan & Lamand, 1981). Due to the fact that appreciable amounts of ^{65}Zn were still present in the blood stream, mainly in the erthrocytes, 10 d after dosing (Ivan & Lamand, 1981), the actual proportion of ^{65}Zn dose which was secreted into the stomach and intestinal tract regions in the present study could not be precisely determined. Nevertheless, the ratio 1:2·1 for 10 d recoveries of ^{65}Zn in the digesta flowing from the stomach and in the faeces from intravenously dosed sheep showed the relative magnitude of recycling of Zn into the stomach and the intestinal tract regions.

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