A model for zinc metabolism in sheep given a diet of hay

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1. Four pairs of Scottish Blackface ewes were given a basal diet of hay, providing 8 mg zinc/d, and one of four levels of supplementary Zn (0, 75, 150 or 225 mg/d) continuously by intraruminal infusion.

2. ^{65}Zn (59 μCi) was given intravenously after stabilization for 14 d and the changes in specific radioactivity (SR) of plasma and faeces were monitored for a further 10 and 13 d respectively. The faecal endogenous losses and absorption of Zn were estimated, using the principle of isotope dilution, by two methods using the whole or latter parts of SR:time curves.

3. Faecal endogenous losses were estimated by the part area method to be 4·9, 6·4, 5·1 and 6·3 $\pm$ 0·35 mg/d at 0, 75, 150 and 225 mg supplemental Zn/d i.e. largely unaffected by Zn intake and averaging 0·11 mg/kg live weight (LW). The whole area method gave similar results.

4. Urinary excretion was negligible (probably $< 0{\cdot}2$ mg/d) for all sheep.

5. The amount of Zn absorbed was assumed to equal the irreversible loss of Zn under steady-state conditions and found to remain constant at 7·6 $\pm$ 0·39 and 10·3 $\pm$ 0·6 mg/d when calculated by 'whole' and 'part area' methods. Zn retention did not increase with Zn intake and homoeostasis was achieved primarily by control of Zn absorption which fell from 0·75 to 0·03 or from 0·96 to 0·05 of intake, depending on method of calculation, as intakes increased.

The control of zinc metabolism has been the subject of much recent controversy. Some workers have suggested that Zn homoeostasis in the rat is maintained primarily by the control of Zn secretion into the intestine (Evans *et al.* 1979) while others have reported that control over absorption plays an important role in the regulation of Zn metabolism (Smith & Cousins, 1980; Starcher *et al.* 1980). These contrasting results may be reconcilable in the light of Weigand & Kirchgessner's (1980) work which showed that as Zn intakes decreased, the influence of endogenous loss on homoeostasis decreased while that of absorption increased. The endogenous secretion of Zn in saliva has been found to remain constant in rats despite severe Zn depletion (Everett & Apgar, 1979).

Errors associated with the use of ^{65}Zn in short-term experiments can give misleading quantitative information on Zn metabolism. Failure to take account of differential dilution of ^{65}Zn by stable Zn in the digesta and tissues has been shown to cause underestimates of Zn absorption in rats on Zn-adequate diets when vascularly-perfused intestinal preparations are used (Smith & Cousins, 1980) and over-estimates in those on Zn-deficient diets when whole-body counting is used (Evans *et al.* 1979). The involvement of such errors in the few ^{65}Zn balance studies with ruminants (Miller *et al.* 1966, 1967) suggests that at best they give only qualitative information on the absorption and endogenous loss of Zn. Experiments with large animals given ^{65}Zn can however afford good opportunities for the continuous monitoring of SR in the same individual over long periods. These opportunities have therefore been exploited to assess the effects of Zn intake on the absorption and endogenous loss of Zn, using sheep as the animal model.

MATERIALS AND METHODS

Experimental design

Eight Scottish Blackface ewes, weighing 45–63 kg and equipped with rumen cannulas, were housed in the individual metabolism crates described by Grace & Suttle (1979). They were

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allocated in pairs to one of four supplementary Zn intakes, nominally 0, 75, 150 and 225 mg/d and were given a basic diet of chaffed pasture hay (0·7 kg/d) which provided 5–11 mg Zn/d. The supplementary Zn was given by infusing appropriate amounts of zinc sulphate into the rumen using double-channel peristaltic pumps. Slight differences in flow-rate between channels caused actual Zn infusion rates to differ slightly from the nominal rates. On average 2 l of solution were infused into each sheep daily. The hay had a low crude protein (nitrogen × 6·25) content (60 g/kg) and urea (4 g/d) and sodium sulphate (0·12 g/d) were added to the infusion solution to improve microbial protein synthesis.

Portions of the daily hay allowance were dispensed at intervals of 2 h by automatic feeders. The infusate provided most of the water intake; only 0·5 l/d were available for drinking and the sheep were not observed to consume any of the water offered. The treatments continued for 14 d before the administration of isotope to establish steady-state conditions. On day 15, 59 μCi ^{65}Zn together with 67 μg carrier Zn as zinc chloride (The Radiochemical Centre, Amersham, Bucks.) were injected via a temporary catheter into the jugular vein in 10 ml saline (9 g sodium chloride/l) and the catheter was flushed with an equal volume of saline. Before injection, the $ZnCl_2$ was brought to approximately pH 7·0 with 0·01 M-sodium hydroxide and made up to volume with sterile physiological saline.

Faeces and urine were collected in polythene buckets via separators made of stainless steel, fibre glass and nylon mesh and the daily collections sampled for the determination of stable Zn and ^{65}Zn balances. Urine was collected from days 15 to 24 and faeces from days 15 to 27 inclusive.

Sampling and analytical methods

Venous blood samples for plasma Zn and ^{65}Zn determinations were obtained by jugular venepuncture, using heparinized evacuated containers with minimal trace element content (Beckton Dickinson UK Ltd) at 0·1, 0·5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 48, 72, 96, 114, 192 and 240 h after injection. The homogeneity of plasma Zn was examined by separating the plasma protein by ultracentrifugation at 120000 ***g*** for 16 h and comparing the SR of the protein-enriched pellet (containing 75% of plasma protein) with that of whole plasma. Ten 2·5 ml samples were selected from those available on the basis of freedom from haemolysis and ample sample volume and radioactivity: they covered the range from 2 to 72 h after dosing and were diluted 1:1 with saline prior to spinning.

Two samples of crude rumen contents were taken from each sheep on two occasions (days 22 and 25) via the rumen fistulas under vacuum (Suttle, 1974); one sample was freeze-dried and its moisture and Zn contents determined and the other was used for determining ^{65}Zn content.

Duplicate samples of freeze-dried faeces, rumen contents and diet (0·5 g) and single samples of 'plasma protein' (fraction 5, Grace & Suttle, 1979) were wet-washed with concentrated perchloric–nitric acids (1:4, v/v), the dry digest was dissolved in 10–20 ml warm, 0·125 M-hydrochloric acid, and Zn determined by atomic absorption spectrophotometry (AAS). Plasma was diluted 1:5 with butanol (60 ml/l) and Zn concentration determined by AAS.

Counting procedures

Duplicate samples of approximately 15 g fresh faeces and rumen contents or 1–20 ml plasma (sample volume was increased as the experiment progressed) were counted in an automatic gamma-well scintillation counter fitted with 75 mm sodium iodide crystal. Counting standards of appropriate geometry were prepared from the dose solution. Each day's total urine collection (approximately 2 l) was made up to constant volume in Winchester bottles of 2·5 l capacity and counted in the whole-body counter described by Smith & Field (1973) using standards of comparable geometry.

Calculations

Faeces contain Zn of exogenous (dietary) and endogenous origin and the latter portion was estimated, using the principle of isotope dilution, by two methods. Both involved comparisons of areas under the SR:time curves for faeces and plasma (Shipley & Clark, 1972) and assumptions that the SR of faecal endogenous Zn was the same as that of plasma Zn at a given time. The two methods used either the whole or the latter part of the areas under the curves. The part area method ignored results from the first 3 d to avoid possible complications associated with the heterogeneity of the plasma Zn pool over that period. Compatibility of faecal endogenous and plasma Zn SRs was further ensured by using the time taken for faecal ^{65}Zn to reach a peak (36–60 h) as the lag between secretion and excretion (after Lofgreen, 1960). Areas were calculated as a series of contiguous trapezoids summed for all but one sheep over the period 4–10 d for plasma and 5·5–11·5 d for faeces.

The absorption rate for Zn was assumed to equal the irreversible loss rate under steady-state conditions and irreversible loss was estimated (whole area method) by compartmental analysis of the plasma SR curve, using curve-peeling techniques to obtain approximate intercept and rate-constant values for the four compartments (White *et al.* 1969). The equation is; plasma Zn SR $= Ae^{-K_1t} + Be^{-K_2t} + Ce^{-K_3t} + De^{-K_4t}$. Since the inclusion of the early plasma SR values seemed likely to introduce errors, an additional estimate of irreversible loss was obtained (part area method) from the pool size and fractional turnover of the last compartment (D, mean values 157·5 mg and 0·00293/d, respectively).

Rough estimates of the secretion of Zn into the rumen were obtained by the method described briefly for molybdenum secretion by Suttle & Grace (1978), except that plasma (P) rather than urine SR was used as the marker for the SR for Zn secreted into the rumen. Assuming that rumen volume remained constant and there was no absorption of Zn from the rumen (R), rumen (SR) is determined by the flow of radioactive, secreted (S, mg/d) and non-radioactive, dietary (D)+infused (I) Zn into the rumen, the equation being:

$$R_{SR} = \frac{S \times P_{SR}}{S + Zn_D + Zn_I}.$$

Re-arrangement of this equation allows the unknown S, to be calculated from the known values thus:

$$S(\text{mg/d}) = \frac{R_{SR}(Zn_D + Zn_I)}{P_{SR} - R_{SR}}.$$

Plasma SR values on the day of rumen sampling were used in the equation.

RESULTS

Sheep health

The sheep were generally healthy throughout the experiment and food refusals were low at all but the highest Zn intake. The poor consumption of hay at the highest Zn intake did not improve when Zn infusions ceased. The dry matter and Zn intakes over the collection period for each treatment are given in Table 1. One sheep given 150 mg Zn/d was affected by diarrhoea on days 7, 8 and 9 of the collection period. The mean (±SE) digestibility of the dietary dry matter (DM) was 0·616±0·015 with no apparent effect of Zn intake.

Plasma Zn

There were no effects of level of infused Zn upon the rate of disappearance of ^{65}Zn from the plasma and only the extreme intake groups are illustrated in Fig. 1. The levels of non-radioactive Zn in the plasma were also unaffected by Zn intake and the mean (±SE)

Table 1. *Mean daily intakes and digestibility of dry matter (DM) and intakes of zinc in the food or by intraruminal infusion in individual ewes on a hay diet for 13 d following the intravenous injection of ^{65}Zn*

Sheep	Intake (g)	DM digestibility	Zn input (mg) In food	Zn input (mg) By infusion	Zn input (mg) Total
1	602	0·568	10·4	0	10·4
2	453	0·688	7·8	0	7·8
3	629	0·598	10·8	81·6	92·4
4	592	0·612	10·2	79·2	89·4
5	363	0·589	6·2	122·8	129·0
6	602	0·588	10·4	160·6	171·0
7	289	0·609	5·0	244·8	249·8
8	301	0·678	5·2	216·0	221·2

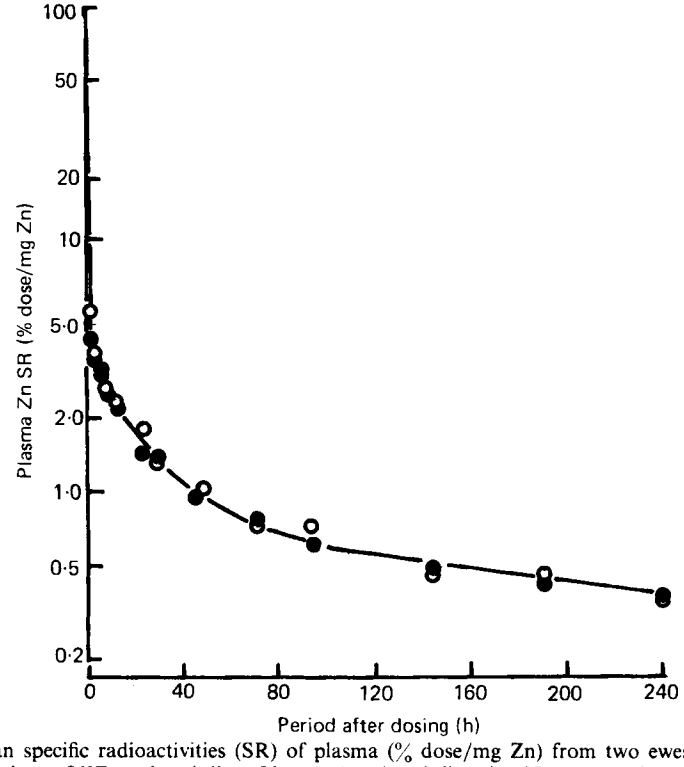

Fig. 1. Mean specific radioactivities (SR) of plasma (% dose/mg Zn) from two ewes given a single intravenous dose of ^{65}Zn, a basal diet of hay (○) or basal diet plus 225 mg supplementary Zn/d (●) by intraruminal infusion.

levels at the four Zn intakes were 0·69, 0·61, 0·68 and 0·73 ± 0·05 mg/l. The mean (± SE) percentage of the plasma ^{65}Zn which was in the protein pellet increased with time, 63·7 ± 2·8% being found in samples taken 2–14 h after dosing and 87·4 ± 5·2 in those taken between 22–72 h (n 5). The differences between the SR of protein pellet Zn and whole plasma Zn (D, % dose/mg) diminished with time (t, h) according to the relationship

Table 2. *Estimates by 'whole- or part-area' methods of the faecal endogenous excretion and irreversible loss of zinc in individual ewes on various Zn intakes*

Sheep no.	Zn intake	Faecal excretion* of Zn (mg/d)	Proportion of faecal Zn of endogenous origin		Faecal endogenous Zn excretion (mg/d)		Irreversible loss (mg/d)	
			Whole†	Part†	Whole†	Part†	Whole†	Part†
1	10·4	19·7	0·35	0·255	6·90	4·9	8·3	10·5
2	7·8	14·6	0·30	0·345	4·38	5·0	5·4	7·1
3	92·4	112·7	0·057	0·056	6·42	6·34	7·4	10·0
4	89·4	111·7	0·047	0·0575	5·25	6·42	8·4	12·7
5	129·0	124·3	0·029	0·0382	3·60	4·75	7·3	9·6
6	171·0	194·2	0·026	0·0277	4·45	5·37	7·7	11·6
7	249·8	238·0	0·020	0·0288	4·76	6·86	9·0	11·8
8	221·2	250·9	0·021	0·0232	5·27	5·8	6·9	9·4

* Mean for period 6 to 13 d after administration of ^{65}Zn.
† For details of method, see p. 107.

$D = 0{\cdot}66 \log t - 1{\cdot}08$ ($P < 0{\cdot}001$; r 0·90) i.e. D went from markedly negative to marginally positive between 2 and 72 h.

Faecal Zn

The total recoveries (%) of ^{65}Zn in faeces for the pairs of ewes after 13 d were 49·5, 40·2; 52·4, 53·1; 60·2, 61·8; 51·6, 50·4 in order of increasing Zn intake. Maximal excretion occurred on the 2nd day after dosing for all but one sheep, when values ranged from, 7·7 to 13·3% dose, and declined exponentially thereafter. The effect of Zn intake on stable Zn excretion in faeces is shown in Table 2. Faecal Zn (F_{Zn}) increased linearly with increases in Zn intake (I_{Zn}) and the regression coefficient was not significantly different from unity, the equation being $F_{Zn} = 13{\cdot}1 + 0{\cdot}99 \pm 0{\cdot}066\, I_{Zn}$. There was therefore no detectable increase in Zn retention as Zn intake increased. The proportion of the faecal Zn which was of endogenous origin decreased with increases in Zn intake but the total endogenous faecal Zn remained remarkably constant despite a wide range of Zn intakes (Table 2); the mean ($\pm$SE) values by the 'part-area' calculation were 0·096, 0·114, 0·108 and 0·110 mg/kg live weight $\pm$0·005 in order of increasing Zn inputs. Using the 'whole-area' method decreased the estimates of endogenous loss by 20% at the highest Zn intake but the effect disappeared as Zn intakes diminished.

Zn absorption

The irreversible losses estimated by the 'whole-area' method showed no significant treatment effects (Table 2). The efficiency of Zn absorption, calculated by dividing irreversible loss by Zn intake, was the only factor affected by Zn intake; mean ($\pm$SE) values for the absorption coefficient using whole-area data were 0·75, 0·087, 0·051 and 0·034 $\pm$ 0·027 in order of increasing Zn intakes. Using the 'part-area' method invariably increased the estimates of irreversible loss and hence of absorption: one absorption coefficient exceeded unity suggesting that the 'whole-area' values were the more appropriate.

Urine Zn

The percentage of the ^{65}Zn dose excreted in urine over the entire 10 d collection period amounted to only 0·21% and was unaffected by Zn intake. Mean ($\pm$SD) stable Zn output

in urine was apparently $0{\cdot}83 \pm 0{\cdot}17$ mg/d and was also independent of Zn input. However, contamination of the urine was suspected when urine SR values at the end of the experiment were found to be several fold lower than those in plasma. Contamination was proved by obtaining samples of urine from five of the ewes immediately after they were voided during brief suffocation; these samples contained only $0{\cdot}11 \pm 0{\cdot}05$ mg Zn/l compared with $0{\cdot}56 \pm 0{\cdot}1$ mg/l from the same animals during days 15–25 of the experiment.

Rumen contents

From levels of radioactive and stable Zn found in rumen contents on days 22 and 25, mean secretion rates for Zn into the rumen were estimated to be 0·8, 1·5, 1·3 and 1·7 mg/d for the four groups on increasing Zn intakes. Although the level of Zn secreted into the rumen was apparently higher when Zn was infused into the rumen the increase was small relative to the increment in Zn intake (225 mg/d). There was no evidence therefore that appreciable amounts of Zn were recycled via the saliva under the particular conditions of the experiment. However, the estimates must be regarded as tentative in view of the unknown lag between plasma and rumen SR values, the physical heterogeneity of the sampled pool and the unknown distribution of salivary Zn between liquid and solid phases.

DISCUSSION

The results provide clear qualitative evidence that the sheep in this experiment controlled their retention of Zn by changing absorption rather than endogenous loss as Zn intakes increased. That some control was exercised was evident from the fact that the supplementary Zn was not retained but quantitatively recovered in the faeces. If absorptive control had not been the predominant homoeostatic factor, then the flux of absorbed stable Zn through the plasma into urine or faeces would have increased the rate of clearance of ^{65}Zn from plasma: this did not occur (Fig. 1). The low urinary Zn excretion and small changes in ^{65}Zn excretion in faeces confirmed that secretory pathways were of minimal importance in achieving homoeostasis. Our sheep, therefore, differed from the growing rats studied by Weigand & Kirchgessner (1980) and Evans *et al.* (1979) which showed marked increases in faecal endogenous secretion over a narrower range of Zn inputs. In several studies with ruminants the apparent absorption of ^{65}Zn has been shown to decrease when Zn intakes were increased (e.g. Miller *et al.* 1967; Neathery *et al.* 1973). Endogenous losses of ^{65}Zn have been shown to decrease in goats and calves given Zn-deficient diets (Miller *et al.* 1966, 1967) but neither the absorption nor endogenous loss values from these experiments could be converted appropriately to quantities of stable Zn.

The results from our experiments can be used to generate quantitative values for Zn absorption and endogenous loss provided that certain assumptions are valid. The crucial assumption in computing endogenous loss from our 'single-shot' tracer experiment is the value attributed to the SR of secreted Zn. Our original intention was to use urine SR to represent the SR of Zn in the transport compartment and Zn secreted from it (e.g. Compère *et al.* 1965). That intention was frustrated by clear evidence of contamination of urine by stable Zn. The alternative of using plasma SR is associated with certain problems. The low SR of 'protein-pellet Zn' relative to whole plasma Zn in the first 24 h after dosing indicates that plasma Zn consisted initially of at least two compartments. The relatively high SR of that same fraction after 72 h probably reflects the flux of unlabelled dietary Zn through a more labile protein-free compartment. Errors in using whole plasma SR to indicate the SR of secreted Zn in faeces would therefore arise if secreted Zn came predominantly from one compartment and would be likely to be greatest early in the experiment. When endogenous loss was calculated by comparing areas under the plasma and faeces SR *v.* time curves, for the time-period 0–10 d rather than 4–10 d with a 36 h delay for faecal excretion,

endogenous losses were decreased at the higher Zn intakes by as much as 30% (Table 2): the latter were assumed to be nearer to the true values and were remarkably uniform when expressed on a live-weight (LW) basis (91–127 μg/kg LW) and not significantly affected by Zn input.

Irreversible loss should exceed faecal loss to the extent that there are additional secretory losses via urine and skin, accumulations of Zn in wool and slowly-exchangeable Zn pools ('sinks') which supply predominantly unlabelled Zn into the transport compartment during the isotope study. The latter are potentially important as they are 'exchange' rather than 'loss' phenomena and would lead to over-estimates of the amount of Zn absorbed. The difference between faecal endogenous and irreversible loss varied with the method of calculation but was generally around 2 mg/d. Of this 1·2 mg/d would be accounted for if wool was growing at 10 g/d and contained 115 mg Zn/kg (Burns *et al.* 1964; Healy & Zielman, 1966). The closeness of the values for endogenous loss and irreversible loss – wool Zn suggests that irreversible loss gives a reasonable measure of absorption: the derived values, like those for endogenous loss were relatively constant for a given method of calculation (Table 2) despite the wide range of Zn inputs.

It would appear that there was either a low limit to the sheep's capacity to absorb Zn or that by humoral control mechanisms, the sheep absorbed Zn according to requirement. The latter seems more likely since the limit observed in our sheep would be insufficient to meet the requirement of 14 mg/d for lactation (Agricultural Research Council, 1980). There is evidence in rats that the efficiency of Zn absorption increases during late pregnancy and early lactation (Davies & Williams, 1977). If the sheep absorbs Zn according to requirement, absorption values become progressively an animal attribute rather than a dietary or plant one as Zn intakes increase above the required level. The influence of dietary factors which limit the availability of Zn must be studied at Zn intakes close to the requirement level. There were clearly no major constraining plant factors in the low-quality hay which yielded 75% or more of its Zn to the absorptive process.

The observation of constant faecal endogenous losses in the presence of divergent absorptive efficiencies can only be explained in one of two ways: either Zn is secreted largely posteriorly to the principal sites of absorption or it is secreted anteriorly and remains unaffected by the processes influencing the absorption of dietary Zn. Our estimates of salivary Zn secretion show that it is relatively small but the extent and fate of Zn in gastric and duodenal secretions is a matter of controversy (Miller & Cragle, 1965; Grace, 1975).

The Zn concentration in the hay used in the present experiment was low (17 mg/kg DM) and yet it must have met the ewes' requirements for maintenance and wool growth since no fall in plasma Zn was observed after 1 month. The adequacy of an intake of 9 mg Zn/d suggests that faecal endogenous loss, the major component of the maintenance requirement, was unlikely to have exceeded 9 mg/d i.e. to have been over-estimated by our methods.

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