

Studies on the effects of dietary zinc dose on ^{65}Zn absorption in vivo and on the effects of Zn status on ^{65}Zn absorption and body loss in young rats

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1. Weanling male rats were maintained on diets containing 5, 10, 20, 40, 80 or 160 mg zinc/kg for 14 d. On day 15 they received ^{65}Zn either by intraperitoneal injection or in a test meal containing 20 mg Zn/kg. After dosing, the rats were again maintained on the diets they had received previously.

2. Whole-body ^{65}Zn retention was measured immediately after dosing and daily for a further 9 d. From regression analysis of the semi-logarithmic plots of ^{65}Zn retention from 0 to 192 h after ^{65}Zn administration, the true extent of ^{65}Zn absorption and the biological half-life ($t_{1/2}$) of body ^{65}Zn stores were calculated.

3. At the end of the experiment, the rats were killed and the entire small intestines of some rats from each group were rapidly flushed out to remove food and faecal residues, frozen in liquid nitrogen and stored under an atmosphere of N_2 at -20° before separation of cytosolic Zn-binding fractions by gel filtration on Sephadex G-75.

4. The results suggest that rats which received diets that were either deficient (5 mg Zn/kg), marginal (10 mg Zn/kg) or adequate (20–80 mg Zn/kg) in Zn achieved homeostatic regulation of body Zn by changes in both the extent of Zn absorption and excretion. However, when Zn supply was excessive, increasing from 80 to 160 mg Zn/kg, no further changes were seen in Zn absorption, and homeostatic control appeared to be effected entirely by changes in rates of body Zn loss.

5. Gel chromatography of intestinal cytosol on Sephadex G-75 revealed that Zn was associated with two major fractions. The first (peak 1) had a molecular weight (MW) > 75 kdaltons and the second (peak 2), a MW of approximately 10 kdaltons and was assumed to be metallothionein.

6. There was no obvious relation between the amount of Zn bound to peak 1 and dietary Zn content. In contrast, the amount of Zn recovered in peak 2 increased linearly with increasing dietary Zn content.

7. Comparisons between the effect of dietary Zn content on Zn bound to peak 2 and ^{65}Zn retention may, depending on the range of Zn intakes, indicate possible roles for intestinal metallothionein in the control of Zn absorption or excretion.

8. A study of the effects of dietary dose of ^{65}Zn on the extent of ^{65}Zn absorption in rats of normal Zn status indicated a possible biphasic relation. At low doses (5–40 mg Zn/kg) ^{65}Zn absorption appeared to exhibit a curvilinear response to increasing ^{65}Zn dose, indicating possibly a saturable process. At higher doses (40–160 mg Zn/kg) the capacity of this process appeared to be exceeded and ^{65}Zn absorption increased in a linear fashion.

Previous studies in rats have indicated that zinc absorption occurs most rapidly in the duodenum with lesser amounts being absorbed by the jejunum and ileum (Davies, 1980). Virtually no Zn is absorbed in either the stomach or the caecum (Van Campen & Mitchell, 1965; Methfessel & Spencer, 1973). Zn absorption from ligated loops of duodenum in vivo has been shown to be rapid with 1% of the dose being transferred to the carcass within 1 min of duodenal dosing. It has also been reported, using the same preparation, that the mechanism of Zn absorption is a saturable process, indicative of a carrier-mediated or enzymic process (Davies, 1980).

Although it has been suggested that mucosal metallothionein might play a role in Zn homeostasis (Richards & Cousins, 1976, 1977*a, b*; Smith *et al.* 1978; Cousins, 1979*a, b*; Smith & Cousins, 1980), this view has been contested (Olafson, 1983; Flanagan *et al.* 1983).

The current study was undertaken to investigate the homeostatic control of Zn absorption and excretion, the possible roles of mucosal metallothionein and the effects of different doses of dietary Zn on Zn absorption.

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MATERIALS AND METHODS

In all experiments, male rats of the Rowett Hooded Lister strain were used. Weanling rats were randomly assigned to different groups.

Series 1. Homeostatic studies

Six groups, each of five male weanling rats, received a semi-synthetic diet similar to that described by Williams & Mills (1970). The basal diet contained (g/kg): spray-dried egg albumin 200, sucrose 660 and arachis oil 100. In addition it was supplemented with minerals to supply (g/kg): calcium 6, potassium 5.08, magnesium 0.5, sodium 2.3, phosphorus 5 and chloride 0.5; and salts of trace elements to contain (mg/kg): copper 5, iron 50, manganese 50, silicon 100, iodine 1, fluorine 2.5, nickel 1, tin 2, selenium 0.1 and chromium 5. The vitamin contents of the basal diet were (mg/kg): thiamin 10, pyridoxine 10, riboflavin 10, *p*-aminobenzoic acid 10, nicotinic acid 30, calcium pantothenate 20, folic acid 5, biotin 5, inositol 400, α -tocopherol acetate 200, retinyl acetate 8, calciferol 0.25, vitamin B₁₂ 0.0025, menadione 5 and choline hydrochloride 1000. The rats were fed twice daily between 10.00 and 11.00 hours and 16.00 and 17.00 hours. During these periods the rats were allowed to feed *ad lib*. For the first 14 d, each group received a diet which contained 5, 10, 20, 40, 80 or 160 mg Zn/kg. On the 15th day, the rats were offered a single test meal containing 20 mg Zn/kg and ⁶⁵Zn (1 μ Ci). This meal weighed 1 g less than that consumed by the rats from the meal offered 24 h previously. They were then maintained on their previous diets *ad lib*. for a further 8 d.

Series 2. Studies on the effect of dietary Zn dose on Zn absorption

Six groups, each of five male weanling rats, received a semi-synthetic diet containing 40 mg Zn/kg on a meal-feeding regimen as described previously. On the 11th day the rats were offered a single test meal containing 5, 10, 20, 40, 80 or 160 mg Zn/kg and ⁶⁵Zn (1 μ Ci). The rats were then returned to their previous diet *ad lib*. for a further 8 d.

Series 3. Control groups for studies on the effects of Zn dose on Zn absorption and homeostasis

Six additional groups of rats (three male weanling rats per group) received a semi-synthetic diet for 14 d as previously described. The diets contained 5, 10, 20, 40, 80 or 160 mg Zn/kg. On the 15th day, each rat received an intraperitoneal (i.p.) injection of 1 μ Ci ⁶⁵Zn in saline (9 g sodium chloride/l) and was offered a meal containing no radioactive Zn. They were then returned to their previous diets *ad lib*. for 8 d except for three rats from each group which were offered their respective diets *ad lib*. for 6 d and then returned to the meal feeding regimen described above for the final 2 d of the experiment.

Following ⁶⁵Zn administration, all rats were monitored daily for radioactivity by whole-body counting.

On the 9th day after ⁶⁵Zn administration, all animals were killed between 14.00 and 15.00 hours. Animals that had been returned to the meal-eating regimen were killed 4 h after receiving their final meal, their intestines were flushed with ice-cold saline and then rapidly frozen in liquid N₂ and stored deep-frozen under an atmosphere of N₂.

Throughout the entire experimental period, the body-weights of the rats were recorded weekly. Deionized water was offered *ad lib*. throughout.

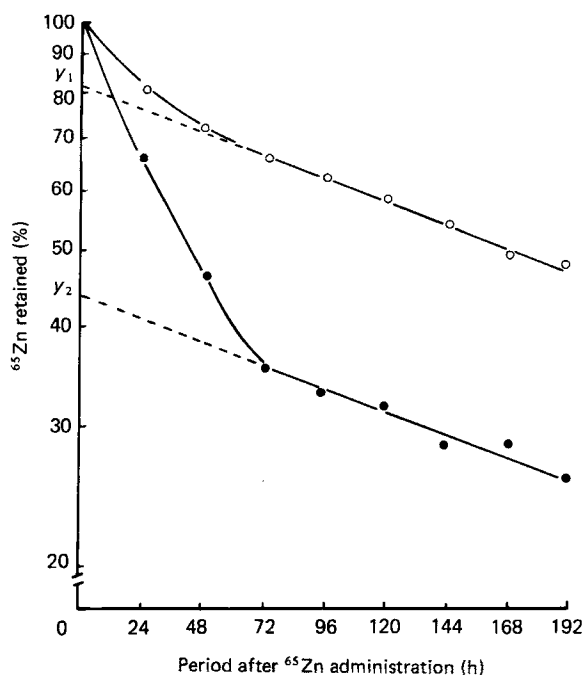


Fig. 1. Semi-logarithmic plot of the ^{65}Zn retained (%) post-administration over 192 h by rats which were maintained on diets containing 40 mg Zn/kg. The rats received the ^{65}Zn either in one test meal which contained 20 mg Zn/kg (●-●) or by intraperitoneal injection (○-○) while receiving a test meal of 20 mg Zn/kg without ^{65}Zn . ^{65}Zn retention by the rats was determined by whole-body counting immediately post-administration of ^{65}Zn and then at 24-h intervals thereafter. Each point represents the mean of three to five rats.

Estimation of the true percentage absorption of dietary Zn

The true percentage Zn absorption after orally administered ^{65}Zn was estimated by the method of Heth & Hoekstra (1965). Semi-logarithmic plots of the percentage retention of ^{65}Zn after oral administration were drawn for each animal. The first phase over the initial 72 h represents the loss of unabsorbed ^{65}Zn in the faeces together with loss of absorbed ^{65}Zn rapidly excreted into the intestinal lumen, at early times post-administration, whereas the second phase (72–192 h after administration) is a measure of the biological turnover of absorbed ^{65}Zn (Fig. 1). For all dietary treatments the rates of decline in body ^{65}Zn activity during this second phase were identical whether the ^{65}Zn was administered orally or by i.p. injection, indicating once the ^{65}Zn was in the body it was handled in the same manner irrespective of the route of entry. Thus the intercepts on the ordinate of the extrapolated linear components after 72 h for the orally dosed animals (Y_2) and the animals dosed by i.p. injection (Y_1) represented, for each dietary treatment, the proportion of the ^{65}Zn initially retained. The true percentage absorption of ^{65}Zn can thus be calculated by $(Y_2 \div Y_1) \times 100$. The intercepts Y_2 and Y_1 were calculated by linear regression. For each dietary treatment, individual values of Y_2 were divided by the mean value of Y_1 to calculate mean percentage absorption and standard errors. Analysis of this type corrects for the excretion of absorbed ^{65}Zn into the intestinal lumen at early time-intervals post-administration. As a measure of turnover of body Zn stores, the biological half-life ($t_{1/2}$) was calculated for each rat from regression analysis of \log_{10} ^{65}Zn retained 72–192 h post-administration. Within each dietary treatment group, values for rats dosed either orally or by injection were pooled for calculation of mean values and standard errors.

Separation of soluble intestinal Zn metallo-proteins

The separation of soluble intestinal Zn-metallo-proteins was carried out by a procedure similar to that described for rat liver by Bremner & Davies (1975). The pooled, entire small intestines of three rats from each dietary treatment group were homogenized in ice-cold Tris-acetate buffer (10 mM, pH 7.4) (1 g tissue in 2.5 ml buffer) using an Ultra Turrax homogenizer (Janke & Kunkel, K. G., Staufen Br., F.D.R.). The homogenates were centrifuged at 75000 g for 90 min at 4 °. The supernatant fraction was decanted and 3.5 ml applied to a G-75 Sephadex (Sigma Chemical Co., St Louis, Mo) column (15 × 600 mm) previously equilibrated with Tris-acetate buffer (10 mM, pH 7.4). Separation of the proteins was carried out at 4 °. The proteins were eluted with the same Tris-acetate buffer and the Zn in each fraction estimated by atomic absorption flame spectrophotometry as previously described (Bremner & Davies, 1975).

Statistical methods

Results are expressed as means with their standard errors. Levels of significance were tested by analysis of variance as indicated in the text. Lines were fitted by linear regression or other polynomial functions, again as indicated in the text.

RESULTS

Homeostatic studies

Comparison of the body-weight gains of the groups of rats which had received diets containing different Zn contents showed that those which had received the diet with the lowest Zn concentration (5 mg Zn/kg) had lower body-weight gains than those of the other groups over a period of 2–3 weeks (Fig. 2).

When diets previous and subsequent to ⁶⁵Zn administration contained between 5 and 40 mg Zn/kg the proportion of orally administered ⁶⁵Zn absorbed varied inversely with dietary Zn (Table 1). However, when dietary Zn was 80 or 160 mg/kg the efficiency of ⁶⁵Zn absorption (58 and 56% respectively) was not apparently influenced by dietary Zn. Over the range of dietary Zn contents tested, Zn absorption (y ; % of dose absorbed) was related to dietary Zn content (x ; mg Zn/kg) by a power curve described by the equation:

$$y = 4.488x^{-0.73}, r^2 0.96.$$

The turnover and body loss of ⁶⁵Zn appeared to be particularly slow at low dietary Zn concentrations; the biological half-life ($t_{1/2}$) being > 1000 h at dietary Zn contents of 5–10 mg/kg. When dietary Zn was increased to 20 mg Zn/kg the $t_{1/2}$ was markedly reduced and it continued to decline steadily as Zn concentrations increased to 160 mg Zn/kg. The relation between ($t_{1/2}$) (y ; h) and dietary Zn content (x ; mg Zn/kg) could be described by the equation:

$$y = 135.5x^{-0.18}, r^2 0.95.$$

Such results suggest first that Zn is avidly retained when dietary Zn is adequate to meet requirements for growth (approximately 12–15 mg/kg diet, Williams & Mills, 1970), and second that rapid turnover and excretion modulates body Zn burden when supply greatly exceeds requirements.

Tissue Zn

The distribution of Zn between soluble intestinal proteins was determined by Sephadex G-75 gel fractionation of supernatant fractions of homogenized intestinal tissue. Zn was associated with two principal fractions (Table 2). Peak 1 represented Zn bound to ligands of apparent molecular weight (MW) > 75 kdaltons, while peak 2 represented Zn associated

Effects of dietary Zn on Zn absorption

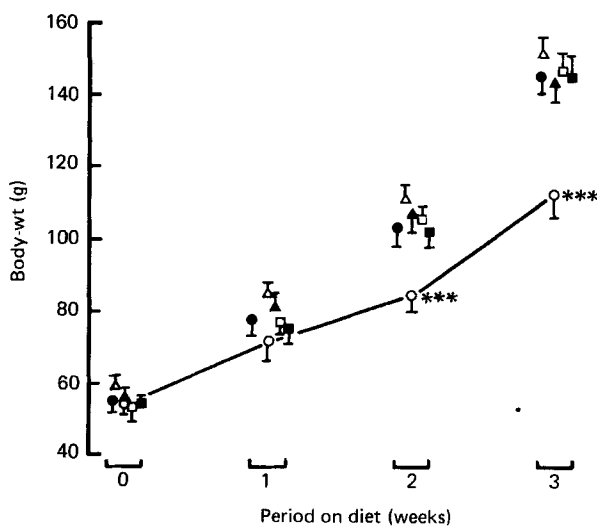


Fig. 2. Body-weights of rats maintained on semi-synthetic diets of different zinc concentrations (mg/kg): (○) 5, (●) 10, (△) 20, (▲), 40, (□) 80, (■) 160. Over the first 14 d the rats were fed twice daily between 10.00 and 11.00 hours and 16.00 and 17.00 hours. On the 15th day, the rats received one test meal containing 20 mg Zn/kg. After this meal, they were returned to their previous diets *ad lib*. All rats were singly housed and received deionized water *ad lib*. Results are mean values with 1x standard errors represented by vertical bars for seven to eight rats. ***Significant effects due to treatment (analysis of variance) $P < 0.001$.

Table 1. The biological half-life ($t_{1/2}$) of ^{65}Zn and the percentage absorption of ^{65}Zn from a test meal containing 20 mg Zn/kg, labelled with 1 μCi , by rats maintained before and after ^{65}Zn dosing on diets of different Zn contents

(Mean values with their standard errors. Methods of Heth & Hoekstra (1965); see p. 37. The $t_{1/2}$ (h) of body ^{65}Zn was calculated for each rat from the linear portion obtained by regression analysis, of the semi-logarithmic plot of \log_{10} of percentage ^{65}Zn retention *v.* time from 72–192 h following ^{65}Zn administration either orally or by intra-peritoneal injection. The mean percentage absorption of ^{65}Zn (four or five rats) and biological $t_{1/2}$ of ^{65}Zn (seven or eight rats) were calculated as described in Materials and Methods)

Dietary Zn content (mg/kg)	$t_{1/2}$ of ^{65}Zn (h)		Zn absorbed from test meal (%)	
	Mean	SE	Mean	SE
5	1266	144	99	5
10	1115	62	89	3
20	413	29	86	3
40	268	14	68	7
80	226	24	58	4
160	105	9	56	7

with species with an apparent MW of 10 kdaltons (Bremner & Davies, 1975). The latter has been identified in intestinal mucosa as metallothionein (Richards & Cousins, 1976, 1977a). The distribution of Zn between these two peaks is shown in Table 2 and the relation between the Zn contents of the diets offered before and after dosing with ^{65}Zn , and Zn associated with peak 2, is shown in Fig. 2.

There was no clear relation between dietary Zn content and Zn bound to peak 1 (Table

Table 2. The distribution of zinc ($\mu\text{g/g}$ wet weight intestinal tissue) between the major peaks of the elution profiles from a column of Sephadex G-75 of the cytosol from the pooled intestinal tissue from rats receiving diets of different Zn contents

(The tissue was homogenized in, and the cytosol eluted with, Tris-acetate buffer (pH 7.4) (for details, see p. 38). Peak 1 represents species with an apparent molecular weight > 75 kdaltons and peak 2 represents Zn bound to metallothionein)

Dietary Zn content (mg/kg)	Zn contents of eluted peaks ($\mu\text{g Zn/g}$ wet wt of intestinal tissue)	
	Peak 1	Peak 2
5	3.97	0
10	5.66	0
20	7.34	0.31
40	5.85	1.65
80	6.67	2.73
160	8.65	5.10

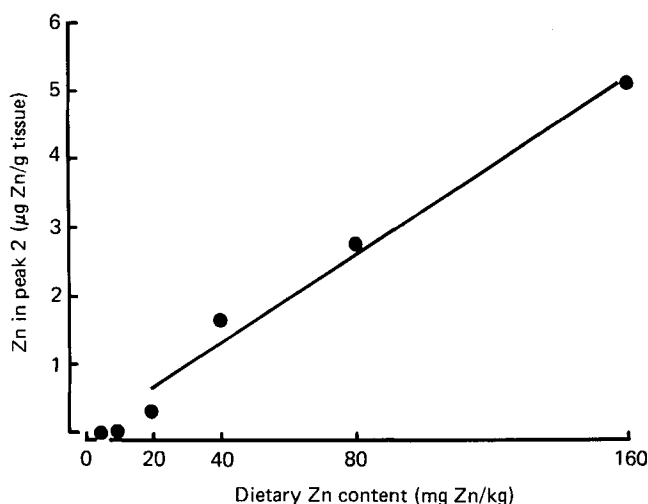


Fig. 3. The relation between the amount of intestinal cytosolic zinc recovered in peak 2 and dietary Zn content. The line for dietary Zn contents of 20–160 mg Zn/kg was fitted by linear regression analysis (r 0.98, P < 0.02). Pooled rat small intestines were homogenized in Tris-acetate buffer (pH 7.4) and samples of the cytosolic fraction were applied to a column of Sephadex G-75 and eluted with the same buffer (for details, see p. 38). Peak 2 represents the Zn content of fractions containing proteins with an apparent molecular weight of 10 kdaltons.

2). In contrast, as dietary Zn content increased from 20 to 160 mg/kg, there was a linear increase in the Zn content of peak 2. (Fig. 3). Thus, the Zn content of peak 2 (y , $\mu\text{g Zn/g}$ tissue) was related to the dietary Zn content (x , mg Zn/kg) by the expression:

$$y = 0.0324x + 0.0191, r 0.99, P < 0.002.$$

Studies on the effects of dietary Zn dose on Zn absorption

To examine the effects of dietary doses of Zn on Zn absorption in rats of normal and equal Zn status, groups of rats all previously maintained on a diet containing 40 mg Zn/kg received one test meal labelled with ^{65}Zn . The dietary Zn content of the test meals was 5,

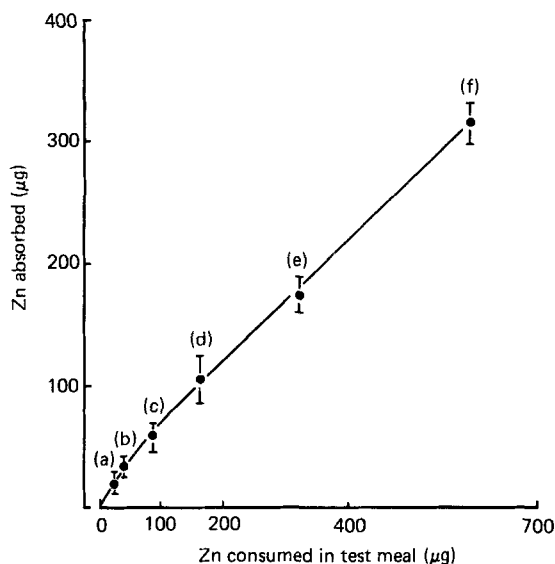


Fig. 4. The relation between the amount of ^{65}Zn absorbed from a test meal and the total Zn content of the meal. Male weanling rats were maintained on a diet containing 40 mg Zn/kg. After 14 d, they received a single test meal containing (a) 5, (b) 10, (c) 20, (d) 40, (e) 80 or (f) 160 mg Zn/kg and ^{65}Zn . The rats were returned to their previous diet and the whole-body retention of ^{65}Zn retention was monitored. The true amount of Zn absorbed was monitored by the method of Heth & Hoekstra (1965) (see p. 37). Values are means, with 2 standard errors of the mean represented by vertical bars, for four or five determinations.

10, 20, 40, 80 or 160 mg Zn/kg. The true percentage of the Zn absorbed was again estimated by the method of Heth & Hoekstra (1965).

A plot of the amount of Zn absorbed against the Zn content of the test meal is shown in Fig. 4. When dietary Zn content increased from 5 to 40 mg Zn/kg, the fractional absorption of Zn appeared to decrease although curvilinearity was not established statistically. Regression analysis of amount of Zn absorbed against Zn content of the meals showed that when dietary Zn content was increased from 40 to 160 mg Zn/kg, the amount of Zn absorbed increased linearly with increasing dose of dietary Zn ($P < 0.001$). These results are not inconsistent with the proposal that when dietary Zn concentration is < 40 mg Zn/kg, Zn may be absorbed by a mechanism exhibiting saturation kinetics. The linear relation between Zn absorption and dietary Zn content when dietary Zn is > 40 mg/kg may indicate that the capacity of the saturable process was exceeded and at high dietary Zn contents absorption occurred by a mechanism exhibiting kinetics compatible with a diffusion process.

DISCUSSION

It has been demonstrated that Zn absorption is decreased when Zn status has been elevated above normal levels and, conversely, is increased in states of Zn deficiency (Weigand & Kirchgessner, 1980). It is therefore clear that there are mechanisms controlling Zn absorption but, as yet, such mechanisms have not been defined fully.

The results of the present study indicate that Zn homeostasis is achieved by changes in both absorptive and excretory processes. Rats previously maintained on diets containing from 5 to 80 mg Zn/kg over a 14 d period absorbed decreasing proportions of Zn from an identical test meal given on the 15th day as dietary Zn content increased. However, rats

previously maintained on diets providing as much as 80 or 160 mg Zn/kg exhibited very similar fractional efficiencies of Zn absorption and thus appeared to exercise no discrimination against absorption of an excess of dietary Zn. It was also evident, first, that previous exposure to low or marginal dietary Zn (e.g. 5 or 10 mg/kg) greatly increased the biological $t_{1/2}$ of absorbed Zn and that a previous dietary excess of Zn accelerated body Zn loss. In the latter situation, it was thus clear that excretory rather than absorptive mechanisms were involved in regulating body Zn burden when Zn intake was excessive.

These results are essentially in agreement with those of Weigand & Kirchgessner (1980) who studied Zn absorption by a Zn balance and isotope dilution procedure using weanling rats maintained on diets of differing Zn contents. However, their techniques did not allow differences in absorption induced by homeostatic regulation and differences due to the possible saturable kinetic properties of the Zn absorptive process to be distinguished.

Evans *et al.* (1979), also using an isotope dilution procedure, examined the effects of dietary Zn status on Zn absorption and excretion. They concluded that Zn homeostasis was achieved primarily by changes in excretion since the intestinal mucosa from rats offered a higher dietary Zn content did not contain proportionally more ^{65}Zn than the mucosa from rats offered a lower dietary Zn content, 2.5 h after enteral administration of ^{65}Zn . However, these authors did not consider the possibility that the initial entry of ^{65}Zn into the mucosa may be at the point at which the amount of ^{65}Zn absorbed was regulated.

During the past few years, much interest has centred on the possible role of the protein, metallothionein, in Zn absorption. On the basis of a series of investigations including studies of ^{65}Zn absorption by isolated vascularly and lumenally perfused intestines, and parallel studies of ^{65}Zn -binding to mucosal metallothionein in rats whose Zn status was altered either by dietary manipulation or previous Zn loading by injection (Richards & Cousins, 1976, 1977*a, b*; Smith *et al.* 1978; Cousins, 1979*a, b*; Smith & Cousins, 1980), Cousins (1979*b*) has proposed that metallothionein is involved in the regulation of Zn homeostasis. The essential features of this concept are first that the metallothionein content of the intestinal mucosa reflects in some, as yet ill-defined manner, the total body Zn burden, and second that the fractional absorption of Zn presented to the intestinal mucosa is inversely related to the mucosal metallothionein content. Thus, when body Zn stores are high, Zn taken up by the mucosa is preferentially sequestered by the high concentration of thionein and only a small proportion is available for transfer across the basolateral membranes of the mucosal cells into the carcass. Conversely, when body Zn stores are low, as in Zn deficiency, little or no thionein is present in the absorptive cell and a higher fraction of the Zn entering the mucosal cells from the intestinal lumen is available for transcellular transfer.

While at first sight this is an attractive hypothesis, it should be pointed out that in most of these studies the doses of Zn labelled with ^{65}Zn presented to the intestinal lumen were low and no account was taken of possible differences in isotope dilution by different concentrations of endogenous tissue Zn in Zn-deficient normal and Zn-loaded rats (Evans *et al.* 1979). In contrast to the proposals of Cousins (1979*a, b*), Starcher *et al.* (1980) observed that Zn absorption in mice appeared to be directly proportional to intestinal metallothionein content when Zn-thionein was induced either by previous i.p. injection of increasing doses of Zn or by stress. Studies of Olafson (1983) of Zn absorption and Zn binding to intestinal metallothionein in Zn-deficient and Zn-replete mice also failed to provide evidence in support of Cousins' (1979*a*) hypothesis.

The results of the present study agree with the findings of Cousins and his co-workers (reviewed by Cousins, 1979*b*) in so far that Zn bound to intestinal metallothionein was apparently related to the Zn status of the rats (Fig. 3). When dietary Zn supply was deficient or only marginally adequate (5–10 mg Zn/kg) little or no Zn-thionein was detectable. However, as Zn supply increased from adequate to excessive (20–160 mg Zn/kg) there was a linear increase in Zn associated with the peak corresponding to metallothionein.

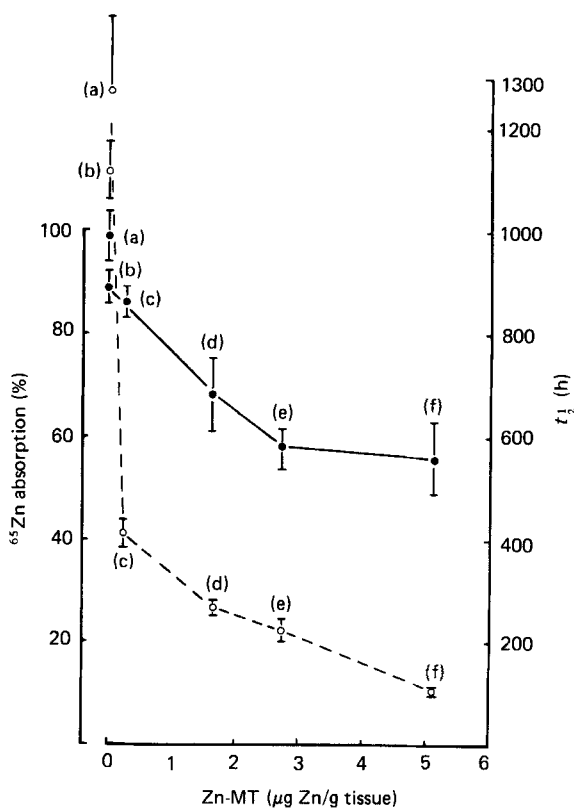


Fig. 5. The relation between zinc bound to intestinal metallothionein (Zn-MT) and percentage absorption of ^{65}Zn administered in a test meal containing 20 mg Zn/kg (●—●) and biological half-life ($t_{1/2}$) of body ^{65}Zn stores (○—○) in rats maintained before and subsequent to ^{65}Zn dosing on diets containing (a) 5, (b) 10, (c) 20, (d) 40, (e) 80 or (f) 160 mg Zn/kg (for details of methods, see p. 37). Values are means, with standard errors of the mean represented by vertical bars for four to eight determinations.

These findings are at variance with those of Flanagan *et al.* (1983) who reported that intestinal Zn-thionein levels exhibited only a transient decrease in mice receiving a Zn-deficient diet for 5 d, but after 10 d Zn-thionein levels rose to a level similar to that found in Zn-adequate controls. The authors suggested that this increase may have been due to 'stress-induced' synthesis of metallothionein in response to the deficiency state.

The results of the present study neither confirm nor conflict with Cousins' (1979*a*) hypothetical role for metallothionein in the homeostatic regulation of Zn absorption. The plot of percentage absorption of ^{65}Zn from the test meal *v.* Zn recovered in intestinal metallothionein in the rats maintained on the different dietary treatments indicated an apparently linear inverse relation when dietary Zn was varied between 5 and 80 mg Zn/kg (Fig. 5), as predicted by Cousins' (1979*a*) model. However, as dietary Zn was increased from 80 to 160 mg Zn/kg, this relation was no longer apparent; thus whereas the amount of Zn bound to metallothionein doubled, the extent of Zn absorption was unaffected (Fig. 5).

A possible role for metallothionein in modulating Zn excretion when dietary Zn contents exceed requirements for growth may be equally well inferred from our findings. Thus when dietary Zn contents were varied between 20 and 160 mg Zn/kg, $t_{1/2}$ of body Zn stores appeared to be inversely related to the intestinal Zn-metallothionein content, i.e. increased turnover and loss of body Zn may be a function of intestinal Zn-thionein levels (Fig. 5).

However, it should be pointed out that the estimates of intestinal Zn bound to metallothionein were of the steady-state levels of unlabelled Zn at the end of 24 h of dietary treatment. In order to distinguish between possible functional roles of metallothionein in intestinal Zn absorption or excretion, detailed isotope studies would be needed of changes in specific activity of luminal and body Zn pools and measurements of Zn flux rates through metallothionein and the absorptive and excretory pathways.

The effects of dietary Zn dose on Zn absorption

The apparent biphasic relation between Zn absorption and dietary dose of Zn, i.e. a possible curvilinear increase in the amount of Zn absorbed as the dietary Zn dose was increased from 5 to 40 mg Zn/kg and a linear relation in the dose range 40–160 mg Zn/kg, is similar to the kinetics of Zn binding to intestinal tissue observed by Davies (1980). In this latter study, ligated loops of rat duodenum were injected with saline containing increasing concentrations of Zn labelled with ^{65}Zn . Doses from 1 to 50 μg Zn exhibited saturation kinetics with an apparent Michaelis–Menten constant of 48.2 μg Zn/ml.

The results of the present study overall have shown that as dietary Zn supply in the rat is varied, Zn homeostasis in vivo is effected by changes in both Zn absorption and the rate of Zn loss from body stores. However, when dietary Zn content is excessive (> 80 mg Zn/kg), body Zn burden appears to be modulated entirely by changes in Zn excretion. The amount of intestinal Zn bound to metallothionein appears to be linearly related to dietary Zn supply although the results of the present study do not allow its possible roles, if any, in the control of Zn absorption or excretion to be distinguished.

In rats of normal Zn status, the extent of Zn absorption indicated that in the range 5–40 mg Zn/kg, Zn may have been absorbed by a saturable process. However, when dietary Zn content was > 40 mg Zn/kg, the capacity of this process appeared to be exceeded and the amount of Zn absorbed was linearly related to dietary Zn content.

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