

## Relationship Between Plasma Zinc, Angiotensin-converting Enzyme, Alkaline Phosphatase and Onset of Symptoms of Zinc Deficiency in the Rat

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### Abstract

Recent evidence suggests that changes in plasma zinc concentration may play a central role in the development of early lesions of zinc deficiency. The aim of the following work was to better understand events occurring in plasma during the onset of zinc deficiency, and to investigate biochemical mechanisms by which plasma zinc may exert its effects. Fifty male weanling rats of 90 g weight were allocated to five treatment groups of ten rats each. Treatments were: 1, zinc deficient, mixed diet (1-2 mg Zn per kg); 2, zinc deficient, self-select diet; 3, zinc repleted; 4, control, pair fed; 5, control, *ad libitum* fed. With the exception of treatment 1, which consisted of a 25% casein diet, all rats were offered protein as a separate component of the diet. Control rats received zinc in the drinking water (100 mg l<sup>-1</sup>). The sequence of events following initiation of zinc deficiency were: reduced plasma zinc concentration (2 days), reduced plasma angiotensin-converting enzyme and alkaline phosphatase activities (3-4 days), reduced feed intake and growth (5-6 days) and reduced percentage protein intake (12 days). Plasma zinc concentration in the deficient rats was inversely correlated with the growth rate of the rat over the previous 24 h. Zinc repletion resulted in marked overshoot in plasma zinc concentration (300%) and converting-enzyme activity (150%) within 24 h, but a return to normal within 72 h. Alkaline phosphatase activity responded likewise, albeit more slowly. Protein self selection had no effect on the manifestations of zinc deficiency, although reduced protein intake was associated with lower plasma zinc concentration. The results provide evidence of a role for plasma zinc in the development of early clinical signs of zinc deficiency, possibly acting biochemically through reduced activity of zinc-dependent peptidases such as angiotensin-converting enzyme.

**Keywords:** Zn-deficiency, zinc, alkaline phosphatase, angiotensin-converting enzyme, protein self selection, growth, appetite, protein intake.

### Introduction

Zinc deficiency in rats is characterized initially by loss of appetite and poor growth (Underwood 1977). A search for biochemical mechanisms to explain these defects in metabolism has been largely unsuccessful, despite the discovery of a large number of zinc metalloenzymes (Chesters 1982). Part of the problem in understanding how zinc exerts its effects is that zinc is firmly retained within the cells of most soft tissues during zinc deficiency, and the anorexia and reduced growth occur at a time when zinc concentration and zinc-metalloenzyme activities in these tissues are still normal (Underwood 1977). In most cases, only plasma zinc concentrations are reduced at these early stages of zinc deficiency, and this observation led O'Dell to postulate that plasma and extracellular zinc are necessary for cell membrane stability, and as such play a central role in the development of early lesions of zinc deficiency (O'Dell 1981). An alternative explanation, however, is provided by

the recent discovery that plasma angiotensin-converting enzyme (ACE) (EC 3.4.15.1) and kininase I require zinc for normal activity *in vitro* and *in vivo* (White *et al.* 1985, 1986; Reeves and O'Dell 1985). On this evidence it can be argued that a failure to maintain plasma zinc concentrations within the normal range will result in disturbances in hormone metabolism, since many circulating peptide hormones are metabolized by metallopeptidases in plasma and extracellular fluids. For example, ACE and kininase I will act on angiotensin I, bradykinin, substance P and enkephalins (Erdos and Gafford 1983). While it is doubtful that a reduced activity of ACE alone could explain all the symptoms of zinc deficiency, it is noteworthy that in humans the administration of captopril, an ACE inhibitor, results in symptoms typical of zinc deficiency, such as dysgeusia and skin rashes (Rotmensch *et al.* 1982). In addition, zinc-deficient rats show many symptoms that could be related to defects in the renin-angiotensin system and the kallikrein-kinin system. These include reduced blood pressure in pregnant (O'Dell *et al.* 1977) and male (White *et al.* 1985) rats, abnormal haemostasis (O'Dell *et al.* 1977) and a disturbed water and electrolyte balance (O'Dell 1981).

The aim of the present work was to investigate the temporal relationships between plasma zinc concentration, the activities of angiotensin-I-converting enzyme and alkaline phosphatase, and time of onset of early manifestations of zinc deficiency. Self selection of protein was also examined because others have shown that zinc-deficient rats offered diets containing either 10 or 50% soybean protein were able to increase their level of feed intake by selecting more of the low protein diet (Reeves and O'Dell 1981). It was of interest to know if this process worked in rats fed a casein-based diet, and if the changes in protein intake resulted in changes in total zinc status or in plasma zinc or zinc-dependent enzymes.

**Table 1. Treatment groups**

Abbreviations for the diets and self-select components are: M, 25% casein (1.6 mg Zn per kg); CF, casein-free (1.2 mg Zn per kg); C, 90% casein (1.9 mg Zn per kg)

Treatment group	Zn in drinking water (mg/L)	Dietary regimen
1 LoZn, mixed diet	0	M, <i>ad libitum</i>
2 LoZn, self select	0	C+CF, <i>ad libitum</i> , self select
3 Lo/HiZn, self select	0/100	C+CF, <i>ad libitum</i> , self select
4 HiZn, pair fed	100	C+CF, pair fed to 2
5 HiZn, <i>ad lib.</i> self select	100	C+CF, <i>ad libitum</i> , self select
To (killed on day 1, pretreatment)	—	C+CF, <i>ad libitum</i> , self select

## Materials and Methods

### *Treatments and Animals*

Following a 6-day period of dietary adjustment, 50 male SPF Sprague-Dawley rats weighing 90–100 g were allocated to five treatment groups of ten rats each (Table 1). An additional group of eight rats was killed on day 1 of the experiment to serve as a pretreatment control. During the adjustment period, rats were offered casein as a separate component of the diet (see Table 2). The diets were zinc deficient (containing 1–2 mg Zn per kg), and ZnSO<sub>4</sub> was provided at 30 mg Zn per L in the distilled drinking water during the adjustment period.

The experimental period lasted 15 days and the treatment groups were as follows (see Table 1): 1, zinc deficient, 25% casein diet; 2, zinc deficient, self-select regimen; 3, zinc deficient for 10 days followed by zinc repletion, self-select regimen; 4, control, pair fed both casein and casein-free component of treatment 2; 5, control, *ad libitum*, self-select regimen. Repleted and control rats received 100 mg Zn per L in the distilled drinking water.

All rats were kept at 25°C and 55% relative humidity in individual plastic cages with stainless steel grid lids.

### Diets

The composition of the diets is shown in Table 2. The diets and self-select components were prepared as dry biscuits. On the basis of previous experience, cellulose (10%) was added to the casein to make it more friable and acceptable to the rats. The total diet and the protein and protein-free components all contained between 1 and 2 mg Zn per kg, as determined by atomic absorption spectroscopy after dry ashing (Roth and Kirchgessner 1974). The casein-free and 90% casein diets were coloured with amaranth blue and red food dye, respectively, to assist in the separate collection of residues. These dyes did not contribute any zinc to the diets. The casein contained 96% crude protein (Kjeldahl N  $\times$  6.25) and the casein diet contained 18.2 kJ g<sup>-1</sup> gross energy (bomb calorimetry). The mixed diet contained 20 kJ g<sup>-1</sup>, and the casein-free component 23 kJ g<sup>-1</sup>, gross energy.

**Table 2. Dietary composition (%)<sup>A</sup>**

	Mixed diet (M)	Components for self-select regimen	
		Casein-free (CF)	90% Casein (C)
Casein	25	—	90
Sucrose	29	38.7	—
Starch	28.3	37.7	—
Fat (Palmin, coconut oil)	8.7	11.6	—
Cellulose (B.OO)	3	4.0	10
Vitamins	2	2.7	—
Minerals	4	5.3	—
Gross energy (kJ/g)	20	23	18.2

<sup>A</sup> See Pallauf and Kirchgessner (1971) for a description of the method of preparation of the EDTA-extracted casein and the mineral and vitamin mixtures.

### Sampling and Analysis

Rats were weighed daily at the same time as feed residues were collected. Plastic water bottles were weighed and refilled twice weekly.

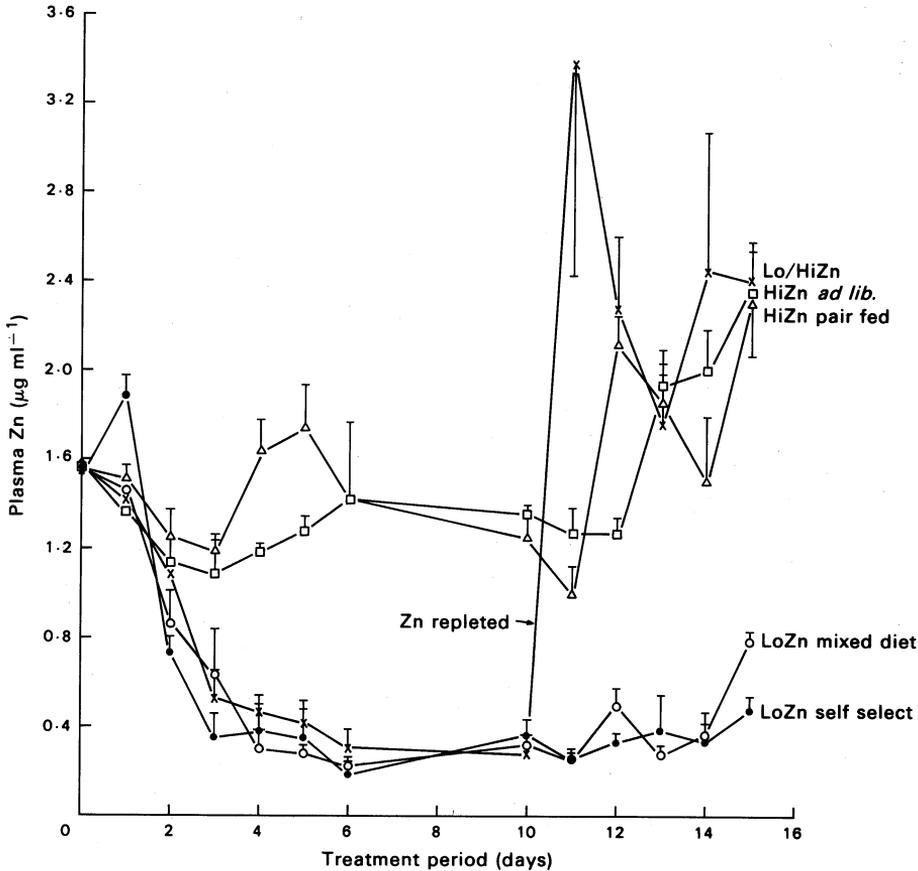
Blood samples of 50–200  $\mu$ L were taken from the retro-orbital site at 10 a.m. on days 1–6 and 10–14 from each of three rats per group. The samples were taken using a hematocrit tube moistened with heparin–saline solution (1600 USP mL<sup>-1</sup>) (Carl Roth; Karlsruhe) after the rats were anaesthetized with ether. In some cases feed intake was reduced during the 24 h following blood sampling and so growth and feed-intake data for all bled rats were statistically assigned as missing for the day of sampling. Blood was immediately centrifuged (1500  $\times$  g for 4 min) and plasma was removed and stored in 50 or 100  $\mu$ L aliquots at  $-20^{\circ}$ C prior to enzyme assays. On day 1, the eight pretreatment control rats, and on day 15 all experimental rats, were killed by decapitation under ether anaesthesia following a partial overnight fast (50% of the average daily intake over the previous 5 days). Blood was collected from the neck into heparinized 1.5 mL Eppendorf tubes and immediately processed as described above. The gastrointestinal tract, pelt, paws, head and tail were removed and the remaining carcass was freeze dried, finely ground in a stainless steel coffee grinder and analysed for fat (7-h ether extraction of 3 g dry material in a Soxhlet apparatus) and zinc (Roth and Kirchgessner 1974).

Zinc was determined directly on diluted plasma samples (1 : 6 in distilled water) using flame atomic absorption spectroscopy, and read against aqueous standards containing 5% glycerol. Plasma alkaline phosphatase activity was assayed at 25°C using p-nitrophenylphosphate as substrate (Boehringer-Mannheim kit No. 415278). One unit of activity represents 1 micromole of nitrophenol formed per minute per litre at 25°C.

Plasma ACE activity was determined spectroscopically using a modification of the method of Cushman and Cheung (1971) with hippuryl-L-his-L-leu as substrate (Rosenthal *et al.* 1984). One unit of plasma ACE activity is the amount of enzyme required to catalyse formation of 1 nanomole of hippuric acid per mL per min at 37°C. Insufficient blood was available on every occasion for analysis of each of the three rats at each of the daily blood samplings. However, all rats were assayed on day 15.

### Statistical Analyses

One-way analysis of variance (AOV) and regression analysis were used to test for significant treatment effects. Comparison between means was by Duncan's Multiple Range Test ( $P < 0.05$ ) (Snedecor and Cochran 1967).



**Fig. 1.** Concentration of zinc in plasma of zinc-deficient, repleted and control rats. Each point represents three rats ( $\bar{x} + \text{s.e.m.}$ ) and symbols represent the following treatments: ○, 1; ●, 2; ×, 3; △, 4; □, 5.

## Results

### Plasma Zinc and Enzymes

With low zinc diets, the concentration of zinc in plasma fell rapidly during the first 3 days of the experiment and reached a constant low level after 4–6 days (Fig. 1). There was no significant effect of protein self selection on the rate of decline or equilibrium level of plasma zinc up until day 14. However, on day 15, plasma sampled from slaughtered rats fed the mixed diet (LoZn, mixed diet) had a 63% higher concentration of zinc than plasma from those fed the self-select diets (LoZn, self select) ( $P < 0.05$ ).

Until day 6, the variation in plasma zinc concentration between the 30 individual rats fed the zinc-deficient diets could not be explained on the basis of previous 24-h feed intakes or growth rates. However, for the zinc-deficient rats between day 10 and day 14 (inclusive) there was a significant negative relationship between the concentration of zinc in plasma

(Z,  $\mu\text{g mL}^{-1}$ ) and the previous 24-h growth rate (G,  $\text{g day}^{-1}$ ). The regression equation was  $Z = 0.3 - 0.02G$ ;  $r = -0.60$ ,  $\text{s.e.}(x) = 0.006$ ,  $P = 0.003$ .

Repletion with zinc on day 10 caused an immediate increase in plasma zinc concentration (up to three-fold those of continuously supplemented control rats), eventually declining to control values after 48–72 h (Fig. 1). There was evidence of a gradual increase in the concentration of zinc in the plasma of control rats over the duration of the experiment, especially after day 10. Values at day 15 were 50% higher than at the beginning of treatment (day 0). This increase is attributed to the relatively high zinc intakes from drinking water (Table 3).

**Table 3. Main effects of zinc deficiency on growth rate, DMI, water and protein intake ( $\bar{x} \pm \text{s.e.m.}$ )** Efficiency of feed conversion (EFC) is calculated as weight gain/DMI. Zinc intake calculated using water intakes. Values within rows with common superscripts are not significantly different ( $P > 0.05$ )

	Treatment number and type of diet					
	1 LoZn Mixed diet	2 LoZn Self select	3 LoZn Self select	3 HiZn (Self select)	4 HiZn Pair fed (Self select)	5 HiZn <i>ad libitum</i> (Self select)
Growth rate ( $\text{g d}^{-1}$ )						
Day 1–10	$2.7 \pm 0.1^a$	$2.6 \pm 0.2^a$	$2.8 \pm 0.2^a$	—	$2.6 \pm 0.3^a$	$4.7 \pm 0^b$
Day 10–15	$0.7 \pm 0.4^{ab}$	$0.1 \pm 0.4^a$	—	$4.8 \pm 0.5^c$	$1.4 \pm 0.4^b$	$4.4 \pm 0.2^c$
Dry matter intake ( $\text{g d}^{-1}$ )						
Day 1–10	$10.1 \pm 0.2^a$	$9.5 \pm 0.3^a$	$10.0 \pm 0.4^a$	—	$9.5 \pm 0.3^a$	$11.3 \pm 0.3^b$
Day 10–15	$9.2 \pm 0.4^a$	$7.9 \pm 0.4^a$	—	$12.6 \pm 0.6^a$	$8.1 \pm 0.5^a$	$12.4 \pm 0.4^b$
Casein selected (%)						
Day 1–10	(25)	$22.0 \pm 1.2$	$23.1 \pm 1.9$	—	$21.9 \pm 1.1$	$21.9 \pm 1.2$
Day 10–15	(25) <sup>a</sup>	$19.1 \pm 1.8^b$	—	$23.9 \pm 1.6^a$	$19.0 \pm 1.8^b$	$27.9 \pm 0.8^a$
Water intake ( $\text{ml d}^{-1}$ )						
Day 1–10	$16.9 \pm 0.7^{ac}$	$21.2 \pm 1.4^b$	$24.8 \pm 3.1^b$	—	$15.2 \pm 1.0^c$	$17.2 \pm 1.7^a$
Day 10–15	$18.8 \pm 1.7^{ab}$	$26.0 \pm 3.0^b$	—	$24.8 \pm 4.1^b$	$15.6 \pm 1.5^a$	$36 \pm 6.0^c$
EFC ( $\text{g g}^{-1}$ )						
Day 1–10	$0.27 \pm 0.01^a$	$0.27 \pm 0.02^a$	$0.28 \pm 0.02^a$	—	$0.27 \pm 0.03^a$	$0.41 \pm 0.01^b$
Day 10–15	$0.08 \pm 0.04^{ab}$	$0.01 \pm 0.06^a$	—	$0.38 \pm 0.05^c$	$0.17 \pm 0.04^b$	$0.36 \pm 0.01^c$
Zinc intake ( $\text{mg per kg DM}$ )						
Day 1–10	—	—	—	—	160	150
Day 10–15	—	—	—	200	190	290
Carcass Zn ( $\text{mg per kg}$ fat-free DM)	$74 \pm 1^a$	$73 \pm 2^a$	—	$106 \pm 3^b$	$112 \pm 1^c$	$112 \pm 1^c$

Changes in the activity of plasma AP tended to reflect changes in the concentration of zinc in plasma (Fig. 2). However, there was a major exception to this during the last 2 days of sampling, where AP activity decreased in the plasma of pair-fed control rats while zinc concentration remained high. There was no significant correlation between zinc concentration and AP activity in plasma from rats killed on day 15, nor was there any significant correlation between the activity of AP in plasma and the previous 24-h feed intake during the period from day 10 to 14.

The activity of ACE in plasma was reduced by 50% after 4 days of zinc depletion, regardless of feeding situation (Fig. 3). In contrast to the results for AP, changes in the

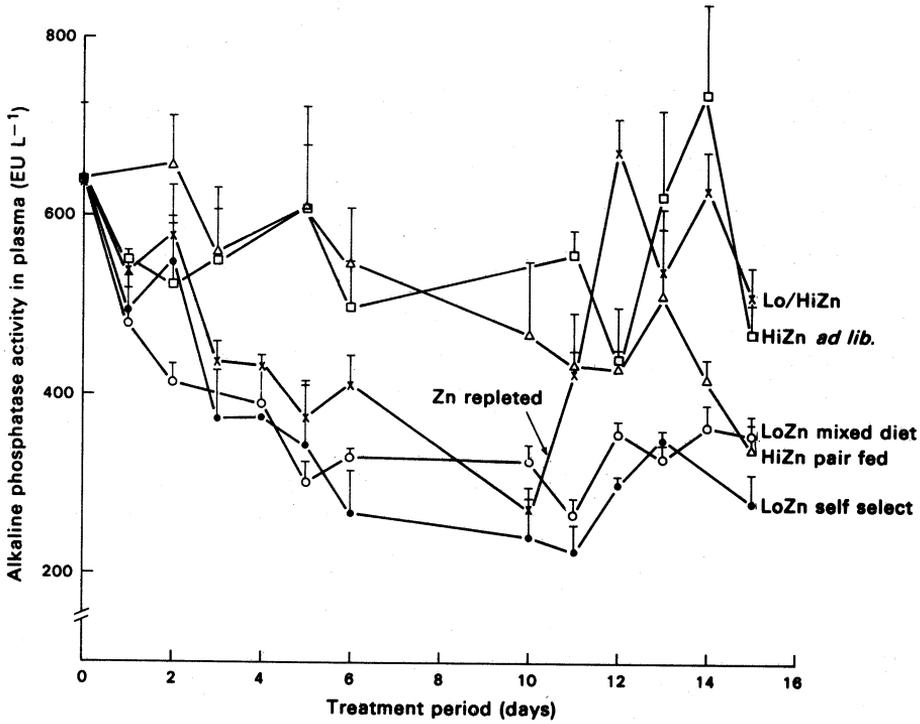


Fig. 2. Alkaline phosphatase activity in plasma of zinc-deficient, repleted and control rats. Each point represents three rats ( $\bar{x}$  + s.e.m.) and symbols are as for Fig. 1.

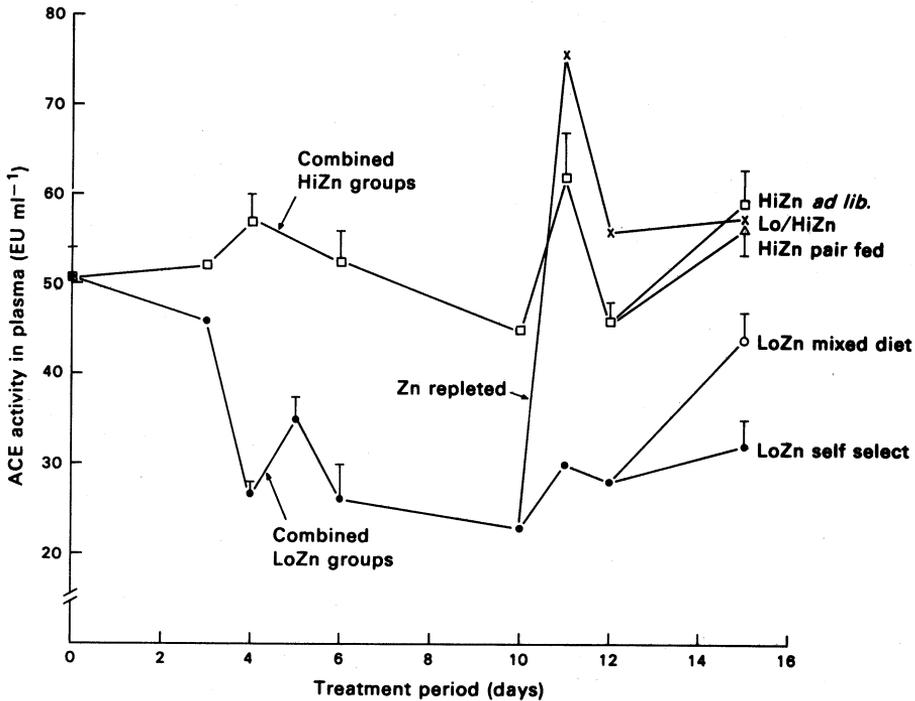


Fig. 3. Angiotensin-converting enzyme activity (ACE) in plasma of zinc-deficient, repleted and control rats. Prior to day 15, results were pooled for the two zinc-deficient groups and for the two control groups. Symbols are as for Fig. 1.

activity of ACE closely reflected temporal changes in plasma zinc. For example, on day 15 deficient rats offered the self-select diets had ACE activities that were 33% lower than deficient rats fed the mixed diet—a situation similar to that for plasma zinc concentration. A plot of the concentration of zinc in plasma versus the activity of ACE (on day 15) was best described by a Mitscherlich equation, with a plateau in activity at plasma zinc concentrations above 1 mg L<sup>-1</sup> (Fig. 4).

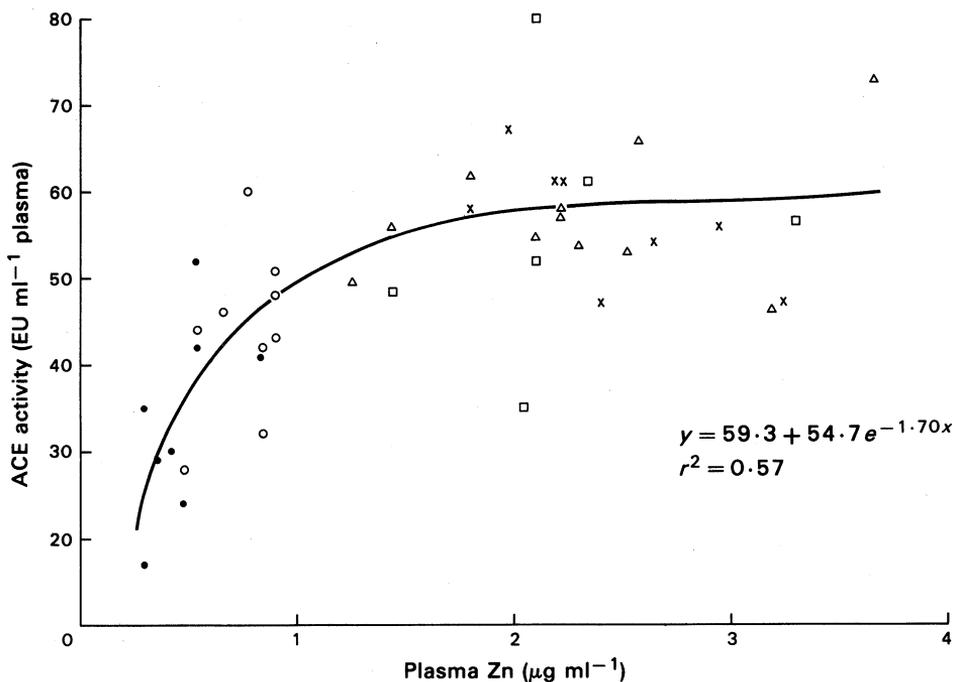


Fig. 4. Relationship between ACE activity and zinc concentration in plasma from rats at slaughter. Symbols are as for Fig. 1.

#### Growth Rate, Feed and Protein Intake

The main effects of zinc deficiency on feed intake and growth are summarized in Table 3. Zinc-deficient rats apparently drank more water than pair-fed controls but variation due to type of diet and time on treatments were greater than the effects of zinc deficiency. It was therefore difficult to draw any firm conclusions about the effects of zinc deficiency on water intake. Self selection of protein had no effect on fat-free carcass zinc concentration in zinc-deficient rats. Rats killed on day 1 had carcass zinc concentrations of  $105 \pm 1$  mg kg<sup>-1</sup>, and over the 15-day experimental period zinc-supplemented rats increased carcass zinc by only 6%, despite a rise in plasma zinc of 50%.

Growth virtually ceased after 6–8 days of zinc deficiency (Fig. 5), although an initial decline in both growth rate and feed intake (dry matter, DMI) was evident by day 5 (Figs 6–8). This effect of zinc deficiency on growth and feed intake occurred irrespective of whether rats were offered the mixed diet or were allowed to self select. Taken over the 15-day experimental period, the reduced growth rate of zinc-deficient rats was primarily associated with a reduced level of feed intake (Table 3). However, efficiency of feed conversion (EFC, growth ÷ DMI) was also reduced as shown by the fact that a test for homogeneity of slopes for the regression of dry matter intake (DMI, g day<sup>-1</sup>) on growth rate (GR, g day<sup>-1</sup>) for days 10–14 of the experiment indicated that treatment had no effect on slope ( $P = 0.85$ ), but that intercepts were significantly different between zinc-deficient

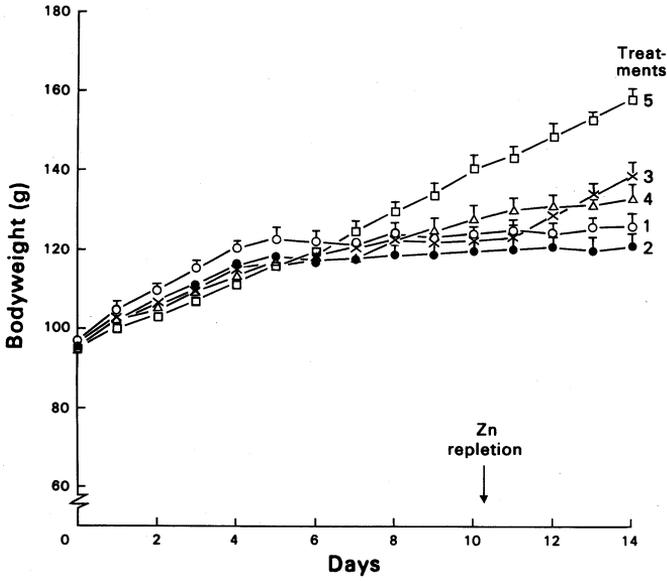


Fig. 5. Growth of rats. Symbols are as for Fig. 1.

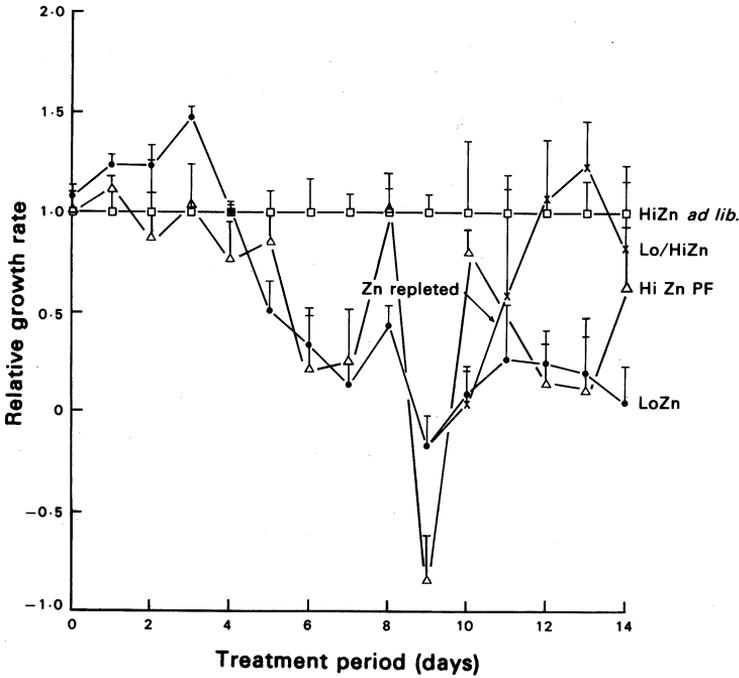


Fig. 6. Growth rate of rats as a proportion of the corresponding daily mean growth rate of *ad libitum* controls (i.e. treatment 5 has a relative growth rate of 1.0). Points are ( $\bar{x}$  + s.e.m.) for all animals in each group (excluding bled rats). Up until day 10, zinc-deficient rats are represented as a single group (treatments 1-3). After day 10, treatments 1 and 2 are combined. Symbols represent the following treatment groups: ●, 1 and 2; ×, 3; △, 4; □, 5.

and control groups ( $P < 0.001$ ). It was estimated from these regression equations that zinc-deficient rats met maintenance requirements for energy (when  $GR = 0$ ) at a DMI of  $8.2 \pm 0.4 \text{ g day}^{-1}$ , whereas *ad libitum* and pair-fed control rats were able to meet maintenance requirements with  $6.3 \pm 0.7 \text{ g day}^{-1}$ . Metabolic size of the *ad libitum* control rats was 11% greater than that of pair-fed controls, and so the *ad libitum* rats should have shown a higher maintenance energy (intake) requirement. However, the pair-fed rats had large variations in day-to-day growth rates over the 4-day sampling period, and this masked any differences in liveweight.

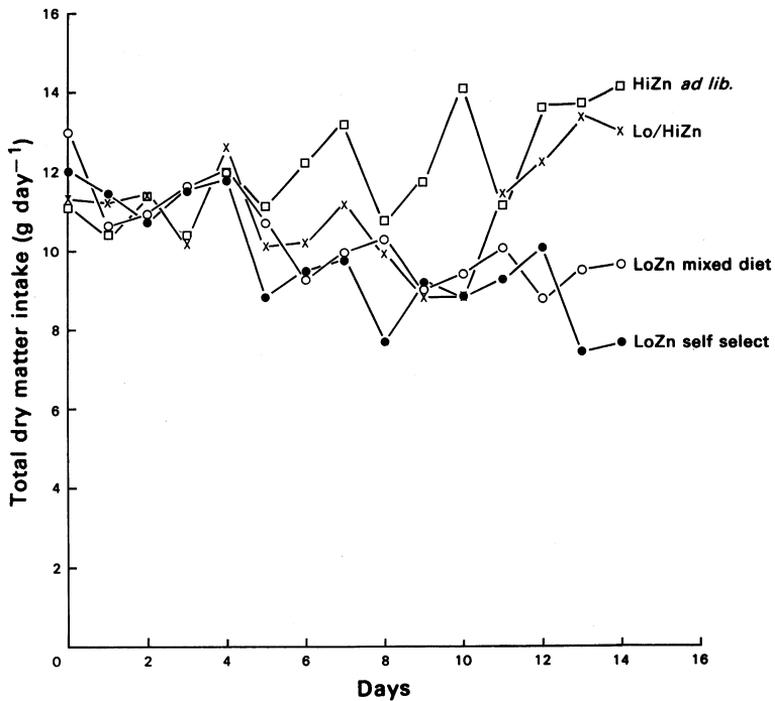


Fig. 7. Dry matter intakes. Symbols are as for Fig. 1.

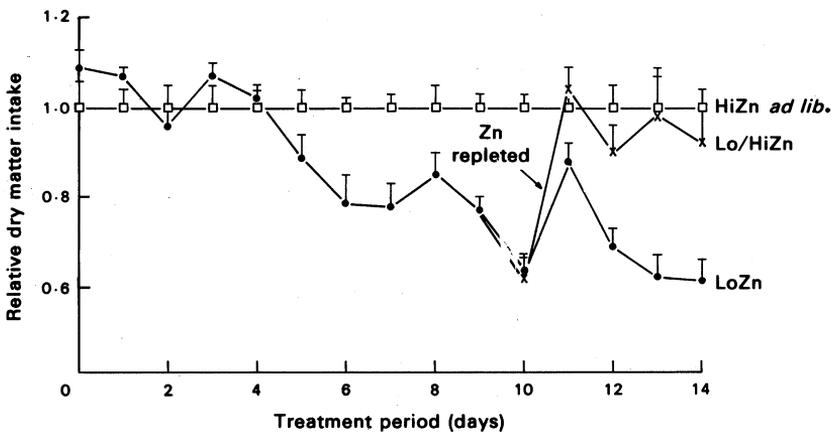


Fig. 8. Dry matter intake of rats as a proportion of the daily mean intake of *ad libitum* controls (treatment 5 = 1.00). Symbols are as for Fig. 6.

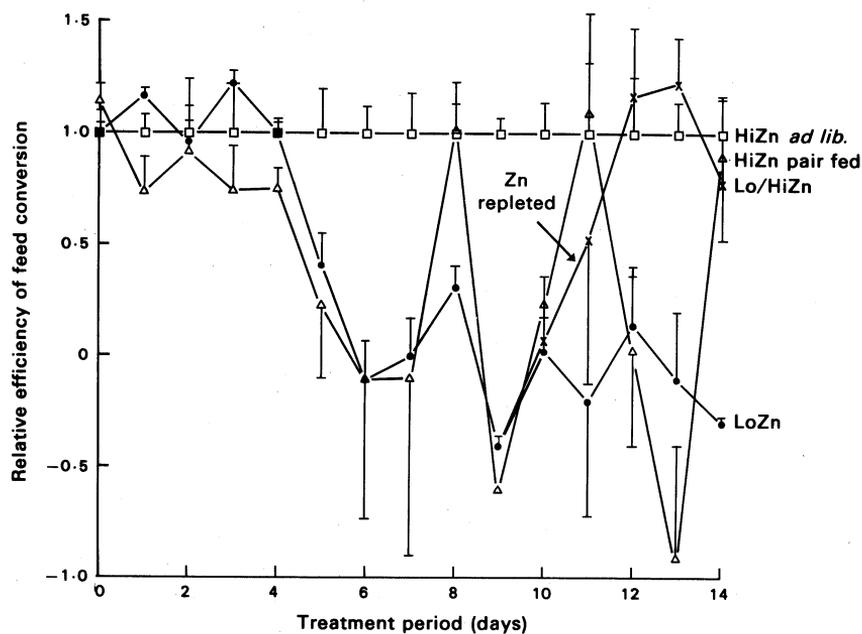


Fig. 9. Relative efficiency of feed conversion (treatment 5 = 1.00). Symbols ( $\bar{x}$  + s.e.m.) are as for Fig. 6.

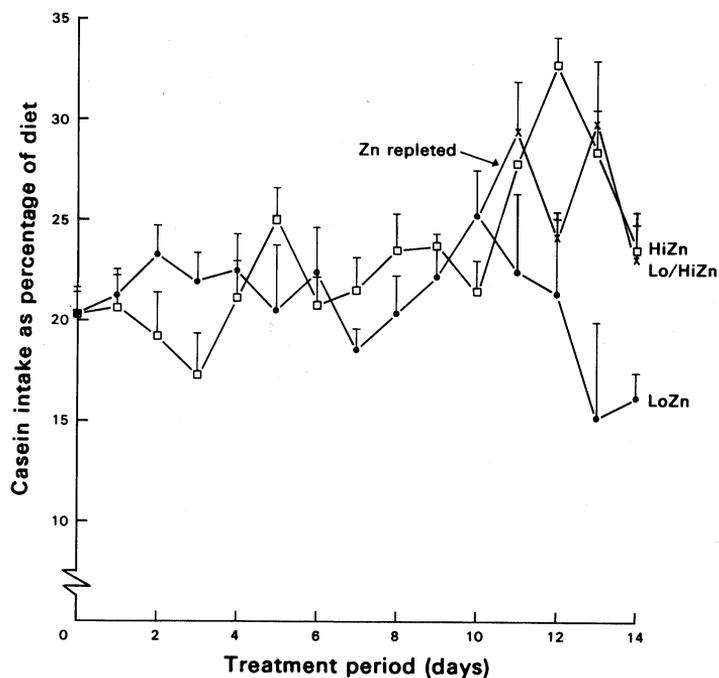


Fig. 10. Casein intake expressed as percentage of total diet. Symbols represent the following treatments: ●, 2; X, 3; □, 5.

The first appearance of a reduced efficiency of feed conversion was seen by day 5 (Fig. 9), and this was coincident with a reduction in feed intake. Pair-fed control rats showed a similar decline in EFC to that of zinc-deficient rats at this time. Repletion of group-3 rats with zinc on day 10 resulted in an immediate increase in feed intake (Figs 7 and 8) and EFC (Fig. 9). During the 5-day repletion period, repleted rats grew at the same rate as controls (Table 3).

For rats allowed to self select, the initial loss of appetite due to zinc deficiency was characterized by a reduction in intake of both the 90% casein and casein-free diet, such that per cent protein intake was unaffected. Protein selection averaged about 22% of the diet for all self-select groups from day 1 until day 10, with no treatment differences being apparent (Fig. 10). After day 10, zinc-deficient rats tended to reduce their protein intake (both in absolute and percentage-of-diet terms) while control rats increased theirs. The result was a significant reduction in per cent protein selected by day 15 in deficient rats compared with control rats ( $P < 0.05$ ).

## Discussion

The results provide evidence of a causal relationship between changes in plasma zinc concentration and the initial appearance of pathological manifestations of zinc deficiency. In deficient rats, plasma zinc concentration decreased to 50% of control values within 2–3 days and reached a stable level of 25% of control values by day 6. The initial sharp decrease in plasma zinc concentrations preceded the fall in plasma enzyme activities by about 2 days, with ACE and AP activities falling to 50% of control values by days 4 and 5, respectively. Feed intake, growth rate and EFC all decreased simultaneously after day 4, coincident with the plasma enzyme activities reaching a low plateau. The close temporal relationships and the significant correlation between plasma zinc concentration and ACE activity are evidence of a possible biochemical link between changes in plasma zinc concentration and the early clinical signs of zinc deficiency. Thus, the reduced ACE activity together with a reduced activity in plasma of other zinc-dependent peptidases such as kininase-I and carboxypeptidases A and B (Mills *et al.* 1967) may be directly responsible for the reduced feed intake and reduced growth seen early in zinc deficiency. Both ACE and kininase-I are known to catalyse reactions involving bradykinin, substance P and enkephalins (Erdoş and Safford 1983). Among other functions, enkephalins are involved in the maintenance of meal eating (Della-Fera and Baile 1984), while bradykinin acts on prostaglandin synthesis and interconversion (Liebermann and Arold 1980). Thus any disturbance in bradykinin or enkephalin metabolism might be expected to result in reduced feed intake and abnormal protein metabolism, hence reduced growth and efficiency of feed conversion. The question as to whether the decline in the activity of ACE during zinc deficiency is sufficient to cause changes in the level of circulating peptide substrates is partly answered by the work of Ercan *et al.* (1979), who showed a marked decrease in the rate of conversion of angiotensin-I to angiotensin-II in the lung of zinc-deficient rats. Reduced blood pressure in zinc-deficient male (White *et al.* 1985) and female (O'Dell *et al.* 1977) rats gives further evidence of a role for peptidases in the etiology of symptoms of zinc deficiency.

In the current experiment, ACE activity responded rapidly to zinc depletion and repletion. This temporal relationship, together with previously reported results (White *et al.* 1986) concerning the *in vitro* activation of ACE by zinc repletion, indicates the existence of an apoenzyme in plasma the synthesis of which is independent of zinc supply, but with activity dependent on levels of circulating plasma zinc. In fact, the overshoot in ACE activity seen in replete rats suggests a feedback may be operating to increase synthesis or secretion of ACE apoenzyme in plasma of zinc-deficient animals. This is in contrast to AP activity, which was affected by level of feed intake and responded relatively slowly to changes in zinc intake. This supports the observations of others that AP requires zinc for synthesis (Luecke

*et al.* 1968) and that plasma AP activity is affected by factors other than zinc intake (Luecke *et al.* 1968; Macapinlac *et al.* 1966). The lack of correlation between AP activity and growth rate suggests that AP is not directly involved in the early symptoms of zinc deficiency.

The highly significant inverse correlation between plasma zinc concentration and the previous day's growth rate in zinc-deficient rats indicates that plasma zinc pools are labile and respond rapidly to changes in the rate of tissue accretion. Thus, the results show that daily variations in plasma zinc levels in deficient rats are primarily a function of the balance between catabolic and anabolic processes occurring in tissues, and not due to changes in daily feed (and hence zinc) intakes. There was some indirect evidence that protein intake may be involved in this phenomenon, since there was a 40% difference in plasma zinc levels between self-select zinc-deficient rats and deficient rats fed the mixed diet only at the end of the experiment when mean protein intakes in the self-select group fell to 15% of the diet. Prior to that, self-select rats chose a diet of greater than 20% casein, and plasma zinc levels were not significantly lower than in deficient rats fed the 25% casein diet.

A possible direct effect of zinc deficiency on appetite could be distinguished from an indirect effect (via some defect in protein synthesis, for example), by showing whether anorexia or reduced EFC occurred first. The experiment of Faraji and Swenseid (1983) clearly showed that zinc deficiency has little effect on EFC, and that its effects on growth are almost entirely due to inappetence. This was in contrast to the results of Weigand and Kirchgessner (1977), who demonstrated a reduction in growth rate in zinc-deficient rats before feed intake declined. In the current experiment both events occurred simultaneously and it was not possible to differentiate between the timing of the appearance of reduced growth and reduced feed intake. Since force feeding was not undertaken, it was not possible to determine a cause and effect relationship between anorexia and reduced growth in zinc deficiency. This aspect of zinc deficiency requires further investigation. It should be pointed out that the diets used in this experiment, and that used by Weigand and Kirchgessner (1977), are likely to be low in essential fatty acids. However, no symptoms of fatty acid deficiency were seen in control animals in this experiment, and none were reported by Weigand and Kirchgessner (1977).

With regard to effects of zinc deficiency on protein intake, a decline in protein intake did not begin to occur until after day 10, suggesting that this effect was secondary to the effects on feed intake and growth. There was no difference between the carcass zinc concentration of rats fed the 25% casein diet and those allowed to self-select protein, and there was no difference in plasma zinc concentration or enzyme activities until the last day of the experiment. At this sampling, rats fed the mixed diet had 40% higher plasma zinc and ACE levels. This change in plasma zinc concentration in response to reduced protein intake is the opposite of that seen in a previous self-selection experiment (White 1985), and therefore is difficult to interpret. Reeves and O'Dell (1981) suggested that the reduced protein intake in zinc deficiency may represent an example of an adaptation to a direct appetite-depressing effect of protein, presumably through increased levels of circulating amino acids. In the current experiment, zinc-deficient rats offered a free choice of protein did not increase their level of feed intake when protein intake decreased, and it is therefore unlikely that protein alone was contributing to the anorexia. The non-growing zinc-deficient rat fed a diet of 25% casein is ingesting six times its protein requirements (Anon. 1972), and there is little doubt that the disposal of the excess nitrogen as urea represents an unnecessary energy cost (3 mol ATP per mol urea formed from ammonia) (McGilvery 1970) and would contribute to the reduced EFC. There are several other possible reasons for the observed effects, but further discussion of this aspect of the experiment would be speculative in the absence of more appropriate data.

In conclusion, the results provide evidence of a possible biochemical role for plasma zinc in the early clinical signs of zinc deficiency. The proposed mechanism(s) involve the activation of zinc-dependent peptidases such as ACE.

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