

The similarity between alkaline phosphatase (EC 3.1.3.1) and phytase (EC 3.1.3.8) activities in rat intestine and their importance in phytate-induced zinc deficiency

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1. The activities of alkaline phosphatase (EC 3.1.3.1) and phytase (EC 3.1.3.8) were similarly distributed in the small intestine of rats. Regional differences in activity were reflected by similar differences in the capacity of ligated intestinal segments to hydrolyse phytate *in vivo*. Activities were greatest in the duodenum and lowest in the terminal ileum.
2. Specific activities of both enzymes were tenfold greater in the brush border fraction of duodenal mucosa compared with entire mucosal homogenates.
3. Brush-border alkaline phosphatase and phytase activities required both magnesium and zinc ions for maximal activity.
4. Zn deficiency induced by feeding a diet low in Zn (0.5 mg Zn/kg) caused similar reductions in activity of both enzymes.
5. Zn deficiency induced by feeding diets marginally adequate in Zn (12 mg/kg) and phytate (10 g/kg) caused reductions in alkaline phosphatase, phytase activities and phytate hydrolysis *in vivo*.
6. It is suggested that phytase activity is a manifestation of alkaline phosphatase and the significance of this in relation to phytate-induced Zn deficiency is discussed.

The nutritional importance of phytic acid (*myo*-inositol hexaphosphate) in limiting the availability of essential dietary minerals calcium, zinc, manganese, iron and copper has recently been emphasized (Davies & Nightingale, 1975; Oberleas, 1973). Few studies however, have been made on the metabolism of phytate by the intestinal mucosa. Patwardhen (1937) demonstrated that the mucosa of rat intestine can hydrolyse phytic acid with a concomitant release of inorganic phosphate, albeit at a very slow rate. Incubation for up to 32 h was employed before appreciable amounts of phosphate were released. The author was unable to determine whether the hydrolysis was catalysed by a specific 'phytase' (*myo*-inositol hexaphosphate phosphohydrolase; EC 3.1.3.8) or by non-specific phosphatases present in the mucosa. Reinhold, Pascoe, Arslanian & Bitar (1970) studying a number of Zn-dependent enzymes in rat intestine, showed a relationship existed between the mucosal Zn content and 'phytase' activity although this was not so close as that between Zn content and alkaline phosphatase (EC 3.1.3.1).

Intestinal mucosal alkaline phosphatase activity is known to be Zn-dependent and in the rat, Zn deficiency brings about a marked reduction in enzyme activity (Williams, 1972). It has been suggested that 'phytase' activity may be a manifestation of alkaline phosphatase (Pileggi, 1959) and if so the ability of dietary phytate to affect Zn availability may itself be influenced by the Zn status of the animal. The studies reported here were therefore conducted to determine first whether these two activities are expressions of the same enzyme and secondly whether Zn deficiency induced by dietary phytate could modify the metabolism of this compound by the intestinal mucosa.

MATERIALS AND METHODS

Animals

Male Hooded Lister rats of the Rowett strain weighing between 120 and 165 g were used in all experiments.

Diets

In preliminary studies involving measurement of 'phytase' and alkaline phosphatase activities and phytate metabolism by intestinal mucosa *in vivo*, rats were maintained on a commercial cube diet (Oxoid; H. E. Styles, Bewdley, Worcs.). In subsequent studies, in which the Zn status of the animals was modified, the basal Zn-deficient diet of Williams & Mills (1970) was used. This diet contained 0.5 mg Zn/kg. When fed as a Zn-supplemented diet, ZnSO₄·7H₂O was added to give a Zn content of 50 mg Zn/kg. When rats were given diets containing phytate, sodium phytate (Sigma Chemical Co., St Louis, USA) was added to give a phytate content of 10 g/kg and the Ca content adjusted by the addition of CaCO₃ to supply 13 g Ca/kg. The Zn contents of the diets were adjusted by addition of ZnSO₄·7H₂O so that they contained either 0.5 (unsupplemented), 12 or 100 mg Zn/kg.

Measurement of 'phytase' and alkaline phosphatase activities

To enable direct comparisons to be made between these two enzyme activities, standard conditions were employed with regard to substrate concentration and pH. Preliminary experiments showed the pH optimum for 'phytase' activity was between pH 7.2 and 7.6 and hence in all experiments the pH was maintained at pH 7.4. Since each molecule of phytic acid contains six phosphate moieties, equivalence between β -glycerophosphate and phytate was maintained with respect to phosphate by using one-sixth molar concentration of phytate compared with that of β -glycerophosphate.

Preparation of tissue

Samples of mucosa from the duodenum (0–160 mm distal to the pyloric sphincter), jejunum (320–480 mm distal to the pyloric sphincter) and ileum (terminal 160 mm proximal to the ileo-caecal junction) were prepared by everting the appropriate segment and scraping away the mucosa from the underlying muscle layers with a glass microscope-slide. The mucosal samples were homogenized separately in 5 vol. 0.25 M-sucrose (adjusted to pH 7.4 by minimal volumes of Tris-HCl buffer) with ten strokes of a Teflon pestle, glass-tube homogenizer (concentrated homogenate). For determination of alkaline phosphatase activity a portion of these homogenates was diluted in a further 5 vol. 0.25 M-sucrose (dilute homogenate).

'Phytase' activity

Incubations were carried out at 37° for 30 min in duplicate. Each tube contained sodium phytate (0.83 mM; equivalent to 5 mM-phosphate) Tris-succinate buffer (50 mM; pH 7.4) and magnesium ions (0.5 mM as MgCl₂) in a final volume 3 ml. The inorganic phosphate content of the substrate was > 1 g phosphorus/kg sodium phytate and at the concentrations used in this assay it was below the level of detection by the method used for phosphate analysis (Sumner, 1944). The reaction was started by the addition of 0.2 ml concentrated homogenate. Preliminary experiments showed that the rate of reaction was linear with respect to time for periods up to 1 h and thus an incubation period of 30 min was therefore used in all assays. The reaction was terminated by the addition of 1 ml trichloroacetic acid solution (200 g/l). After centrifuging, a sample of the supernatant fraction was analysed for

phosphate by the method of Sumner (1944). Reagent 'blanks' (no enzyme added) and enzyme 'blanks' (no substrate added) were subjected to the same procedure and the values obtained were subtracted from the experimental assay values. Results were expressed as μmol phosphate liberated/mg protein per h.

Alkaline phosphatase

A similar procedure to that described for 'phytase' was employed except the tubes contained 5 mM- β -glycerophosphate instead of phytate, and 0.2 ml dilute homogenate, and the reaction was carried out for only 15 min. This time was chosen after preliminary experiments had shown the reaction was linear with respect to time for 30 min, after which the rate declined. Results were corrected for reagent and enzyme 'blank' values and expressed as phosphate liberated/mg protein per h.

Protein concentration of the homogenates were measured by the Biuret method (Miller, 1959) using bovine serum albumin as a standard.

'Phytase' and alkaline phosphatase activity in mucosal brush borders

Mucosal brush-border fractions of duodenal mucosa were prepared by a method similar to that of Miller & Crane (1961). Briefly, the scraped mucosa of the duodenal segment was dispersed in 20 ml 5 mM-EDTA which had been adjusted to pH 7.4 with Tris buffer and was then homogenized in a top-drive blender (Measurement and Scientific Instruments Ltd, London SW1) at half-speed for 1 min. The homogenate was spun for 10 min at 850 g in a refrigerated centrifuge (MSE High Speed 18; Measuring and Scientific Instruments Ltd). The pellet was washed by resuspension in 20 ml 5 mM-EDTA-Tris buffer, homogenized a second time and centrifuged. The washed pellet was finally resuspended in 2 ml 0.25 M-sucrose (pH 7.4). 'Phytase' and alkaline phosphatase activities were measured on 0.2 and 0.02 ml portions respectively using the same incubation condition as described for mucosal homogenates. When included, magnesium was added as MgCl_2 and Zn ions as ZnSO_4 (see p. 311). Results were expressed as μmol phosphate liberated/mg protein per h after corrections for enzyme and reagent 'blanks'.

Phytate hydrolysis in vivo

The rate at which phytate was metabolized by different regions of the rat intestine in vivo was measured using a ligated-loop technique. Loops of either duodenum, jejunum or ileum (corresponding to the same regions as described previously) were prepared as described by Davies & Nightingale (1975). The loops were filled with 1 ml physiological saline (9 g NaCl/l) containing 2 μmol sodium phytate, adjusted to pH 7.4 with hydrochloric acid. After either 15 min (duodenum) or 30 min (jejunum and ileum) the loops were excised, emptied and the lumen flushed through with 5 vol. saline. Following protein precipitation with 1 ml trichloroacetic acid (200 g/l), duplicate portions of the supernatant fraction were analysed for phytate by the method of Holt (1955). The loss of phytate was calculated by difference between the amount injected and that recovered and the results expressed as μmol phytate/loop per h.

Statistical treatment of results

All comparisons were made by Student's *t* test for unpaired samples except in dietary studies in which Zn-adequate control rats were pair-fed to Zn-deficient rats when a *t* test for paired samples was used.

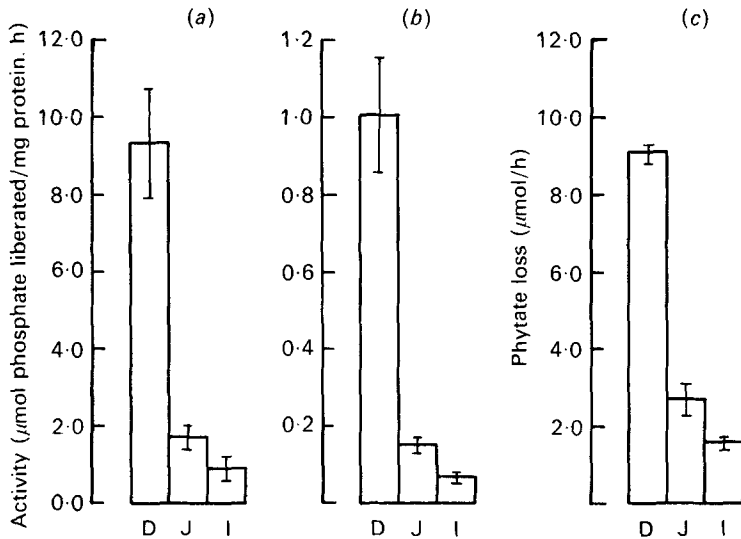


Fig. 1. Regional distribution of (a) alkaline phosphatase (*EC* 3.1.3.1) and (b) phytase (*EC* 3.1.3.8) and (c) phytate loss ($\mu\text{mol}/\text{h}$) along the small intestine of rats. Alkaline phosphatase and phytase activities ($\mu\text{mol phosphate liberated}/\text{mg protein per h}$) were measured on intestinal homogenates, and phytate loss from ligated loops of duodenum (D), jejunum (J) and ileum (I). Results are mean values of four rats with their standard errors ($\times 2$) represented by vertical bars.

RESULTS

Regional distribution of 'phytase' and alkaline phosphatase and rate of loss of injected phytate

It is well established that alkaline phosphatase activity is greatest in the proximal regions of the intestine and is considerably lower in the more distal regions (Moog, 1968). The results of measurements of 'phytase' and alkaline phosphatase activities in mucosal homogenates, prepared from different regions of the intestine are shown in Fig. 1. It should be noted that in all regions studied, alkaline phosphatase activity was eight to ten times greater than 'phytase' activity. However, the profile of activities were similar, thus the activity ratio, ileal:jejunal:duodenal for alkaline phosphatase was 1:1.9:10.2 compared with 1:2.2:15.7 for 'phytase'. Similarly the relative rates at which phytate was lost from different regions of the intestine when injected into isolated loops in situ showed a broadly similar distribution (Fig. 1); the value for ileal:jejunal:duodenal was 1:1.7:5.8.

Localization of alkaline phosphatase and 'phytase' in mucosal brush borders

Hubscher, West & Brindley (1965) showed that most intestinal alkaline phosphatase activity is present in the brush-border fraction. This finding is supported by the present study where the specific activity of alkaline phosphatase in the brush-border fraction was approximately ten times greater than that in the whole homogenate (Table 1). Similarly the specific activity of 'phytase' was ten times greater in the brush-border fraction than of the whole homogenate, indicating a similar subcellular distribution of these two activities as well as the same regional distribution along the length of the small intestine.

Ionic requirements of alkaline phosphatase and phytase

Intestinal alkaline phosphatase requires the presence of both Zn^{2+} and Mg^{2+} for maximal activity. The effect of these two ions added separately and together on both alkaline phos-

Table 1. Alkaline phosphatase (EC 3.1.3.1) and 'phytase' (EC 3.1.3.8) activities $\mu\text{mol phosphate liberated/mg protein per h}$ of duodenal homogenates and duodenal isolated brush-border fractions of rats

(Mean values with their standard errors for four rats. Individual values were the average of duplicate determinations for each enzyme; both enzymes were assayed in the same homogenate or brush-border preparation. For details of assay, see p. 308)

Preparation	Alkaline phosphatase		Phytase	
	Mean	SE	Mean	SE
Homogenate	5.72	0.02	0.53	0.04
Brush border	51.0	3.51	5.38	0.63

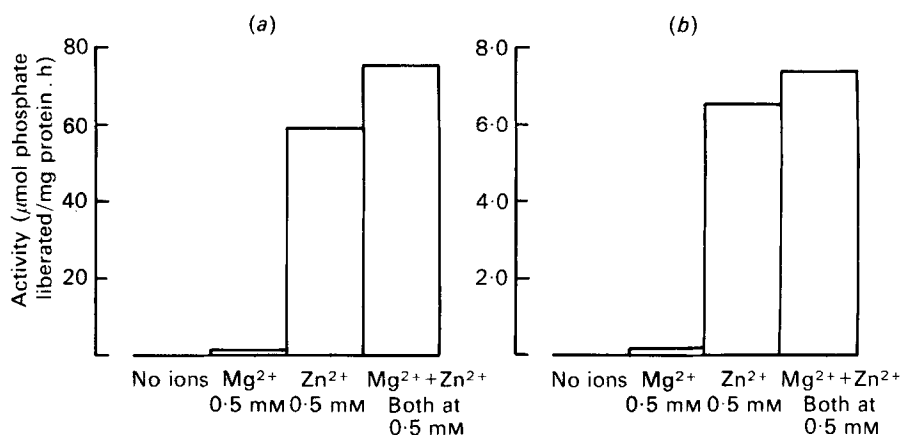


Fig. 2. Duodenal brush-border alkaline (a) phosphatase (EC 3.1.3.1) and (b) phytase (EC 3.1.3.8) activities ($\mu\text{mol phosphate liberated/mg protein per h}$) of rats at various concentrations of magnesium and zinc ions. Results are mean values for duplicate determinations of brush-border fractions prepared from two rats.

phatase and 'phytase' activity in a duodenal brush-border preparation is shown in Fig. 2. Both enzymes exhibited almost no activity in the absence of both Zn^{2+} and Mg^{2+} . Slight activity was present for both substrates when Mg^{2+} alone was present (0.5 mM) and considerably greater activity was found when Zn^{2+} alone was present (0.5 mM). However, the greatest activity towards both substrates was when both ions were present together at a concentration of 0.5 mM. The relative increases in activities of both alkaline phosphatase and 'phytase' due to these various additions were very similar, again indicating pronounced similarities between these two enzymes.

It has been reported that although Zn is required for intestinal alkaline phosphatase, the concentration required for maximal activity is fairly critical and if an excess is added an inhibition is observed (Williams, 1972). Accordingly studies were made on the effects of different concentrations of Zn^{2+} on the activities of alkaline phosphatase and 'phytase' of the brush-border fraction of duodenal mucosa. The results are shown in Fig. 3. Increasing the Zn^{2+} concentration from 0.05 mM to 0.25 mM caused increases in both activities. However, no further increases were observed when the Zn^{2+} concentration exceeded 0.25 mM and there was an indication that at the highest concentration tested (1 mM) there was an inhibition of both activities.

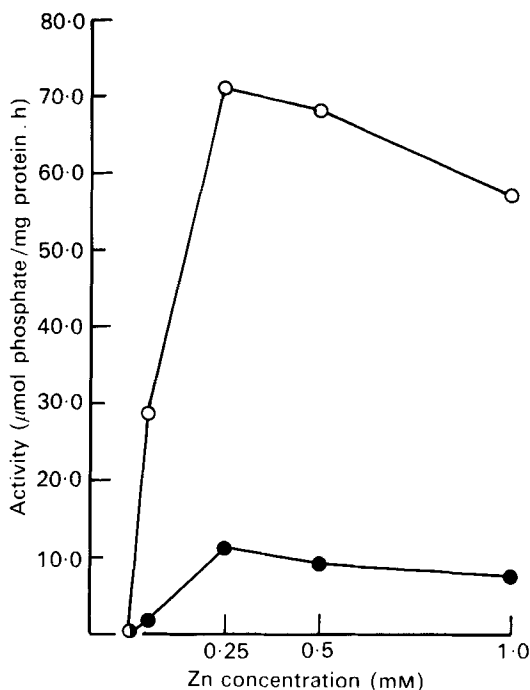


Fig. 3. The effects of different zinc ion concentrations on alkaline phosphatase (*EC* 3.1.3.1) (○—○) and 'phytase' (*EC* 3.1.3.8) (●—●) activities ($\mu\text{mol phosphate liberated/mg protein per h}$) of duodenal mucosal brush borders of rats. Results are mean values for three rats.

Effect of Zn deficiency on duodenal alkaline phosphatase and 'phytase' activity

A marked reduction in intestinal alkaline phosphatase activity has been reported to be an early consequence of Zn deficiency in the rat (Williams, 1972; Leucke, Olman & Baltzer, 1968). It was therefore of interest to discover whether a reduction of 'phytase' activity paralleled changes in alkaline phosphatase when rats were fed a Zn-deficient diet.

Young male rats were transferred to a Zn-deficient diet (0.5 mg Zn/kg) or a Zn-supplemented diet containing 50 mg Zn/kg. Two groups of Zn-supplemented control rats were used in this study; an *ad lib.*-fed and a pair-fed group whose food intakes were matched to those receiving the Zn-deficient diet. After 4 d on these diets the rats receiving the Zn-deficient diet suffered a check in growth rate which was accompanied by a decrease in food consumption followed by a 'cyclic' pattern of food intake characteristic of Zn deficiency in this species (see Williams & Mills, 1970; Chesters & Will, 1973; Davies & Nightingale, 1975). At 6 and 14 d after initiating these treatments duodenal mucosal homogenates were prepared and assayed for alkaline phosphatase and 'phytase' activities. The results are shown in Table 2. Clearly, Zn deficiency caused comparable reductions in the activities of these two enzymes for both treatment periods studied. In the rats killed on the sixth day of treatment the alkaline phosphatase and 'phytase' activities were reduced by 58% and 38% respectively and on the fourteenth day of treatment the activities were reduced by 72% and 76% respectively. A comparison of the activities of the 'pair-fed' controls and the controls fed *ad lib.* measured on the sixth day of treatment showed no significant difference for either enzyme indicating the reductions observed in the Zn-deficient groups were a direct consequence of the Zn deficiency and not secondary to a reduction in food consumption.

Table 2. The effect of Zn deficiency on alkaline phosphatase (EC 3.1.3.1) and phytase (EC 3.1.3.8) activity ($\mu\text{mol phosphate liberated/mg protein per h}$) of duodenal mucosal homogenates of rats

(Mean values with their standard errors for five rats/treatment. Individual values for each rat were the average of duplicate determinations for each enzyme; both enzymes were assayed in the same homogenates)

Dietary treatments	Period on diet (d)	Alkaline phosphatase		Phytase	
		Mean	SE	Mean	SE
Zn-deficient	6	6.58	0.60*	0.78	0.13*
Zn-supplemented, 'pair-fed'	6	15.54	1.90	1.26	0.07
Zn-supplemented, <i>ad lib.</i>	6	14.72	2.40 ^{NS}	1.35	0.14 ^{NS}
Zn-deficient	14	3.60	0.45†	0.36	0.05†
Zn-supplemented, 'pair-fed'	14	12.76	1.61	1.50	0.18

NS, Values for Zn-supplemented *ad lib.* rats were not significantly different from those of pair-fed Zn-supplemented rats: $P < 0.05$.

* Values for Zn-deficient rats were statistically significantly different from those of pair-fed Zn-supplemented rats: $P < 0.01$.

† Values for Zn-deficient rats were statistically significantly different from those of pair-fed Zn-supplemented rats: $P < 0.01$.

The effect of dietary phytate on alkaline phosphatase and phytase activity of duodenal mucosal homogenates and phytate metabolism in vivo

A previous study from this laboratory showed that when rats were given a diet containing marginally-adequate Zn (15 mg Zn/kg) and phytate (10 g/kg) they exhibited symptoms of Zn deficiency indistinguishable from those induced by feeding a phytate-free, Zn-deficient diet containing 0.5 mg Zn/kg. Studies were therefore carried out to determine whether under similar conditions 'phytase' and alkaline phosphatase activities would be reduced, due to a 'phytate-induced' Zn deficiency and, furthermore, if the ability of the intestine to metabolize phytate in vivo was similarly affected.

Experiment 1. In the first experiment rats were given diets containing 10 g phytate/kg. Zn was added to the diets to give contents of either 12 mg Zn/kg (Zn-deficient) or 100 mg Zn/kg. This latter concentration of Zn completely overcomes the deleterious effects on growth and food intake induced by this level of dietary phytate (N. T. Davies, unpublished observations). The animals receiving the diet containing the higher Zn content were pair-fed to those receiving the Zn-deficient diet. After 12 d on the diets, some rats from each group were killed and the duodenal mucosa assayed for alkaline phosphatase and 'phytase' activities. The remaining rats were anaesthetized and the rate of loss of phytate from ligated duodenal loops was measured.

The rats receiving the diet containing the lower Zn content exhibited reduced growth rates accompanied by a reduced food consumption from the fifth day of treatment indicating these rats were Zn-deficient. Significant reductions in alkaline phosphatase and 'phytase' activities were observed in these rats compared with those receiving the diet containing the higher Zn content. Similarly, the rate at which injected phytate disappeared from ligated duodenal loops in situ was significantly reduced (Fig. 4).

Experiment 2. In a previous paper (Davies & Nightingale, 1975) it was shown that feeding rats a Zn-deficient diet (0.5 mg Zn/kg) containing 10 g phytate/kg caused a far more severe Zn deficiency than that induced by feeding the same diet without added phytate. The rats receiving phytate were in negative Zn balance and suffered a loss in body-weight whereas those receiving the basal Zn-deficient diet just maintained body-weight and a slight positive

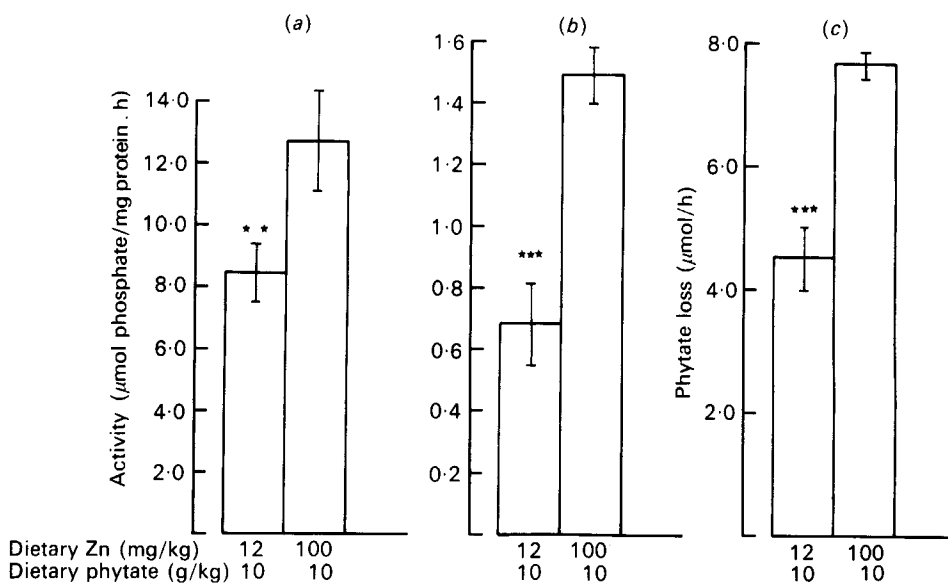


Fig. 4. The effect of phytate-induced Zn deficiency on (a) alkaline phosphatase (*EC* 3.1.3.1) and (b) phytase (*EC* 3.1.3.8) activities ($\mu\text{mol phosphate liberated/mg protein per h}$) and (c) phytate loss ($\mu\text{mol/h}$) from ligated loops of duodenum of rats. Results are mean values for five rats with their standard errors ($\times 2$) represented by vertical bars. Rats offered the diet containing 12 mg Zn/kg and 10 g phytate/kg were fed *ad lib.* for 12 d while those receiving the diet containing 100 mg Zn/kg and 10 g phytate/kg were 'pair-fed' to those on the diet containing the lower Zn content. ** $P < 0.01$, *** $P < 0.001$ (paired-*t* test).

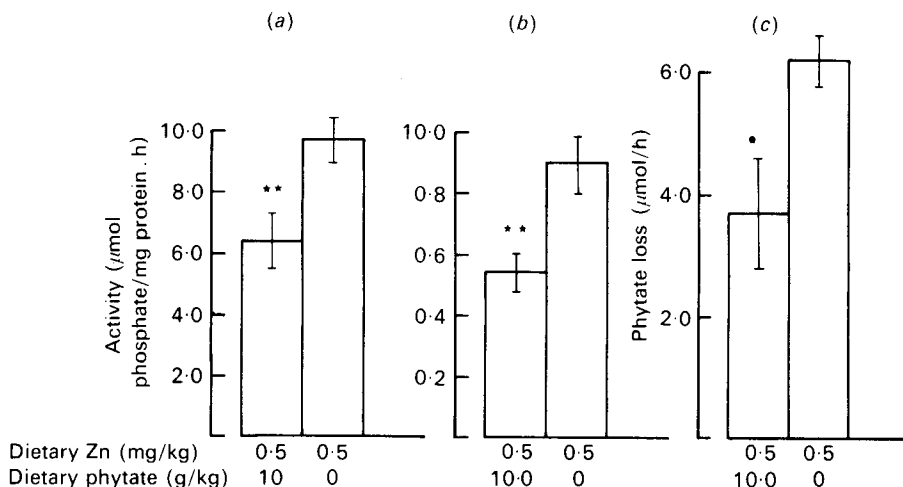


Fig. 5. The effects of phytate inclusion in a Zn deficient diet on (a) alkaline phosphatase (*EC* 3.1.3.1) and (b) phytase (*EC* 3.1.3.8) activities ($\mu\text{mol phosphate liberated/mg protein per h}$) and (c) phytate loss ($\mu\text{mol/h}$) from ligated loops of duodenum of rats. Results are mean values for five rats, with their standard errors ($\times 2$) represented by vertical bars. Rats were offered the diets *ad lib.* for 12 d. * $P < 0.05$, ** $P < 0.01$ (unpaired-*t* test).

Zn balance. In view of these findings a study was made of the effects of phytate inclusion in a Zn-deficient diet on alkaline phosphatase, 'phytase' and phytate disappearance from ligated duodenal loops.

The results of this experiment are shown in Fig. 5. Rats offered the Zn-deficient diet *ad lib.* for 12 d had significantly lower duodenal alkaline phosphatase and 'phytase' activities and had an impaired ability to hydrolyse duodenally injected phytate compared with those receiving the same diet without added phytate. These results may be interpreted as showing that the more severe Zn deficiency induced by phytate resulted in greater reductions in alkaline phosphatase and 'phytase' activities, which in turn resulted in the decrease in the rate at which phytate was metabolized by the duodenal mucosa *in vivo*.

DISCUSSION

Phytic acid is a hexaphosphate ester of inositol and it would seem likely that it may act as a substrate for alkaline phosphatase since this enzyme brings about the hydrolysis of organic phosphate esters. The results of the present study fully support this hypothesis since under all conditions studied 'phytase' activity paralleled alkaline phosphatase activity. Thus the regional distribution of these two phosphatases along the intestine, their ionic requirements, inhibition by excess Zn^{2+} added *in vitro* and subcellular localization were qualitatively similar. In agreement with the findings of Williams (1972) and Leucke *et al.* (1968), intestinal alkaline phosphatase activity was reduced in Zn-deficient rats compared with either pair-fed Zn-adequate controls or controls fed *ad lib.* and, similarly, phytate-hydrolysing activity of the same homogenates was reduced. In view of these findings we support the proposals of Pileggi (1959) and Maddaiah, Kurnick, Hulett & Reid (1969) that intestinal 'phytase' activity may be a manifestation of alkaline phosphatase activity. A similar conclusion was reached by Davies, Ritcey & Motzok (1970) and Davies & Motzok (1972) who conducted a similar series of studies on chick intestine. In agreement with the present study on the rat, Zn deficiency reduced the activities of both phosphatases in the chick and, in addition, when extracts of the mucosa were subjected to gel-filtration chromatography, the two enzymes showed broadly similar activity profiles. This view has been challenged by Bitar & Reinhold (1972) who showed some divergence of activities during a four-stage partial purification of alkaline phosphatase of duodenal mucosa from rat, chicken and cow. Similarly, partial separation of 'phytase' and alkaline phosphatase activities of chicken mucosa were obtained when mucosal extracts were chromatographed on DEAE-cellulose. However, most activity for both enzymes resolved into two overlapping zones.

Alkaline phosphatase activity of the intestine is not due to a single enzyme but to a number of isoenzymes which are characterized by an ability to hydrolyse phosphate-ester linkages at pH values greater than 7. Their substrate specificities and pH optima for β -glycerophosphate or *p*-nitrophenol phosphate differ markedly (reviewed by Moog, 1968) and it would seem likely, therefore, that at least some of the isoenzymes which contribute to alkaline phosphatase activity may function to a greater or lesser extent as 'phytases'.

The results of experiments in which rate of loss of injected phytate from ligated intestinal loops was measured indicate that this aspect of intestinal activity parallels 'phytase' distribution along the intestine and both of these activities are reduced to comparable extents when rats suffer different extents of Zn deficiency induced by dietary phytate. Since phytic acid is not absorbed (Oberleas, 1975) it seems likely that the loss of injected phytate represents hydrolysis *in vivo* of phytate in contact with alkaline phosphatase of mucosal brush-border membranes.

A role for phytic acid in reducing Zn availability has been well established since O'Dell & Savage (1957) first showed that Zn in some plant proteins was poorly available compared

with the Zn in protein of animal origin. Although phytate has been shown similarly to reduce the availability of a number of essential divalent metals, i.e. Fe (Widdowson & McCance, 1942), Cu, Mn (Davies & Nightingale, 1975), Mg (Seelig, 1964) and Ca (Bruce & Callow, 1934), our present findings high-light how its effects on Zn nutrition may be of greater significance. This study shows that the metabolism of phytate both in vitro and in vivo depends upon an adequate supply of dietary Zn. Thus if the dietary Zn intake is marginal and the diet contains phytate the reduction in availability of Zn can lead to a Zn deficiency state which in turn decreases the ability of the intestinal mucosa to hydrolyse phytate. One can postulate that the lowered ability of the intestine to metabolize dietary phytate would result in an increased concentration of phytate in the intestinal contents which in turn could bind more Zn and further reduce dietary Zn availability.

In conclusion, the results of this current study again emphasize the importance of dietary phytate in relation to Zn nutrition. If man increases his dietary supply of protein from cereal and soya-based products, both of which are rich in phytate, due regard must be paid to the effect this may have on the supply of adequate amounts of Zn in an available form.

REFERENCES

- Bitar, K. & Reinhold, J. G. (1972). *Biochim. biophys. Acta* **268**, 442.
 Bruce, H. M. & Callow, R. K. (1934). *Biochem. J.* **28**, 517.
 Chesters, J. K. & Will, M. (1973). *Br. J. Nutr.* **30**, 555.
 Davies, M. & Motzok, I. (1972). *Comp. Biochem. Physiol.* **42**, 345.
 Davies, M., Ritcey, G. M. & Motzok, I. (1970). *Poult. Sci.* **49**, 1280.
 Davies, N. T. & Nightingale, R. (1975). *Br. J. Nutr.* **34**, 243.
 Holt, R. (1955). *J. Sci. Fd Agric.* **6**, 136.
 Hubscher, G., West, G. R. & Brindley, D. N. (1965). *Biochem. J.* **97**, 629.
 Leucke, R. W., Olman, M. E. & Baltzer, B. V. (1968). *J. Nutr.* **94**, 344.
 Maddaiah, V. T., Kurnick, A. A., Hulett, B. J. & Reid, B. L. (1969). *Proc. Soc. exp. Biol. Med.* **115**, 1054.
 Miller, D. & Crane, R. K. (1961). *Biochim. biophys. Acta* **52**, 293.
 Miller, G. L. (1959). *Analyt. Chem.* **31**, 964.
 Moog, F. (1968). In *Handbook of Physiology: Alimentary Canal* vol. 3. *Intestinal absorption*. Washington, DC: American Physiological Society.
 Oberleas, D. (1973). *Toxicant Occurring Naturally in Foods*, p. 363. Washington, DC: National Academy of Sciences.
 Oberleas, D. (1975). In *Proceedings Western Hemisphere Nutrition Congress IV 1975*, p. 156 [P. L. White and N. Selvey, editors]. Acton, Massachusetts: Publishing Sciences Group, Inc.
 O'Dell, B. L. & Savage, J. E. (1957). *Fedn Proc. Fedn Am. Socs. exp. Biol.* **16**, 394.
 Patwardhen, V. N. (1937). *Biochem. J.* **31**, 560.
 Pileggi, V. J. (1959). *Archs. Biochem. Biophys.* **30**, 1.
 Reinhold, J. G., Pascoe, E., Arslanian, M. & Bitar, K. (1970). *Biochim. biophys. Acta* **215**, 430.
 Seelig, M. S. (1964). *Am. J. clin. Nutr.* **14**, 342.
 Sumner, J. B. (1944). *Science, N.Y.* **160**, 413.
 Widdowson, E. M. & McCance, R. A. (1942). *Lancet* **i**, 588.
 Williams, R. B. (1972). *Br. J. Nutr.* **27**, 121.
 Williams, R. B. & Mills, C. F. (1970). *Br. J. Nutr.* **24**, 989.