The effects of phytate on intestinal absorption and secretion of zinc, and whole-body retention of Zn, copper, iron and manganese in rats

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1. The inclusion of phytate (10 g/kg) in a purified diet containing zinc (15 mg/kg) fed to young male rats significantly reduced growth rate and food intake, and promoted a cyclic pattern of food intake characteristic of an uncomplicated Zn deficiency. The decreased growth rate could be accounted for by the reduced food consumption.

2. Rats maintained on a Zn-deficient diet (0·5 mg Zn/kg) were found to have a cyclic pattern of food intake and a very slight weight gain. The addition of phytate (10 g/kg) to the Zn-deficient diet promoted a net loss of mean body-weight.

3. Rats maintained on the Zn-supplemented diet containing phytate excreted significantly more Zn in their faeces than either pair-fed or *ad lib.*-fed control rats. Rats given the Zn-deficient diet supplemented with phytate excreted more Zn in their faeces than Zn-deficient control rats.

4. Dietary phytate significantly reduced the average daily accumulation (μg/d) and whole-body retention (relative to dietary intake) of iron, copper, manganese and Zn, whether or not the diet was supplemented with Zn.

5. The addition of phytate to the lumen fluid of ligated loops of rat duodenum maintained in situ significantly inhibited ^{65}Zn absorption, compared with the control systems without added phytate.

6. Other studies using ligated duodenal and ileal loops indicated that Zn is secreted into the gut lumen and approximately one-third of this is normally reabsorbed. Recycling of endogenous Zn may be a significant process in the over-all body economy of this trace element.

7. The absorption of ^{65}Zn added to the diet was significantly reduced by dietary phytate. Dietary phytate also reduced the biological half-life of body ^{65}Zn from 91 to 211 h post-administration, possibly by inhibiting reabsorption of endogenous ^{65}Zn and thus promoting a more rapid loss from the body.

O'Dell & Savage (1960) were the first workers to suggest that naturally occurring phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) in plant protein might reduce the availability of dietary zinc. This proposal seems amply justified in view of the numerous reports of impairment of Zn utilization when phytate-rich diets have been fed to a number of different species, e.g. chicks (Likuski & Forbes, 1964), pigs (Oberleas, Muhrer & O'Dell, 1962) and rats (Oberleas, Muhrer & O'Dell, 1966 *a, b*). A plausible mechanism suggested by Oberleas *et al.* (1966 *a, b*) proposes that Zn is removed from solution in the intestinal lumen by co-precipitation with calcium and phytate as an unavailable Zn–Ca–phytate complex, although in few studies so far reported have direct effects of phytate on Zn absorption been found in vivo.

In view of these findings, the work reported here was directed towards studying the action of phytate on Zn absorption both in experiments using ligated loops of rat duodenum in situ, and from an assessment of percentage absorption of tracer doses of ^{65}Zn in whole-body retention studies, using the method of Heth & Hoekstra (1965).

The results of recent studies of the intestinal handling of Zn by rats indicate that it is both rapidly absorbed (Davies, 1973; Methfessel & Spencer, 1973 a) and secreted (Methfessel & Spencer, 1973 b) and that the mechanism of secretion and reabsorption may play an active part in the over-all absorption of Zn. Results of experiments reported in the present paper, involving injection of a metal-binding resin into ligated segments of rat intestine, may be interpreted by the suggestion that Zn is continually secreted and reabsorbed across the intestinal mucosa. The possibility that dietary phytate might affect over-all Zn utilization both by affecting the reabsorption of endogenously secreted Zn and by direct action on the absorption of dietary Zn was thus studied.

EXPERIMENTAL

Animals

In all experiments male Hooded Lister rats of the Rowett Institute strain were used. Throughout the treatment periods rats were housed individually in cages constructed of polypropylene and stainless steel.

Diets

In the dietary experiments, rats weighing 66·5–75·5 g were transferred from a commercial rat cube diet to a semi-purified basal diet similar in composition to that of Williams & Mills (1970) containing (g/kg): spray-dried egg albumin 200, sucrose 660, arachis oil 100, plus vitamins and minerals, except that the Ca content was increased to 13 g/kg by addition of extra $CaCO_3$. This basal diet supplied trace metals in the following concentrations (mg/kg): Zn 0·5, copper 25, iron 50, manganese 52. When phytate was added to the diet, the amount of sodium phytate (Sigma Chemical Corp., St Louis, USA) included gave a dietary phytic acid content of 10 g/kg, and for the Zn-supplemented diets, $ZnSO_4$ was added to give a dietary Zn content of 15 mg/kg. In experiments in which [65]Zn was added to diets, a weighed portion of diet (3 g) was thoroughly mixed with 0·5 ml water containing 2 μCi [65]Zn (specific activity (SA) 1·0 mCi/mg; Radiochemical Centre, Amersham, Bucks.). The resulting mixture was freeze-dried and ground to a consistency similar to that of the original diet.

Dietary experiment

Groups of either five or six rats were given five dietary treatments: (1) Zn-supplemented, fed *ad lib.*; (2) Zn- and phytate-supplemented, fed *ad lib.*; (3) Zn-supplemented, pair-fed to treatment 2; (4) Zn-deficient, fed *ad lib.*; (5) Zn-deficient, phytate-supplemented, fed *ad lib.*

Rats were maintained on these treatments for 21 d and body-weights and food intakes were measured daily.

Faeces were collected daily for the first 14 d, oven-dried (24 h at 110°), crushed to fine powder, and duplicate samples (approximately 50 mg) wet-ashed in conc. H_2SO_4–conc. $HClO_4$–conc. HNO_3 (0·5 : 1·0 : 5·0, by vol.). After dilution, the Zn content of the samples was estimated by atomic absorption spectrophotometry (Techtron AA5; Varian-Techtron Pty, Melbourne, Australia).

Estimates of the average daily apparent absorption of Zn were obtained over a 10 d period from the differences between dietary intakes of Zn from day 4 to 13 and the faecal losses of Zn from day 5 to 14.

At the end of the dietary treatment period rats were killed under diethyl ether anaesthesia, the entire intestinal contents flushed out and the whole bodies oven-dried (24 h at 110°). After an initial charring the whole bodies were dry-ashed at 450° for 24 h and the ash residue gently heated with 5 ml conc. HNO_3–H_2O_2 (100 vol.) (50:50, v/v) and evaporated to dryness before a second ashing at 450° for 4 h. The resulting white residue was dissolved in 1 M-HCl and diluted to 1 l for Zn, Cu, Fe and Mn determinations using atomic absorption spectrophotometry.

Whole-body accumulation of each of these elements was estimated from their whole-body contents at slaughter and from the mean metal contents of a group of six rats of similar size and age which were killed at the beginning of the dietary experiment. From these values, whole-body retention of each element was calculated as a percentage of the dietary intake.

For statistical treatment of results the groups maintained on Zn-supplemented diets and those on Zn-deficient diets were considered separately.

Zn absorption from ligated intestinal loops

Male rats weighing 150 g were anaesthetized with Nembutal (Abbot Laboratories Ltd, Queenborough, Kent) (administered intraperitoneally, 45 mg/kg body-weight) and the peritoneal cavity was opened by mid-line incision. The duodenum (150 mm segment distal to the pyloric sphincter) was flushed with 5 ml warm saline solution (9 g NaCl/l) and the fluid and solid material in the loop squeezed into the lower regions of the intestine by gentle finger pressure. Double ligatures were placed quickly and as close as possible to the duodenal wall without restricting blood flow to the intestinal loop.

In order to assess the effect of phytate on Zn absorption, the isolated loops were filled with 0·8 ml saline or saline containing 5·07 μmol phytic acid (as sodium phytate, adjusted to pH 7·0 with HCl) using a plastic syringe with a blunt needle, inserted through the distal ligature. The test dose of 5 μg Zn (as $ZnSO_4$ containing 0·1 μCi ^{65}Zn) in 0·2 ml saline containing 109 μmol Ca^{2+} was subsequently injected into the loop using a fine-bore needle, and the abdominal incision closed with sutures.

The effect of ion-exchange resin on Zn absorption was studied using a similar technique. Isolated duodenal loops in situ were first filled with 0·8 ml of a slurry of Chelex-100 (100–200 mesh, sodium form; Bio Rad Laboratories, Richmond, California, USA) – saline (50:50, v/v) and subsequently injected with 0·2 ml saline containing 5 μg Zn (as $ZnSO_4$ containing 0·1 μCi ^{65}Zn).

After 15 min the loops were rapidly excised, cut open and the contents flushed out with 5 ml warm saline. The loop tissue and loop contents and washings were separately assayed for ^{65}Zn content using a gamma-well counter (Tracerlab, Instruments Division, Hersham, Surrey).

'Zn absorption' from loop to whole body was assessed from the loss of the injected radioactivity (injected radioactivity minus recovered radioactivity in loop contents and

tissue after flushing with saline). 'Zn bound' refers to the radioactivity remaining in the loop tissue after the mucosa was flushed with saline. 'Mucosal Zn uptake' refers to total uptake of Zn from the lumen fluid and was calculated as the sum of Zn bound and Zn absorbed. All results were converted to μg Zn using the initial SA values.

Zinc secretion and reabsorption

Duodenal or ileal loops (150 mm segment proximal to the ileo-caecal junction) were isolated in situ as described previously and filled with 1 ml warm saline or 1 ml of a slurry of Chelex-100 (100–200 mesh, sodium form) – saline (50:50, v/v). After 1 h the loops were removed and their contents and the saline washings were evaporated to dryness in an oven maintained at 110°. The resulting residues were wet-ashed and assayed for Zn using atomic absorption spectrophotometry.

Since Zn was shown to be poorly absorbed when bound to Chelex resin, the difference between the Zn accumulated in the Chelex-filled loops and the saline-filled loops was taken to represent the amount of secreted Zn available for reabsorption.

Effect of dietary phytate on ^{65}Zn absorption and turnover

Twenty male rats in the weight range 65–70 g were transferred from a stock cube diet offered *ad lib.* to the Zn-supplemented, semi-purified diet to which they were allowed restricted access for 1 h periods twice daily. After 10 d all rats were eating at least 4 g food/meal and 10 g food daily compared with intakes of 11–13 g for rats of similar age and weight allowed unrestricted access to the same diet.

Following this initial training period the rats were randomized into four groups of five rats each to receive the following treatments.

Group 1. Rats were offered a meal of 3 g Zn- and phytate-supplemented diet, to which was added 2 μCi ^{65}Zn (SA 1·0 mCi/kg). They were subsequently allowed unrestricted access to the same diet (without ^{65}Zn) for 10 d.

Group 2. Rats were offered a meal of 3 g Zn- and phytate-supplemented diet without added ^{65}Zn. These animals were injected (intraperitoneally) with 2 μCi ^{65}Zn (SA 1·0 mCi/mg) in 0·2 ml saline and subsequently allowed unrestricted access to the same diet.

Group 3. On the day following the administration of ^{65}Zn to groups 1 and 2, rats were offered a meal of 3 g Zn-supplemented diet, to which was added 2 μCi ^{65}Zn (SA 1·0 mCi/mg), and subsequently maintained on this diet (without added ^{65}Zn) and pair-fed with group 1.

Group 4. On the same day as group 3, rats were offered a meal of 3 g Zn-supplemented diet before the injection of 2 μCi ^{65}Zn (SA 1·0 mCi/mg) in 0·2 ml saline. For the remainder of the experiment these animals were pair-fed to those of group 2.

Immediately after either dietary dosing or injection of the radioactive Zn the amount of radioactivity in the rats was measured, using a whole-body counter, to determine the administered dose, and thereafter was measured daily for 9 d. From the ^{65}Zn retention curves obtained for these two methods of administration the amount of the dose that was absorbed and biological half-lives of body ^{65}Zn were calculated by the methods of Heth & Hoekstra (1965).
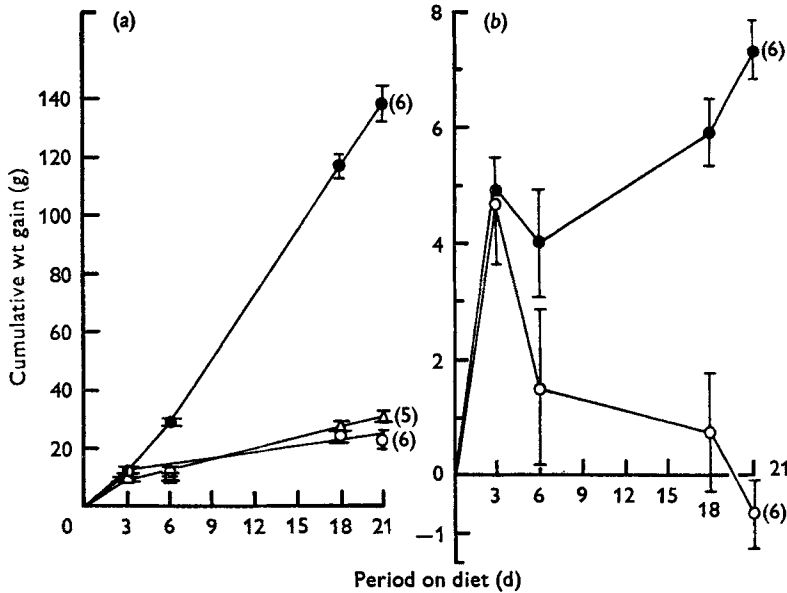
Fig. 1. Mean weight gains for groups of rats maintained on a semi-purified diet with or without zinc and sodium phytate supplements. (*a*) Zn- and phytate-supplemented diet offered *ad lib.* (treatment 2) (○—○); Zn-supplemented diet, rats pair-fed to treatment 2 rats (△—△); Zn-supplemented diet offered *ad lib.* (●—●); (*b*) phytate-supplemented, Zn-deficient diet offered *ad lib.* (○—○); Zn-deficient diet offered *ad lib.* (●—●). For details of diets, see p. 244. Initial body-weights for all rats were between 66·5 and 74·5 g. The no. of rats/treatment is indicated in parentheses; the standard errors are represented by vertical bars.

RESULTS

Influence of dietary phytate on growth and food intake

(1) *Zn-supplemented diets.* The growth rates of rats maintained on the Zn-supplemented diets (treatments 1, 2 and 3) are shown in Fig. 1*a*. The rats given the Zn-supplemented diet *ad lib.* grew at an approximately linear rate of 6·5 ± 0·3 g/d (mean ± SE) throughout the experiment. No significant differences in growth rates between the three groups were found for the first 3 d, but from day 4 to the end of the experiment the phytate-fed animals showed a marked reduction in daily weight gain ($P < 0.001$) compared with the *ad lib.*-fed control rats. As there was a comparable decrease in weight gain for the pair-fed control group, this can be accounted for mainly by a decrease in mean daily food intake.

The average daily food intakes for days 1–3, 4–13 and 14–21 of the experimental period are shown in Table 1. During the first 3 d of the experiment no significant differences in daily food intake were apparent between the phytate-supplemented and control (Zn-supplemented) animals fed *ad lib.* However, the intake for the phytate group decreased after 3 d and thereafter, while eating significantly less food than the *ad lib.*-fed controls, they established a cyclic pattern of intake similar to that reported for Zn-deficient rats by Williams & Mills (1970) (Fig. 2). The greater variability of food intake for the phytate-fed rats was confirmed in statistical analyses of the day-to-day variance of the food intakes of the phytate-fed rats and *ad lib.*-fed controls. The

Table 1. *Average food intakes (g/d) for groups of rats receiving* ad lib. *a semi-purified diet† with or without zinc and sodium phytate supplements*

(Mean values with their standard errors for six rats/group)

Dietary treatment	Average intake (g/d)		
	Days 1–3	Days 4–13	Days 14–21
1 Zn-supplemented	$8 \cdot 3 \pm 0 \cdot 16$	$12 \cdot 6 \pm 0 \cdot 49$	$16 \cdot 5 \pm 0 \cdot 61$
2 Zn- and phytate-supplemented	$8 \cdot 3 \pm 0 \cdot 22^{NS}$	$5 \cdot 8 \pm 0 \cdot 13^{***}$	$6 \cdot 0 \pm 0 \cdot 19^{***}$
4 Zn-deficient	$6 \cdot 4 \pm 0 \cdot 20$	$4 \cdot 8 \pm 0 \cdot 14$	$4 \cdot 7 \pm 0 \cdot 12$
5 Zn-deficient, phytate-supplemented	$6 \cdot 7 \pm 0 \cdot 26^{NS}$	$4 \cdot 6 \pm 0 \cdot 10^{NS}$	$4 \cdot 4 \pm 0 \cdot 14^{NS}$

The two Zn-supplemented groups (treatments 1 and 2) and the two Zn-deficient groups (treatments 4 and 5) were considered separately, for each time period, for statistical analysis by Student's t test. The statistical significance of the difference between mean values was: NS, not significant ($P > 0 \cdot 05$); *** $P < 0 \cdot 001$.

† For details of diets, see p. 244.

residual variation in food intake from day to day over the 21 d period, after removing any linear trend, was measured for each rat. All six values for the *ad lib.*-fed, Zn-supplemented group were substantially greater than the maximum of the six values for the *ad lib.*-fed, Zn- and phytate-supplemented group. This treatment difference was clearly significant, and a statistical significance of $P < 0 \cdot 001$ was obtained with a t test done using log residual variances (*ad lib.*-fed, Zn-supplemented $0 \cdot 17 \pm 0 \cdot 08$, *ad lib.*-fed, Zn- and phytate-supplemented $0 \cdot 59 \pm 0 \cdot 04$).

(2) *Zn-deficient diets.* The growth rates of the rats offered the Zn-deficient, phytate-supplemented, and Zn-deficient diets are shown in Fig. 1*b*. After the 3rd day on the diets for both groups the pattern of daily weight gain changed. The rats offered the Zn-deficient diet maintained a slight daily weight gain whereas the phytate-fed animals suffered a net loss in weight.

There was a voluntary restriction of food intake for both these groups of rats on the 4th day of the experiment and for the remainder of the treatment cyclic patterns of food intake were recorded. The presence or absence of phytate in the Zn-deficient diets had no significant effect on the average daily food intakes throughout the experiment (Table 1) and did not noticeably affect either the frequency or amplitude of the food-intake cycles (Fig. 2).

Influence of phytate on faecal Zn excretion

The faecal excretion of Zn on the 1st day of treatment exceeded that for all subsequent days irrespective of treatment. Since this probably originated from residues of the pre-experiment stock cube diet, results for day 1 were excluded from the cumulative results for Zn output (Fig. 3).

(1) *Effect of adding phytate to Zn-supplemented diets.* The mean cumulative faecal Zn output for days 2–14 for the three groups of rats offered Zn-supplemented diets is shown in Fig. 3*a*. The phytate-fed rats had a significantly higher daily faecal Zn excretion than either the *ad lib.*-fed or the pair-fed control rats ($P < 0 \cdot 001$). No significant differences were found between the two control groups ($P > 0 \cdot 05$).
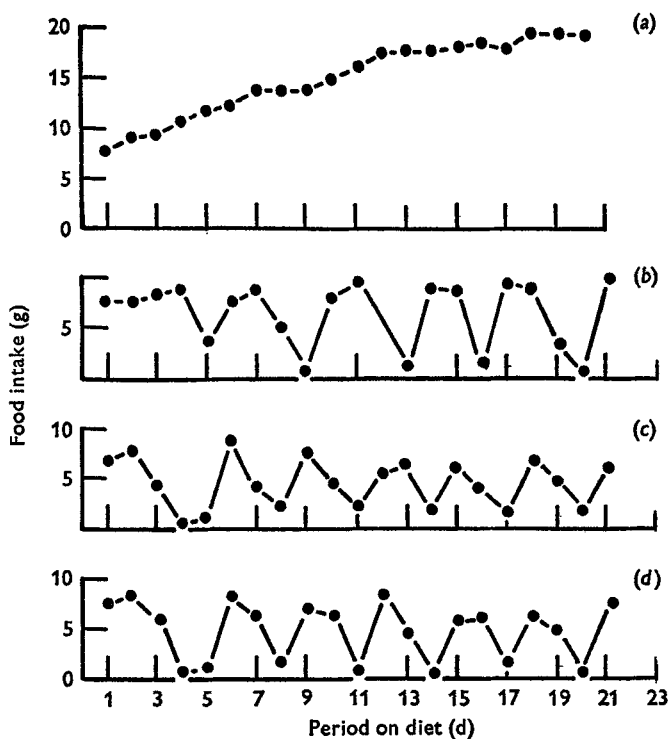
Fig. 2. Examples of daily food intakes for individual rats receiving *ad lib.* a semi-purified diet with or without zinc and sodium phytate supplements. (*a*) Zn-supplemented diet, (*b*) Zn- and phytate-supplemented diet, (*c*) Zn-deficient diet, (*d*) Zn-deficient, phytate-supplemented diet. For details of diets, see p. 244.

The mean ($\pm$ SE) apparent Zn absorption values (μg Zn absorbed/d) calculated as the difference between dietary Zn intake (days 4–13) and faecal Zn output (days 5–14), for the six phytate-fed rats, six *ad lib.*-fed control rats and five pair-fed control rats were: $25 \cdot 3 \pm 3 \cdot 9$, $149 \cdot 0 \pm 7 \cdot 4$, $53 \cdot 2 \pm 4 \cdot 4$ respectively. Dietary phytate significantly ($P < 0 \cdot 001$) reduced the apparent absorption of dietary Zn compared with either the *ad lib.*-fed or the pair-fed control groups.

(2) *Effect of adding phytate to Zn-deficient diets.* The mean cumulative faecal Zn output for days 2–14 for the two Zn-deficient dietary treatments is shown in Fig. 3*b*. The inclusion of phytate in the diet almost doubled faecal Zn output ($P < 0 \cdot 01$) even though the dietary Zn intakes for these two groups were not significantly different.

Calculation of apparent absorption of Zn from the difference between dietary Zn intake for days 4–13 and faecal Zn output for days 5–14 showed that while both groups suffered a net loss of Zn this effect was considerably greater ($P < 0 \cdot 001$) for the phytate-fed rats. The results expressed as μg Zn loss/d (mean $\pm$ SE) were, for the six Zn-deficient control rats $0 \cdot 87 \pm 0 \cdot 28$ and for the six Zn-deficient, phytate-fed rats $7 \cdot 6 \pm 0 \cdot 08$.
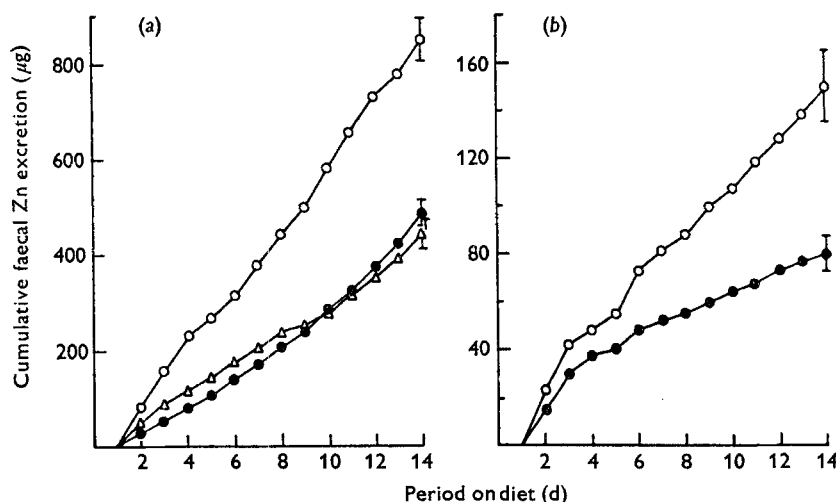
Fig. 3. Cumulative faecal zinc excretion for rats maintained on a semi-purified diet with or without Zn and sodium phytate supplements. (*a*) Zn- and phytate-supplemented diet offered *ad lib.* (treatment 2) (○—○); Zn-supplemented diet, rats pair-fed to treatment 2 rats (△—△); Zn-supplemented diet offered *ad lib.* (●—●); (*b*) phytate-supplemented, Zn-deficient diet offered *ad lib.* (○—○); Zn-deficient diet offered *ad lib.* (●—●). For details of diets, see p. 244. Each point represents the mean value for five rats, with standard errors represented by vertical bars.

Effects of dietary phytate on whole-body contents and retention of Zn, Cu, Fe and Mn

The estimated contents of Zn, Cu, Fe and Mn at the beginning (day 0) and end of the dietary treatments (day 21) are shown in Table 2. These values were used to calculate the average daily accumulation (μg/d) and retention (relative to dietary intake) of these trace metals shown in Table 3.

(1) *Zn-supplemented diets.* Phytate significantly reduced the average daily accumulation of Zn, Cu, Fe and Mn compared with that for both the *ad lib.*-fed and the pair-fed control groups. When differences in dietary intake of these trace metals were taken into account and results were expressed as retention relative to intake, phytate-fed rats again had a significantly reduced retention of all trace metals studied.

(2) *Zn-deficient diets.* For the Zn-deficient animals, phytate significantly reduced both the average daily accumulation and the proportions of the dietary intake of the four trace metals which were retained (Table 3).

Taking the results for the Zn-supplemented and Zn-deficient groups together it is clear that dietary phytate reduces the availability of Cu, Fe and Mn as well as Zn. The results of these studies of apparent absorption and whole-body retention indicate net losses of body Zn that cannot be attributed solely to an effect of phytate in reducing the availability of dietary Zn. A likely explanation for these findings is that dietary phytate may render endogenously secreted Zn unavailable for reabsorption.

Table 2. *Estimated whole-body zinc, iron, copper and manganese contents (μg) at the beginning* (day 0) and after the 21 d feeding period, for rats maintained on a semi-purified diet† with or without Zn and sodium phytate supplements*

(Mean values with their standard errors; no. of rats in parentheses)

Dietary treatment		Zn Day 0 Mean	SE	Zn Day 21 Mean	SE	Fe Day 0 Mean	SE	Fe Day 21 Mean	SE	Cu Day 0 Mean	SE	Cu Day 21 Mean	SE	Mn Day 0 Mean	SE	Mn Day 21 Mean	SE
Zn-supplemented																	
1 *ad lib.*-fed	(6)	1399	19	4328	146	2122	29	4454	196	138·5	1·90	416·8	22·91	41·9	0·59	150·4	24·9
2 Phytate-supplemented, *ad lib.*-fed	(6)	1394	28	1666	31	2115	42	2620	77	138·0	2·80	176·1	3·02	41·8	0·95	35·8	1·05
3 Pair-fed to treatment 2	(5)	1370	12	2661	51	2078	16	3110	31	135·6	1·16	223·2	8·64	41·0	0·36	53·9	6·97
Zn-deficient																	
4 *ad lib.*-fed	(6)	1372	10	1397	22	2082	15	2506	47	135·8	0·85	177·8	6·01	41·1	0·30	62·8	2·11
5 Phytate-supplemented, *ad lib.*-fed	(6)	1404	17	1318	21	2130	25	2376	46	139·0	1·68	158·2	7·89	42·1	0·52	46·1	3·75

* These were derived from the body-wts of the rats on day 0 and the mean metal contents of a group of six rats of similar age and wt which were killed on the 1st day of the experiment. These values were (μg/g body-wt): Zn 20·20, Fe 30·65, Cu 2·00, Mn 0·605.
† For details of diets, see p. 244.

Table 3. *Average daily accumulation, and whole-body retention relative to dietary intake (expressed as a proportion of dietary intake) of zinc, iron, copper and manganese for rats maintained for 21 d on a semi-purified diet§ with or without Zn and sodium phytate supplements*

(Mean values with their standard errors; no. of rats in parentheses)

Dietary treatment		Average accumulation (μg/d) Zn Mean	SE	Fe Mean	SE	Cu Mean	SE	Mn Mean	SE	Whole-body retention Zn Mean	SE	Fe Mean	SE	Cu Mean	SE	Mn Mean	SE
Zn-supplemented																	
1 *ad lib.*-fed	(6)	139·5	6·9	111·0	8·6	13·3	1·1	5·16	1·2	0·689	0·024	0·164	0·010	0·0391	0·0026	0·0075	0·0016
2 Phytate-supplemented, *ad lib.*-fed	(6)	12·9	0·8†††	24·1	4·3†††	1·82	0·22†††	−0·28	0·61†††	0·138	0·008†††	0·077	0·014†††	0·0116	0·0013†††	0·0009	0·0002†††
3 Pair-fed to treatment 2	(5)	61·5	2·4‡‡	49·2	1·1‡‡	4·17	0·44‡‡	0·61	0·34‡‡	0·669	0·022‡‡	0·161	0·004‡‡	0·0273	0·0031‡‡	0·0020	0·0011‡‡
Zn-deficient																	
4 *ad lib.*-fed	(6)	0·36	0·81	20·2	2·0	1·99	0·26	1·03	0·09	0·0078	0·250	0·0813	0·0087	0·0159	0·0018	0·0041	0·0003
5 Phytate-supplemented, *ad lib.*-fed	(6)	−4·08	0·78***	11·7	1·7***	0·91	0·30*	0·19	0·19**	−1·300	0·247***	0·0490	0·0076*	0·0076	0·0026*	0·0008	0·0007**

The Zn-supplemented groups (treatments 1, 2 and 3) and the Zn-deficient groups (treatment 4 and 5) were considered separately for statistical analysis. For comparisons between treatments 4 and 5 results were analysed by variance ratio, and the statistical significance was: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. For treatments 1–3, where there was significant heterogeneity of variance between groups, comparisons were made by a non-parametric test (Mann–Whitney U test). Mean values for treatment group 1 were significantly different from those for treatment group 2: †††$P < 0.001$; those for treatment group 2 were significantly different from those for treatment group 3: ‡‡$P < 0.01$.
§ For details of diets, see p. 244.

Table 4. *The effect of phytate (5·07 μmol) on the absorption, binding and total mucosal uptake of zinc (μg Zn/loop per 15 min) from a test dose of 5 μg ^{65}Zn in 1 ml saline containing 109 μmol Ca^{2+} (as $CaCl_2$) by ligated loops of rat duodenum in situ*

(Mean values with their standard errors; no. of animals in parentheses)

Loop contents	Absorption	Binding	Total mucosal uptake
Saline + ^{65}Zn	0·38 ± 0·08 (4)	1·38 ± 0·09 (4)	1·76 ± 0·05 (4)
Saline + ^{65}Zn + phytate	0·015 ± 0·013 (4)***	0·74 ± 0·17 (4)*	0·76 ± 0·19 (4)***

The statistical significance of the differences between mean values, analysed by Student's t test, were: * $P < 0·05$, *** $P < 0·001$.

Influence of phytate on Zn absorption from ligated duodenal loops

The results of ligated loop experiments in which the effects of phytate on Zn absorption (total transfer of Zn from loop to carcass) and Zn binding to loop tissue were studied, are shown in Table 4. The molar concentration ratio for Zn:phytate:Ca (1:66:1413) was chosen to correspond to the respective molar ratios for the Zn- and phytate-supplemented diet which contained (/kg): Zn 15 mg, phytate 10 g, Ca 13 g, and which was used in the dietary experiments.

Phytate significantly reduced binding of Zn to the loop tissue and virtually stopped Zn absorption. At the end of the 15 min absorptive period the lumen contents of loops to which phytate had been added contained a fine white suspension which, after centrifugation and measurement of radioactivity, was found to contain > 80% of the ^{65}Zn remaining in the loop contents. These in vivo findings are consistent with the conclusion of Oberleas *et al.* (1966 *a, b*), based on in vitro studies, that phytate reduces Zn availability by precipitation of the Zn in the intestinal lumen as a Zn–Ca–phytate complex.

Zn secretion and reabsorption in ligated loops

In order to assess the possible quantitative significance of secretion and reabsorption of Zn across the intestinal mucosa, studies were done involving measurement of the recovery of stable Zn in ligated loops of duodenum and ileum filled with either saline alone or a saline–Chelex cation-exchange resin slurry.

In a preliminary experiment the presence of the cation-exchange resin in isolated duodenal loops virtually stopped the absorption of Zn from a subsequently injected dose of 5 μg Zn labelled with ^{65}Zn. Thus from four saline-filled loops 0·88 ± 0·17 μg Zn was absorbed in 15 min compared with 0·09 ± 0·08 μg Zn absorbed from corresponding loops containing the ion-exchange resin ($P < 0·001$).

In subsequent studies, isolated duodenal or ileal loops were filled with either saline alone or a saline–Chelex resin slurry, and after 1 h the loops were excised and the contents and washings analysed for Zn. Previous work had indicated that ^{65}Zn^{2+} retained by this resin could not be absorbed, and therefore it was considered reasonable to assume that the Zn in the lumen contents of loops containing resin represented the total Zn (as either the free ion or cationic-Zn complexes) present in endogenous secretions produced during the 1 h experiment. The difference between this value and that for Zn present in loops from which resin was omitted was taken to represent

Table 5. *The recovery of zinc in duodenal and ileal loops of rat intestine in situ 1 h after they were filled with either 1 ml saline (9 g NaCl/l) or 1 ml of a slurry of cation-exchange resin (Chelex)–saline (50:50, v/v)*

(Mean values with their standard errors; no. of animals in parentheses)

	Recovery of Zn (μg/loop per h)	Fraction reabsorbed†
Duodenal loops		
Saline + Chelex-filled	1·68 ± 0·10 (6)***	0·36
Saline-filled	1·07 ± 0·15 (6)	
Ileal loops		
Saline + Chelex-filled	1·33 ± 0·14 (5)*	0·34
Saline-filled	0·88 ± 0·13 (4)	

The statistical significance of the differences between mean values, analysed by Student's t test, were:
* P < 0·05, *** P < 0·001.

† For explanation, see p. 252.

the component of endogenous Zn secretion that had been reabsorbed. Under these experimental conditions it would seem that in both regions of the intestine about one-third of the endogenously secreted Zn is normally reabsorbed (Table 5).

Influence of dietary phytate on absorption and retention of tracer doses of ^{65}Zn

Rats previously trained to consume their food in two 1 h periods were dosed with ^{65}Zn either by addition to their food or by intraperitoneal injection. Trained, meal-fed rats were used to ensure complete ingestion of the oral dose of ^{65}Zn. The ^{65}Zn retention curves for the phytate-fed rats dosed either orally or by injection are shown in Fig. 4 *a*, and those for pair-fed control rats not receiving phytate are shown in Fig. 4 *b*. Pair-fed control rats were used in order to eliminate any possible changes in absorption and retention of Zn resulting from the reduction in food intake and low growth rates of rats maintained on the phytate-containing diet (see Table 1 and Fig. 1 *a*).

For each dietary treatment the linear portion of the semi-logarithmic retention curves between 91 and 211 h post-administration had the same slope whether the ^{65}Zn was administered orally or by injection, indicating that these tracer doses of ^{65}Zn, once inside the body, were handled in the same way whether absorbed or injected. Therefore, ^{65}Zn retention results from 91 to 211 h post-administration for both injected and orally dosed rats were pooled within each dietary treatment group for calculation of the biological half-life of the body ^{65}Zn. The true proportion of dietary Zn absorbed by phytate-fed and pair-fed control groups was derived from these values (Fig. 4) as described by Heth & Hoekstra (1965). In brief, the method assumes that extrapolation of the injected-dose retention curve to the ordinate (Y_1) indicates the proportion of the original injected dose present in the linear component under study. As the injected and absorbed doses of Zn are handled in the same way, the intercept on the ordinate of the extrapolated linear component of the oral-dose retention curve (Y_2) must represent the same fraction of the total ^{65}Zn initially absorbed (see Fig. 4). The true proportion of ^{65}Zn absorbed can thus be calculated from $Y_2 \div Y_1$. This analysis
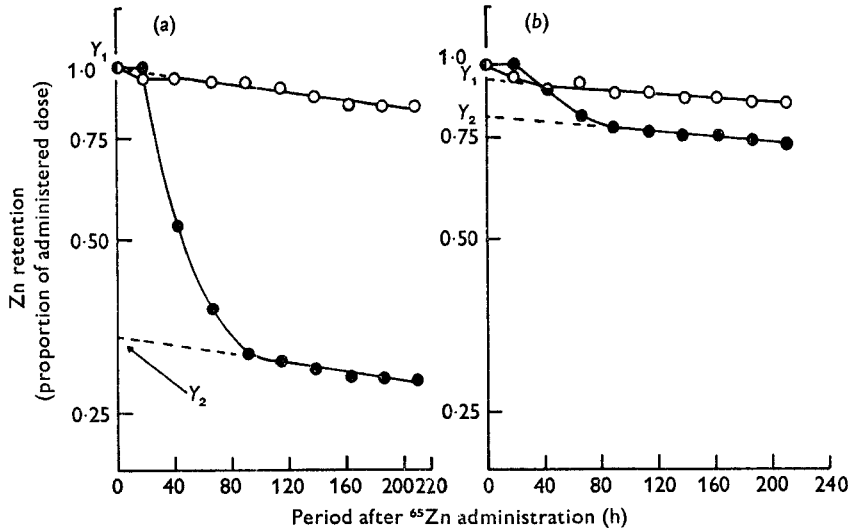
Fig. 4. Retention curves for a tracer dose of ^{65}Zn administered in the diet (●—●) or by intra-peritoneal injection (○—○) to rats maintained on a Zn-supplemented, semi-purified diet, with or without sodium phytate supplements; (a) Zn- and phytate-supplemented diet offered ad lib.; (b) Zn-supplemented diet pair-fed to rats offered the phytate-supplemented diet. Each point represents the mean value for five rats. The slopes and intercepts for the extrapolated retention curves for the injected and orally administered ^{65}Zn (Y_1 and Y_2 respectively) were calculated from the regression equations for the linear components from 91 to 211 h post-administration.

Table 6. *The effect of dietary phytate on the absorption of a tracer dose of ^{65}Zn (2 μCi, specific activity 1·0 mCi/kg), either added to the diet (treatment groups 1 and 3) or administered intraperitoneally in saline (9 g NaCl/l) (treatment groups 2 and 4), and biological half-life 91–211 h after the dose, for meal-fed rats given, with the tracer dose, 3 g Zn-supplemented, semi-purified diet† with or without sodium phytate, and subsequently either offered the phytate-supplemented diet (without ^{65}Zn) ad lib. (treatment groups 1 and 2), or pair-fed the same diet without phytate (or ^{65}Zn) (treatment groups 3 and 4)*

(Mean values with their standard errors; no. of rats in parentheses)

	Proportion of ^{65}Zn absorbed		Biological half-life 91–211 h post-administration (h)	
Treatment group ...	1	3	1 and 2	3 and 4
Dietary treatment ...	Phytate-supplemented, ad lib.-fed	Control (no phytate), pair-fed	Phytate-supplemented, ad lib.-fed	Control (no phytate), pair-fed
	0·344 ± 0·017 (5) ***	0·882 ± 0·035 (5)	1001 ± 156 (10) *	1732 ± 289 (10)

The statistical significance of the differences between mean values, analysed by Student's *t* test, were:
* $P < 0.05$, *** $P < 0.001$.

† For details of diets, see p. 246.

corrects for the absorption and re-excretion of ^{65}Zn into the gut at short periods after administration.

The calculated proportion of ^{65}Zn absorbed and biological half-life for body ^{65}Zn are shown in Table 6. The results indicated that dietary phytate reduced Zn absorption and decreased the biological half-life for body ^{65}Zn. These findings may be explained by the suggestion that as well as inhibiting the absorption of dietary Zn, phytate interferes with the absorption of endogenously secreted Zn, thus promoting a more rapid removal of ^{65}Zn from body stores.

DISCUSSION

Previous studies have shown that the inclusion of phytate in diets fed to many species will result in poor growth rates. Since growth can be improved by increasing the dietary Zn concentration, it has been concluded that this effect results from a reduction in absorption of dietary Zn (reviewed by Becker & Hoekstra, 1971; Oberleas, 1973). The dietary experiments reported in this paper are in full agreement with these findings. Dietary phytate significantly reduced growth rates and food intake of rats given a diet normally adequate with respect to Zn content, and caused a cyclic pattern of food intake characteristic of an uncomplicated Zn-deficiency (Williams & Mills, 1970). Furthermore, the reduction in body-weight of the rats given the Zn-deficient diet supplemented with phytate indicated that these animals suffered a more severe Zn deficiency than the control animals maintained on the Zn-deficient diet without added phytate.

The findings that the addition of phytate to the Zn-supplemented diet resulted in both a substantial increase in faecal Zn excretion, and a reduction in Zn retention assessed both from faecal Zn balance and whole-body analysis, confirmed that phytate had reduced Zn utilization. These results are in full agreement with those of Likuski & Forbes (1965), who found that rats maintained on diets supplying phytic acid (20 g/kg) and Ca (8 g/kg) had reduced growth rates and impaired Zn retention compared with *ad lib.*-fed control animals. Similarly Reinhold, Nasr, Lahimgarzadeh & Hedayati (1973) have recently reported a reduction in Zn (and Ca) retention in human subjects maintained on phytate-rich diets.

A possible mechanism to account for these effects of phytate on the utilization of dietary Zn has been proposed by Byrd & Matrone (1965) and Oberleas *et al.* (1966*a, b*). These workers suggest that Zn combines with phytate and Ca to form an insoluble Zn–Ca–phytate complex from which Zn is unavailable for absorption. In support of this suggestion Oberleas *et al.* (1966*a, b*) found that, within the pH range found in the intestinal lumen, Zn, phytate and Ca in the molar ratio 1:2:1 respectively, form a precipitate in vitro containing 77% of the added Zn. However, these molar proportions are considerably different from those found in practical diets or experimental diets of the type used in this present study, where Ca would be in a 500–1000-fold molar excess and phytate in a 30–100-fold molar excess over Zn. These workers also found that the uptake of ^{65}Zn by strips of jejunal tissue incubated in vitro was reduced by the presence of Ca and phytate in the incubation medium.

The results of studies of Zn absorption from ligated loops of rat duodenum in situ reported here fully support this hypothesis. In these experiments, in which the relative molar ratio of Zn, phytate and Ca present in the loops was the same as their ratio in the phytate- and Zn-supplemented diets, phytate markedly reduced mucosal uptake, binding and absorption of ^{65}Zn (Table 4). The finding that $> 80\%$ of the ^{65}Zn remaining in the lumen contents of the loops injected with phytate was in a suspended particulate form supports the suggestion that Zn is rendered unavailable for absorption by co-precipitation with Ca and phytate.

Further evidence in support of a direct effect of dietary phytate on Zn absorption was obtained in the more physiological measurement of absorption derived from the retention curves subsequent to oral and injected dosing of rats with a tracer amount of ^{65}Zn. In these experiments phytate reduced by 63% the absorption of ^{65}Zn added to the diet. In contrast to these findings Heth & Hoekstra (1965), using the same technique, reported that they were unable to find a direct effect of phytate on Zn absorption when phytate was added to diets containing either protein hydrolysates or animal protein. However, the percentage absorption of dietary Zn has been found to be reduced by increasing the dietary Zn concentration (Becker & Hoekstra, 1971), and the effects of phytate in increasing dietary Zn requirements of rats may be influenced both by the Ca and Zn contents of the diet (Oberleas *et al.* 1966*a, b*) and the phytate concentration (Likuski & Forbes, 1965). Since no details were given by Heth & Hoekstra (1965) of either the Zn, Ca or phytate contents of the diets used, it is difficult to speculate on why their findings are at variance with those reported here.

From the results of this present study it is evident that, while a reduction in absorption of dietary Zn may explain the effects of phytate on faecal Zn output and Zn retention in the rats fed Zn-supplemented diets, it cannot fully account for the net loss of Zn from the rats maintained on the Zn-deficient diet. One possible explanation for these findings is that dietary phytate forms complexes with endogenously secreted Zn and prevents its reabsorption. This proposal seems likely in view of the results of ligated loop experiments in which the recovery of Zn in both duodenal and ileal loops containing cation-exchange resin (Chelex) was 35% higher than the recovery in loops filled with saline alone. Since Zn^{2+} was shown to be unavailable for absorption in the presence of this resin, this extra amount of recovered Zn, amounting to 0.45–0.60 μg Zn/loop per h, may represent a proportion of secreted Zn normally reabsorbed. If the assumptions are made that these rates of secretion and reabsorption are maintained throughout the day, and along the entire length of the intestine, the total amount of Zn reabsorbed daily would correspond to about 90–100 μg. When this amount is compared with the mean daily accumulation of 139.5 ± 6.9 μg Zn/d for the six rats maintained on the Zn-supplemented diet fed *ad lib.* (Table 3), it is clear that recycling of Zn across the intestine may be a significant factor in the over-all body economy of this trace metal.

An alternative explanation for these results is that the presence of a strong Zn-complexing agent in the lumen of the gut, either ion-exchange resin or phytate and Ca, extracts Zn either from the intestinal mucosal cells or across the mucosal tissue from the blood and tissues. Which of these two explanations is correct cannot be

ascertained from these experiments although either can adequately explain how phytate added to a Zn-deficient diet promotes a net loss of carcass Zn.

The same explanations can be advanced to explain the decrease in half-life (increased rate of turnover) of the body ^{65}Zn due to dietary phytate; namely, a more rapid loss of ^{65}Zn due to an inhibited reabsorption of secreted ^{65}Zn or extraction of ^{65}Zn either from or through the intestinal mucosa.

It is over 30 years since Widdowson & McCance (1942) first reported that Fe in phytate-rich diets is relatively unavailable. Since then numerous workers have confirmed this report and have found that dietary phytate reduces Fe absorption. In a recent review on phytate, Oberleas (1973) notes that as ferric phytate is least soluble in dilute acid, but at the pH encountered in the duodenum this complex dissociates, the site of action of phytate in decreasing Fe absorption is probably the stomach, where it may prevent Fe binding to gastroferrin.

The finding in the present study of a reduction in carcass retention of Fe due to phytate addition to both the Zn-supplemented and Zn-deficient diets is surprising in view of the high levels of Fe in these diets (50 mg/kg) in relation to the recommended dietary Fe requirement for the rat ((US) National Research Council, 1972). Even more surprising is the finding that phytate reduced the retention of Cu when the concentration of this trace metal in the diets, at 25 mg/kg, was four to five times the recommended minimum dietary level ((US) National Research Council, 1972).

To the authors' knowledge no studies hitherto reported have shown a reduction in Cu or Mn availability due to the presence of phytate in the diets. However, Davis, Norris & Kratzer (1961) reported that diets containing an isolated soya-bean protein reduced the availability of both trace metals for the chick, and in view of the high phytate content of soya-bean meal (Oberleas *et al.* 1966*a*) it would seem likely that phytic acid was the agent responsible.

The mechanism by which phytate reduces the availability of Cu and Mn probably results from the ability of these trace metals to form metal–phytate complexes which are stable within the intestinal tract (Maddaiah, Kurnick & Reid, 1964; Vohra, Gray & Kratzer, 1965). Whether or not a role for Ca can be implicated in these dietary interactions with phytate, as has been found for Zn (Oberleas *et al.* 1966*a, b*) has yet to be determined. In this context the finding by Oberleas (1973), that the amount of precipitate formed at pH 6–7 when equimolar proportions of Cu and Ca were added to a solution of phytate was more than double the amount formed on addition of Cu alone, may be of relevance.

In addition to its effects on the retention of Zn, Fe, Cu and Mn, dietary phytate has also been shown to reduce the availability of Ca (Bruce & Callow, 1934) and magnesium (Likuski & Forbes, 1965; Seelig, 1964). In view of these findings, the nutritional significance of dietary phytate in relation to the essential trace metals needs further study. In agreement with the speculations of Oberleas (1973), it seems likely that this agent, under the appropriate dietary conditions, may promote a nutritional deficiency of any of these trace metals, depending upon which first becomes limiting in the diet

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