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The availability of zinc in endosperm, whole grain and bran-enriched wheat crispbreads fed to rats on a Zn-deficient diet

BY GÖRAN HALLMANS[1,2], ROLF SJÖSTRÖM[5], LARS WETTER[2,3] AND KENNETH R. WING[4]*

Departments of [1]Nutritional Research, [2]Pathology, [3]Plastic Surgery and [4]Oral Radiology, and [5]Biophysics Laboratory, University of Umeå, S-901 87 Umeå, Sweden

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The hypothesis that factors such as dietary fibre and phytate in wheat bran limit the availability of Zn was tested in growing rats fed on low-Zn diets with different wheat crispbreads as the major source of Zn. Six groups of six weanling male rats each were fed on 5 parts semi-synthetic Zn-deficient diet and 1 part wheat-endosperm crispbread for 1 week. At the beginning of the second week, the crispbread in the diet of five groups was exchanged for crispbread made using one of the following wheat flours: (1) whole grain, (2) bran-enriched whole grain, (3) endosperm with Zn added to the whole-grain level, (4) endosperm with Zn added to the bran-enriched level, (5) whole grain with Zn added to the bran-enriched level. These diets were given *ad lib.* together with deionized water for 2·5 weeks. The relative absorption of Zn was lowest from the three non-supplemented diets (75–82%). All the added Zn was absorbed. As appetite, body-weight increase, Zn absorption, Zn retention and the Zn concentrations in serum and bone differed only slightly among groups fed on diets with similar Zn concentrations, it is concluded that factors such as dietary fibre or phytate in wheat bran limit the availability of Zn in wheat crispbreads very little when all the Zn is needed for growth and development in rats.

Wheat bran: Zinc availability: Zinc deficiency: Rat.

Much information has been presented which appears to support the hypothesis that factors such as dietary fibre or phytate, or both, which are abundant in bran, inhibit the intestinal absorption of minerals such as zinc (Reinhold *et al.* 1973; Davies & Nightingale, 1975; Sandström *et al.* 1980; Sandberg *et al.* 1982; Davies *et al.* 1985). The values obtained for the intestinal absorption of minerals from various foods have been referred to as their bioavailability in those foods. This implies that the mineral which was not absorbed is not available to the animal or to man. If this is the case, the binding factors in the food should limit the amount of mineral available for absorption even when the body's need for Zn is increased due to growth, injury, disease or greater than normal losses. In most studies reported in the literature, the experimental conditions have not been chosen or manipulated in order to increase the body's need for Zn before measuring and comparing the intestinal absorption of Zn from diets with high and low bran concentrations.

In an earlier study of the intestinal absorption of Zn in rats during the early phase of wound healing (Hallmans & Wing, 1978), we found what we interpreted to be an increased intestinal absorption of Zn from wheat-endosperm crispbread compared with that in unoperated controls. In a recent investigation (Hallmans *et al.* 1987) we attempted to increase the need for Zn in rats by administering a parenteral-nutrition solution intraperitoneally, and found the intestinal absorption of Zn from wheat-endosperm bread to be greater than the absorption of Zn from the same bread in controls given physiological saline (9 g sodium chloride/l) intraperitoneally. While this indicates that more Zn is available than that absorbed in control animals, there was no bran in the breads and thus

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Table 1. *Composition of the semi-synthetic zinc-deficient diet (g/kg dry weight)**

Ingredient	
Maize starch	375
Casein†	220
Sucrose	100
Glucose	100
Soya-bean oil	100
Mineral mixture, Zn excluded	40
Cellulose	30
Calcium salts (Ca_2SO_4 18 mg/g, $CaCO_3$ 7 mg/g)	25
Vitamin mixture (with maize starch)	10

* 2·0 μg Zn/g dry weight, 14·2 mg Ca/g dry weight (by atomic absorption spectrophotometry).
† Washed twice with EDTA.

dietary fibre and phytate concentrations can be assumed to have been low. Moreover, as the animals were fed on bread and deionized water only, a diet few humans would choose freely, this is a less than ideal experimental model.

In the present study, the hypothesis that factors such as dietary fibre and phytate in wheat bran limit the amount of Zn in bran available for intestinal absorption was tested in an experimental model in which the need for Zn was high. Growing rats were fed on diets in which the main source of Zn was wheat crispbread and the Zn concentrations were at or below the rats' requirements for growth and development. The amounts of bran and Zn in the diets differed among the experimental groups. Appetite, growth, intestinal absorption and retention of Zn, and tissue and fluid Zn concentrations were determined and compared among the groups.

MATERIALS AND METHODS

Experimental design

Thirty-six male, albino rats of the Sprague-Dawley strain (Anticimex, Stockholm, Sweden) were used in this experiment. At the start they weighed 87·2 (SD 4·6) g. They were assigned by formal randomization to one of six groups of six rats each and they were housed separately in metabolism cages of acrylic resin and stainless steel. During the first week they were all fed *ad lib.* on a diet containing 5 parts semi-synthetic Zn-deficient diet (Astra Ewos, Södertälje; Table 1) and 1 part wheat-endosperm crispbread. From the beginning of the second week until the end of the experiment 18 d later, the wheat-endosperm crispbread in the diets of five of the six groups was replaced with wheat crispbread with different Zn and bran concentrations (Table 2 and Fig. 1). Deionized water was also available *ad lib.*

The dough used to bake approximately 1·1 kg crispbread contained (g): 960 wheat flour (endosperm, whole grain or bran-supplemented whole grain), 68 yeast, 15 salt, 57 sucrose, 88 margarine, and 560 ml water. The wheat-endosperm flour was composed of 70% extraction wheat and the bran-supplemented flour was composed of 3 parts whole-wheat flour and 2 parts wheat bran by weight. Zinc sulphate was added to some of the dough used in baking endosperm and whole-wheat crispbreads in order to achieve the same Zn concentration as that in the bran-enriched whole-wheat bread, and to some of the dough used in baking endosperm bread to achieve the Zn concentration in the whole-wheat crispbread. The phytic acid concentrations were measured using the method of Ellis *et al.* (1977) as modified by Sandberg *et al.* (1982). The dietary fibre concentrations were

Table 2. *The concentrations of zinc, phytic acid and dietary fibre (/g dry weight) in the six diets with different crispbreads as Zn sources* and the phytate:Zn, and calcium × phytate:Zn, molar ratios*

(Zn concentrations are presented as the means and standard deviations of ten 1 g samples)

Crispbread in diet as Zn source	Zn (μg/g)		Phytate (μmol/g)	Molar ratios		Dietary fibre (mg/g)
	Mean	SD		Phytate:Zn	Ca × phytate:Zn (mol/kg)	
Endosperm	3·6	0·2	0·5	9	2·7	48
Whole wheat	7·0	0·4	1·7	16	4·7	60
Bran-enriched whole wheat	10·1	0·4	3·3	22	6·4	75
Endosperm + Zn	6·8	0·2	0·5	5	1·4	48
Endosperm + Zn + Zn	9·5	0·6	0·5	4	1·0	48
Whole wheat + Zn	9·7	0·3	1·7	11	3·4	60

* For details, see Table 1 and Fig. 1.

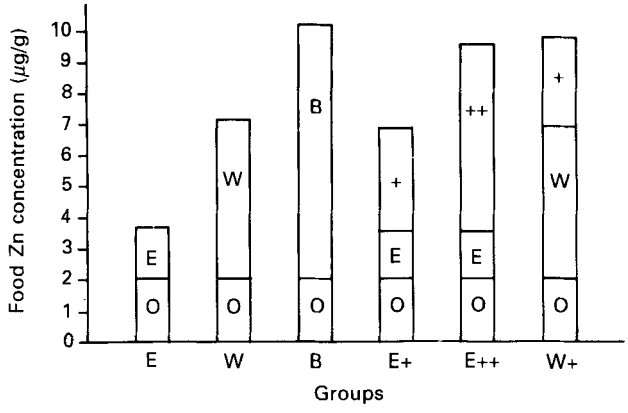

Fig. 1. The zinc concentrations and the sources of Zn in the composite diets given during the last 18 d of the experiment. O, Zn-deficient diet; E, wheat-endosperm; W, whole wheat; B, bran-enriched whole wheat; E+, wheat-endosperm supplemented with Zn to the whole wheat level; E++ and W+, wheat-endosperm and whole wheat supplemented with Zn to the bran-enriched whole wheat level; + and ++, supplements as zinc sulphate.

determined using the method of Asp *et al.* (1983). The calcium concentrations were 11·9–12·0 mg/g composite diet in all six diets.

^{113}Sn-labelled microspheres (NEN-TRAC™, New England Nuclear Corp., Boston, MA, USA) were added to the dough before baking each of the six crispbreads and were used as a marker for the composite diet in the measurements of Zn retention. Microspheres are ion-exchange resin beads coated with a polymeric resin. Those used in the present study were approximately 15 μm in diameter and had a density of 1·3 g/ml. ^{113}Sn emits 0·392 MeV gamma rays and has a half-life of 118 d. The ^{113}Sn activity was 1·5 Bq/bead or 820 Bq/g diet (0·022 μCi/g diet). At 1 week after the change of bread in the diet, all thirty-six rats were injected subcutaneously in the nape of the neck with 5 μCi ^{65}Zn as the chloride (Amersham International plc, Amersham, Bucks) in 0·5 ml physiological saline (9 g sodium chloride/l).

Each day during the 25 d of the experiment, the rats were weighed, the amount of food remaining in the food dispensers and cages was weighed and discarded, and newly weighed portions were put in the dispensers. During day 10 and during the last 5 d of the experiment, days 21–25, the faeces were collected in weighed glass tubes for determination of the amounts of Zn, ^{65}Zn and ^{113}Sn excreted.

Tissue sampling

On day 25 after the start of the experiment the rats were killed by exsanguination under diethyl ether anaesthesia. Samples of blood, serum, liver, kidney, spleen and the entire femur as well as samples of the breads and composite diets were taken in weighed glass tubes. The fresh weights of all samples were recorded. The samples of faeces, blood, tissues, breads and diets were dried to constant weight at 110° for 3 d and weighed again. They were then ashed overnight at 550° and the ash was weighed. After being dissolved in 0·5 ml 3 M-hydrochloric acid and left to stand overnight, they were diluted with 2 ml deionized water.

Gamma scintillation and atomic absorption methods

The ^{65}Zn and ^{113}Sn activities in the serum samples and the dissolved ash of the other samples were measured using a Packard Auto-Gamma Model 5330 spectrometer with a 75 × 84 mm sodium iodide (Tl) through-hole scintillation detector. Portions of the ^{65}Zn injection solution and the ^{113}Sn-labelled diets were used as references. The background, reference and sample counting rates at the 1·119 MeV ^{65}Zn and the 0·392 MeV ^{113}Sn gamma peaks were measured and the ^{65}Zn activity was stripped from the total activity in the 0·392 MeV peak for each sample in order to recover the ^{113}Sn activity.

The ashed samples were further diluted with 0·6 M-HCl as necessary and the serum samples were diluted tenfold with 0·1 M-HCl for the determination of the Zn concentrations using a Varian Techtron Model AA-6DB atomic absorption spectrophotometer at 213·9 nm. Known amounts of Zn dissolved in 0·6 M-HCl were used as references (Hallmans, 1978).

Calculations

The Zn retentions during days 10 and 21–25 were calculated. The amount of diet represented by the faeces is first calculated by dividing the ^{113}Sn activity/g faeces by the ^{113}Sn activity/g diet. The Zn excreted in the faeces is then divided by the amount of diet represented by the faeces and subtracted from the Zn concentration in the original diet:

$$\text{Zn retention } (\mu\text{g/g diet}) = \text{Zn in diet } (\mu\text{g/g diet}) - \text{Zn in faeces } (\mu\text{g/g diet in faeces}).$$

The Zn retention per d or per measurement period can be calculated by multiplying the retention (μg Zn/g diet) by the amount of diet recovered in faeces per d or per measurement period.

The calculation of Zn retention requires that the ^{113}Sn in the microspheres is not absorbed. As no measurable ^{113}Sn was detected in any organ at death after 18 d on the ^{113}Sn-labelled diet, it can be assumed that the microspheres were in fact not absorbed and that no ^{113}Sn leaked from the microspheres.

The amount of Zn absorbed is calculated by adding to the Zn retention (/g diet) the amount of endogenous Zn excreted (/g diet) (Weigand & Kirchgessner, 1976*a*). The excretion of endogenous Zn was calculated by dividing the ^{65}Zn activity in the daily collections of faeces by the time-corrected ^{65}Zn specific activity in serum:

$$\text{Zn absorbed (/g diet)} = \text{Zn retention (/g diet)} + \frac{\text{faeces } (^{65}\text{Zn}:^{113}\text{Sn}) \times (^{113}\text{Sn/g diet})}{\text{serum } (^{65}\text{Zn}:\text{Zn})}$$

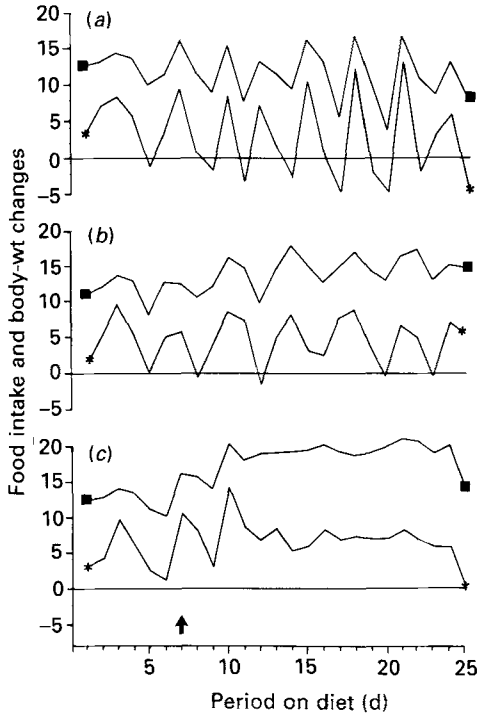

Fig. 2. Diet consumption (g/d, ■) and body-weight changes (g/d, *) of rats fed on a composite diet with wheat crispbread as the main source of zinc. All rats were fed on a diet with wheat-endosperm crispbread (3·6 μg Zn/g diet) during the first week. During the subsequent 18 d they were fed on the same diet with different breads for different groups. For details of diet and breads, see Tables 1 and 2 and Fig. 1. (*a*) A rat from the group fed on a diet with wheat-endosperm crispbread (3·6 μg Zn/g diet), (*b*) a rat from the group fed on a diet with whole wheat crispbread (7·0 μg Zn/g diet), (*c*) a rat from the group fed on a diet with bran-enriched whole wheat crispbread (10·1 μg Zn/g diet). ↑, Start of the different diets.

The time-corrected ^{65}Zn specific activity in serum for a particular collection period is calculated by extrapolating the specific activity in serum at death back in time using the slope of the change in ^{65}Zn specific activity in the faeces according to the method of Weigand & Kirchgessner (1976*b*). In these calculations we have assumed the time interval from the excretion of Zn into the intestinal lumen until its appearance in the faeces to be 12 h.

Statistics

The means for a given variable in the six groups were tested using a one-way analysis of variance (F). If a statistically significant result was obtained ($P < 0{\cdot}01$), *a priori* contrasts of the diet groups were tested with least significant differences (LSD). Pearson product-moment correlation coefficients (r) for pairs of variables were calculated and tested using Student's t test (Snedecor & Cochran, 1967). In all statistical tests we have chosen to reject the null hypothesis at the 1% level ($P < 0{\cdot}01$).

RESULTS

Eating patterns. The eating patterns of all thirty-six rats were cyclical by the end of the first week on the composite Zn-deficient diet with wheat-endosperm crispbread (Fig. 2). During the second week, this cyclical pattern almost disappeared in the three groups with the

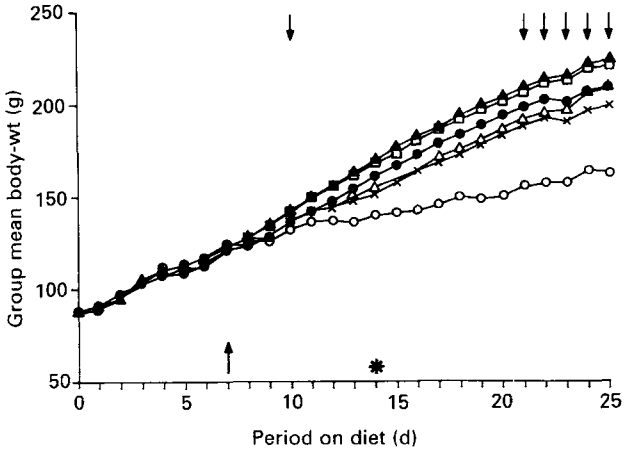

Fig. 3. Group mean daily body-weight of rats fed on a composite diet with wheat crispbread as the main source of zinc. All rats were fed on a diet with wheat-endosperm crispbread (3·6 μg Zn/g diet) during the first week. During the subsequent 18 d they were fed on the same diet with different breads for different groups. ○, Wheat-endosperm (3·6 μg Zn/g diet); △, whole wheat (7·0 μg Zn/g diet); □, bran-enriched whole wheat (10·1 μg Zn/g diet); X, wheat-endosperm supplemented with Zn to the whole wheat level (6·8 μg Zn/g diet); ●, wheat-endosperm supplemented with Zn to the bran-enriched whole wheat level (9·5 μg Zn/g diet); ▲, whole wheat supplemented with Zn to the bran-enriched whole wheat level (9·7 μg Zn/g diet). ↑, Start of the different diets; ✱, day on which ^{65}Zn was injected; ↓, days on which Zn retention studies were made. For details of diet and breads, see Tables 1 and 2 and Fig.1.

highest Zn concentrations in their diets (Fig. 2(*c*)) but continued in the three groups with less Zn (Fig. 2(*a* and *b*)).

Food intake and body-weight gain. The cumulative food intake and the gain in body-weight during the first week were similar in all six groups (Fig. 3). The amount of composite diet consumed and the gain in body-weight during the next 18 d were significantly different among groups with different dietary Zn concentrations (Table 3). The weight gain in individual rats was directly related to the amount of diet consumed (r 0·95, $P < 0·001$). The rats' appetites increased non-linearly with increasing Zn concentration in the diet but they were not affected by the amount of bran. Groups given Zn-supplemented endosperm breads had consistently lower increases in body weight than groups fed on whole wheat or bran-enriched whole wheat breads with similar Zn concentrations, but these differences were not significant (Table 3).

Zn absorption. The absorption of Zn from the six diets (μg Zn/g diet) was significantly different among groups with different dietary Zn concentrations (Table 3). The Zn absorption was not significanly different among groups with similar dietary Zn concentrations, with one exception. In the group given the bran-enriched diet, Zn absorption was slightly less than that in the other two groups with similar dietary Zn concentrations. The relative absorption of Zn was lowest from the three non-supplemented diets (75–82%). The absorption of the Zn supplements was in all cases close to 100%.

Endogenous Zn excretion and Zn retention. The excretion of endogenous Zn was most pronounced in the three groups with the highest dietary Zn concentrations (Table 3). The excretion of endogenous Zn did not differ among groups given diets with similar Zn concentrations. The average Zn retention for days 21–25 was significantly different among groups with different dietary Zn concentrations (Table 3). The Zn retention in the group given the bran-enriched diet was slightly less than that in the other two groups with similar dietary Zn concentrations, while that in the other groups with similar Zn concentrations

Table 3. *The intestinal absorption and retention of zinc and the excretion of endogenous Zn (days 21–25) and the daily food intake and body-weight changes (days 7–25) in six groups of rats given diets with different crispbreads as Zn sources*

(Means for groups of six rats with their standard errors are presented together with the results of one-way analyses of variance (*F*) and least significant differences (LSD))

Crispbread in diet as Zn source	Dietary content (μg Zn/g diet)	Absorption (μg Zn/g diet)		Absorption (%)		Endogenous excretion (μg Zn/g diet)		Retention (μg Zn/g diet)		Food intake (g/d)		Body-wt increase (g/d)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Endosperm	3·6	$2·7^{a}$	0·1	$75·5^{a}$	2·7	$0·6^{a}$	0·1	$2·1^{a}$	0·1	$10·5^{a}$	0·30	$2·2^{a}$	0·2
Whole wheat	7·0	$5·7^{b}$	0·1	$81·9^{bc}$	0·8	$0·6^{a}$	0·1	$5·1^{b}$	0·1	$13·5^{b}$	0·27	$4·9^{bc}$	0·2
Bran-enriched	10·1	$7·8^{e}$	0·2	$77·9^{ab}$	1·6	$1·4^{b}$	0·1	$6·4^{c}$	0·1	$15·3^{c}$	0·58	$5·5^{e}$	0·4
Endosperm + Zn	6·8	$5·8^{b}$	0·1	$85·0^{cd}$	1·1	$0·8^{a}$	0·1	$5·0^{b}$	0·1	$13·5^{b}$	0·49	$4·3^{b}$	0·3
Endosperm + Zn + Zn	9·5	$8·4^{d}$	0·1	$88·4^{d}$	0·6	$1·4^{b}$	0·1	$6·9^{d}$	0·1	$14·2^{bc}$	0·30	$5·0^{bc}$	0·1
Whole wheat + Zn	9·7	$8·5^{d}$	0·1	$87·8^{cd}$	1·3	$1·3^{b}$	0·1	$7·3^{d}$	0·1	$14·6^{bc}$	0·35	$5·6^{c}$	0·1
F		476·9		288·9		29·7		12·1		17·8		28·7	
df		5, 30		5, 30		5, 30		5, 30		5, 30		5, 30	
Statistical significance: *P*		< 0·001		< 0·001		< 0·001		< 0·001		< 0·001		< 0·001	
$LSD_{0·01}$		0·4		0·4		0·3		5·9		1·5		0·9	

^{a,b,c,d} Means with different superscript letters are significantly different ($P < 0·01$).

Table 4. *The fresh weight zinc concentrations in serum, whole blood, liver and femur, and Zn on the basis of the ash weight in femur*

(Means for groups of six rats with their standard errors are presented together with the results of one-way analyses of variance (F) and least significant differences (LSD))

Crispbread in the diet as Zn source	Serum (μg Zn/g)		Blood (μg Zn/g)		Liver (μg Zn/g)		Femur (μg Zn/g)		Femur (μg Zn/g ash)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Endosperm	0·63[a]	0·04	3·67	0·34	30·2	1·1	34·2[a]	1·8	73·6[a]	1·9
Whole wheat	0·52[a]	0·03	3·56	0·10	29·0	0·4	31·6[a]	1·3	74·9[ab]	2·3
Bran-enriched	0·77[b]	0·02	3·66	0·17	30·8	1·0	47·0[b]	2·7	101·4[c]	2·1
Endosperm + Zn	0·58[a]	0·02	3·89	0·16	29·9	0·8	37·8[ac]	2·5	82·6[b]	1·7
Endosperm + Zn + Zn	0·84[b]	0·04	3·96	0·11	29·7	0·7	45·0[bc]	1·7	100·4[c]	1·7
Whole wheat + Zn	0·76[b]	0·01	4·10	0·15	30·3	0·7	49·4[b]	2·4	98·8[c]	2·3
F	17·79		1·20		0·56		11·79		42·50	
df	5, 30		5, 30		5, 30		5, 30		5, 30	
Statistical significance: P	< 0·001		> 0·05		> 0·05		< 0·001		< 0·001	
$LSD_{0·01}$	0·12		—		—		8·3		7·8	

[a, b, c] Means with different superscript letters are significantly different ($P < 0·01$).

did not differ significantly from each other. The Zn retention was directly related to the Zn concentration in the diet and was only 0·6–1·4 μg Zn/g diet less than the absorption. The relations among the retentions of Zn in the six groups on day 10 were nearly the same as those for days 21–25. The variance (SD^2) in the Zn concentrations in each diet (Table 2) was large enough to influence the variance of the Zn retention ($SD^2 = n \times SE^2$; $n6$) in that group (Table 3).

Serum and tissue Zn. The serum and femur Zn concentrations differed significantly among the six groups but not among groups with similar Zn concentrations in their diets (Table 4). The concentrations were highest in the three groups on diets with the highest Zn contents. The Zn concentrations of blood and liver were similar for all six groups.

DISCUSSION

The minimum requirement for Zn for optimal growth in young rats is 12·5–13 μg Zn/g food (Williams & Mills, 1970). The Zn concentrations in the composite diets fed to the rats in the present study were all below that level. The gain in body-weight in each of the thirty-six rats was directly related to the amount of food consumed regardless of the dietary Zn concentration. The lower cumulative consumption of food in the groups with lower Zn concentrations in their diets was not due to an overall reduction in appetite but due to recurrent periods of low food intake (Figs. 2 and 3). This cyclical eating behaviour in rats fed on diets low in Zn has been observed as a symptom of Zn deficiency in earlier studies (Chesters & Quarterman, 1970; Williams & Mills, 1970; Davies & Nightingale, 1975) and it may be the result of Zn deficiency limiting the utilization of amino acids in protein synthesis and causing the amino acids to be used as a source of energy. The rats' appetites will then be reduced until catabolic processes release Zn which can be used in protein synthesis. The incorporation of amino acids into protein will increase the appetite and reduce the amount of Zn available, thus completing the cycle.

Davies *et al.* (1985) have demonstrated a negative linear relation between the gain in

body-weight and the Ca × phytate:Zn molar ratio in the diet at values above 3·5 mol/kg (Zn > 11 μg/g diet). Davies *et al.* (1977), Davies & Olpin (1979) and Morris & Ellis (1980) demonstrated markedly reduced growth rates in rats fed on diets in which the Ca × phytate:Zn molar ratios were extremely high, i.e. 8 or more (phytate:Zn > 40, Ca concentration > 7·5 mg/g). For groups of rats fed on diets with more moderate molar ratios, i.e. about 5 (Morris & Ellis, 1980; Davies *et al.* 1985), the growth rate was reduced only marginally. In the present study, the molar ratios were between 1·0 and 6·4 and there were no demonstrable differences in body-weight in groups given diets with similar Zn concentrations. The Zn concentrations in the bran-enriched diet and in the endosperm and whole wheat diets supplemented with Zn to the bran-enriched level (9·5–10·1 μg Zn/g) were lower than in all but one experimental group in the studies cited previously and were low enough to limit growth slightly, even in the group fed on a diet with a molar ratio of 1·0. It would appear that, at these Zn concentrations, the increase in molar ratio from 1·0 to 6·4 has no further effect on weight gain.

The absorption and retention of Zn and the serum, blood and femur Zn concentrations were also used as measures of the availability of Zn from the different crispbreads. The absorption and retention of Zn (μg/g diet) were slightly but significantly reduced by the addition of bran to the diet. However, as the rats ate more of the bran-enriched diet than either the endosperm or the whole grain diets supplemented with Zn to the bran-enriched level, the daily Zn intake, the average daily weight gain and the Zn concentrations in the serum, blood, liver and femur were nearly equal in these three groups. As unrefined foodstuffs such as whole-wheat flour and wheat bran generally have high concentrations of minerals such as Zn and, at the same time, have less energy, and in some cases protein, than their refined counterparts, it might be more appropriate to relate Zn absorption to the amount of energy or protein consumed than to express it relative to the amount of Zn or diet consumed.

Earlier studies have demonstrated considerable inhibition of Zn absorption when 10 mg phytate/g was added to a basal diet containing 15 μg Zn/g and 13 mg Ca/g (phytate:Zn = 66; Ca × phytate:Zn = 22) (Davies & Nightingale, 1975) or when 20 mg phytate/g was added to a diet containing 33 μg Zn/g diet (phytate:Zn = 60) (House *et al.* 1982). While such experiments show the ability of phytate to impair Zn absorption, the experimental conditions were non-physiological due to the extreme concentrations of phytate and the fact that phytate was added in purified form. The addition of 50, 100 or 150 mg bran/g to a basal diet containing 54 μg Zn/g and 8 mg Ca/g (phytate:Zn < 10; Ca × phytate:Zn < 2) left the relative Zn retention unchanged (Bagheri & Gueguen, 1981). In the present study a diet containing 10 μg Zn/g and 12 mg Ca/g prepared by adding approximately 75 mg bran/g in the form of bran-enriched crispbread to a basal diet (phytate:Zn = 22; Ca × phytate:Zn = 6.4) caused small but significant decreases in relative Zn absorption from 88 to 78% and relative Zn retention from 75 to 63%. Thus, in diets in which the phytate:Zn molar ratio is close to that of high-fibre diets consumed by humans and the phytate is in its natural form, there is little or no demonstrable impairment of Zn absorption in rats. However, diets with bran with phytate:Zn molar ratios of less than 10 have been shown to reduce Zn absorption from composite meals in humans (for example, see Sandström *et al.* 1980). The differences between the results of studies on rats and humans may be the result of species differences but they may also be the result of differences in the experimental conditions and the choice of measurement to represent availability.

In the present study the three groups given diets in which the naturally occurring Zn in the wheat flour used to bake the breads was the source of Zn showed lower relative absorption of Zn than the three groups given diets supplemented with $ZnSO_4$ due to the fact that all the supplemented Zn was absorbed. The absorption of 100% of the

supplemented Zn reflects the body's pressing need for Zn in the rats in the present study. At dietary Zn levels above the requirement level, the absorption of supplemented Zn is incompletely regulated as part of the body's Zn homeostasis (Becker & Hoekstra, 1971; Evans *et al.* 1973; Weigand & Kirchgessner, 1978). If the mineral-binding factors in bran function as ion exchangers, they may act as a mineral reserve when the body's need is near the mineral content of the diet but offer resistance to the free absorption of mineral when the mineral concentration exceeds the body's need. In this way, these factors could complement the body's homeostatic regulation of Zn. If this is the case, it may not be appropriate to use the absorption of Zn as a measure of its availability when the Zn concentration in the food clearly exceeds the body's needs.

The combined results of the present study (weight gain, Zn absorption, Zn retention, and tissue and fluid Zn concentrations) demonstrate that mineral-binding factors such as dietary fibre and phytate in wheat bran do not to any appreciable extent limit the availability of Zn to growing rats from diets in which the main source of Zn is crispbread if the Zn in these diets is below the minimum requirements for growth and development. The dietary fibre and phytate contents of the bran-enriched diet used in the present study were comparable to those in high-fibre diets consumed by humans. The concentration of bran in the bran-enriched bread was as high as baking techniques allowed and the amount of bread in the diet was high for most diets for humans. While it is not possible to use these results to represent the relative availability to humans of Zn from different crispbreads in their diets, it is very likely that differences in the availability to humans of Zn between diets with and without wheat bran will be very small if the dietary Zn content is near or below the body's needs. This and the ability of the body to adjust its growth and metabolism to the availability of Zn in the diet may be the reasons for the rarity of manifest Zn deficiency in the Western population, despite the fact that Zn intake is often below recommended levels and Zn absorption has been shown to be relatively low and easily impaired by many factors in the diet.

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