Cereal Complexes: Binding of Zinc by Bran and Components of Bran[1]

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ABSTRACT

Binding of zinc ions in nonbuffered aqueous media by food-grade white wheat bran, by components of bran, and by substances normally found in gastrointestinal fluid was investigated in vitro at 37°C over the pH range 5–7. Analysis for uncomplexed Zn^{2+} was by means of the metal indicator tetramethylmurexide. Water-soluble components are responsible for about 39% of the total binding power of whole bran, and the principal soluble chelating agent is probably phytate. Bran that has been pretreated with dilute HCl at pH 1.6 contains an even greater concentration of soluble, zinc-binding substances. The combined contributions of cellulose, starch, hemicellulose, and pectin to the observed binding ability of bran are no greater than 10%. Each gram of bran contains about 0.27 mmol of zinc-binding sites, and each site is capable of chelating one zinc ion. Various gastrointestinal components were investigated for their possible role in binding Zn^{2+} in the human small intestine. On one hand, sugar, saliva, amino acids, albumin, and hydrogen carbonate ion at concentrations expected in the intestine have little effect on the concentration of free Zn^{2+}. On the other hand, phosphate ion and mucin do cause significant zinc loss, and this loss might be high enough to influence bioavailability of zinc. The low solubility of zinc salts of certain bile acids may also be important biologically.

Few studies have been done of the in vitro binding of zinc by food fiber. Reinhold et al (1975) studied the binding of Zn^{2+} by wheat bran, whole meal wheat bread, and various components of bran in phosphate-buffered solution (pH 6.8) and reported that the affinity of bran for Zn^{2+} was high and similar to that of whole meal bread, wheat starch, and gliadin (a wheat protein). The affinity of cellulose for Zn^{2+} was also high—approximately half that of bran. Curiously, dephytinized bran had a greater binding power than nondephytinized bran. In another study of zinc binding by bran and its components, these same investigators (Ismail-Beigi et al 1977) found that hemicellulose and lignin, both isolated from wheat bran, had affinities for zinc that were almost the same as those of washed bran. As in their previous study, phosphate buffering was used to maintain a pH of 6.8. Although the results of these studies indicated that fiber was more important than phytate (*myo*-inositol hexaphosphate) in determining bioavailability of polyvalent metals, the results were not in agreement with those of Davies et al (1977), who conducted growth studies on rats and concluded that the phytate in bran, and not the fiber, was the major determinant of zinc availability. The phytate content of the bran diets accounted for almost all reduction in intestinal absorption. Fiber constituents, including pectin, were without significant effect.

Camire and Clydesdale (1981) recently studied the binding of calcium, zinc, magnesium, and iron by wheat bran, cellulose, and lignin in buffered solution. They reported that the interactions were significantly affected by pH and type of cooking. Toasting had no noticeable effect on zinc binding by bran over the pH range 6–7. Boiling had no effect on zinc binding at pH 6, whereas binding at pH 7 was decreased by boiling.

Using a metal-indicator method of analysis and avoiding buffers, which can themselves complex with polyvalent cations, Rendleman (1982) studied in vitro binding of calcium by wheat bran and its components over the physiological pH range 5–8. His work suggested that phytate was the most important calcium-binding agent in wheat bran and that the fiber constituents contributed relatively little toward binding. Because these conclusions were the opposite of those drawn by Reinhold et al (1975) from their in vitro studies of calcium binding and zinc binding, reinvestigation of the binding of zinc by bran was clearly needed.

This article presents results of an in vitro study of the binding of zinc ions by bran, by aqueous bran extract, by individual components of bran, and by various major constituents of gastrointestinal fluid. All reactions were conducted without buffering at 37°C at constant ionic strength ($\mu = 0.165$) and within the pH range 5–7. Except where strong complexing agents were employed, the upper pH limit for these studies was about 6.8. Addition of KOH to increase the pH of a $ZnCl_2$ solution beyond 6.8 often resulted in the formation of finely divided $Zn(OH)_2$. However, careful addition of KOH to adjust the pH of standard 1.0m*M* $ZnCl_2$ to 6.8 does not reduce the concentration of free Zn^{2+}. The tetramethylmurexide (TMM) method used earlier to determine free (uncomplexed) Ca^{2+} (Rendleman 1982) was used to determine free Zn^{2+}. Most of the studies were conducted in 2.0m*M* $ZnCl_2$ solution. Although this concentration is possibly 15–20 times greater than the concentration of postprandial zinc in the stomach of an adult human, the conclusions from these studies at higher zinc concentration should be applicable to systems containing less zinc.

MATERIALS AND METHODS

Zinc chloride was a reagent grade of high purity (Alfa Products). Other materials and the general procedure for studying zinc binding were the same as those described earlier for the study of calcium binding (Rendleman 1982). Pyrex and polyethylene containers were cleaned with detergent and distilled water. The extent to which Zn^{2+} is adsorbed by the walls of polyethylene containers (Laxen and Harrison 1981) was negligible and therefore ignored. Water was distilled and deionized. Clear, nonturbid stock solutions of 4.0m*M* $ZnCl_2$ were prepared by slowly adding small increments of the appropriate weight of granular $ZnCl_2$ to the desired amount of very rapidly stirred water. These solutions were stable for at least one year. For the quantitative determination of free Zn^{2+} by the TMM method, spectrophotometric measurements were made at 460 and 530 nm. The quotient A_{460}/A_{530} was used to determine free Zn^{2+} from a standard curve obtained by plotting zinc concentration against A_{460}/A_{530}. In several studies, zinc in solution phase was determined by both the TMM method and atomic absorption spectrophotometry.

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[2] The mention of firm names or trade products does not imply that they are endorsed or recommended by the USDA over other firms or similar products not mentioned.

RESULTS AND DISCUSSION

Bran and Zn^{2+}

Because the affinity of bran for zinc is about three times greater than that for calcium, the concentrations of bran and divalent metal cation used in the zinc studies were usually much lower than those used earlier in the calcium studies (Rendleman 1982). However, to maintain an acceptable level of analytical precision and accuracy, the initial concentration of Zn^{2+} was rarely less than 1.0m*M*. AACC wheat bran contains only 54.4 ppm of naturally occurring zinc, an amount much too small to cause error in the TMM analysis of bran-$ZnCl_2$ systems. Analysis of the supernatant from a mixture of 1 g of bran in 100 ml of water at pH 6.0 showed no free Zn^{2+}.

Effect of Bran Concentration and Zn^{2+} Concentration on Binding

Figure 1 shows the relationship between bran concentration and extent of zinc binding (expressed as zinc loss) when other variables are held constant. The relationship is essentially linear up to a bran concentration of 0.5 g/100 ml of solution. Table I shows the effect of initial Zn^{2+} concentration on binding in systems of similar pH and identical bran content. Maximum binding of zinc occurred at an initial concentration of Zn^{2+} between 2.0m*M* and 3.0m*M*. The data in Table I indicate that the maximum amount of Zn^{2+} that can be bound by one gram of bran at pH 6.2 is 0.272 (± 0.006) mmol. Maximum binding at higher pH was not investigated.

Effect of pH on Binding

Figure 2 shows the influence of pH on the binding of Zn^{2+} by 0.5 g of bran in 100 ml of 2.0m*M* $ZnCl_2$. Adjustment of pH was made by adding appropriate amounts of KOH or HCl. Unlike calcium binding, which was found to decrease slightly with increasing pH over the pH range 5–7 (Rendleman 1982), zinc binding was constant between 4.4 and about 6.5 and then increased rapidly with increasing pH in the range 6.7–7.3. The great influence of pH in the range 6.7–7.3 cannot be explained readily on the basis of individual binding characteristics of phytate and the various polysaccharide constituents of bran. The soluble protein components might be responsible. In experiments with albumin in zinc chloride solution, unexpectedly large losses of Zn^{2+} occurred when large amounts of KOH were added rapidly to raise the pH from a normally low value of 5.4 to 7.0. In both the bran and albumin experiments, some Zn^{2+} was probably lost through precipitation of $Zn(OH)_2$. Formation of this compound is difficult to avoid when attempts are made to increase pH beyond 6.5. After it is formed, $Zn(OH)_2$ dissolves very slowly at pH 6.5–7.0.

Binding by Acid-Treated, Water-Washed, and Defatted Bran

Table II presents data on untreated, acid-treated, defatted, and water-washed bran; the three former types do not differ significantly in zinc-binding ability.

When a sample of bran is washed with water at pH 6–7, the water-insoluble fraction, which comprises 86.1% of whole bran, has a zinc affinity that is equivalent to about two thirds that of whole, unwashed bran. Because the water-soluble fraction has a zinc affinity less than half that of unwashed bran (Table III), the water-insoluble fraction apparently makes the greater contribution (about 61%) to zinc binding. However, the relative contribution of the soluble fraction toward zinc binding by whole bran probably increases with decreasing pH, as occurred in studies of calcium binding (Rendleman 1982).

Binding by Water-Soluble Components of Aqueous Bran Extract

Water-soluble substances, removed from AACC wheat bran by water extraction at pH 7 (Table II, footnote c), comprise about 13.9% of bran. Thirty-one percent of the phosphorus in bran is

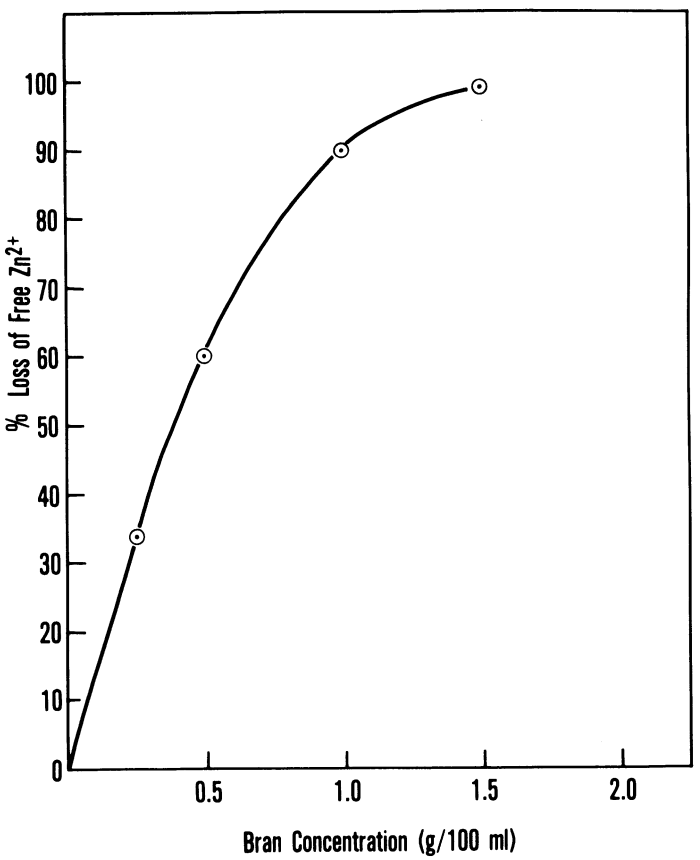

Fig. 1. Effect of bran concentration on loss of free Zn^{2+}. $[Zn^{2+}]_{initial}$ = 2.00m*M*; pH 5.3 (±0.3).

TABLE I
Influence of Initial Zn^{2+} Concentration on Binding by Bran (0.5 g in 100 ml of $ZnCl_2$ Solution)

Zn^{2+} Concentration			
Initial (m*M*)	Final (m*M*)	Loss (mmol)	pH
1.00	0.13	0.087	6.57
2.00	0.74	0.126	6.38
3.00	1.61	0.139	6.19
3.99	2.66	0.133	6.16

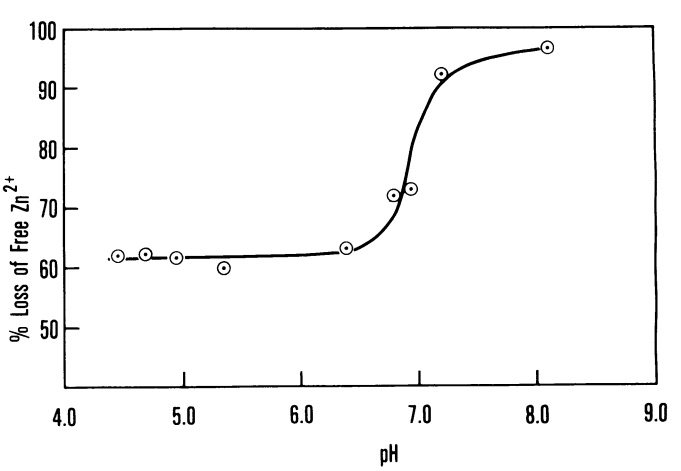

Fig. 2. Influence of pH on binding of Zn^{2+} by bran. $[Zn^{2+}]_{initial}$ = 2.00m*M*; bran concentration, 0.5 g/100 ml.

removed by this extraction. One gram of bran contains approximately 3.25 mg of soluble phosphorus, which would be equivalent to 0.105 mmol of potential binding sites if all phosphorus were in the form of phosphate. However, some of these binding sites may be occupied and not available for binding. Earlier studies (Rendleman 1982) have shown that phytate is the major determinant of calcium binding in bran extract; phytate possibly plays the same role in zinc binding.

Data on binding of zinc by bran extract are provided in Table III and Fig. 3. Phosphorus content of the extract and loss of zinc appear to be closely related. In a sample of extract that is initially 1.0m*M*, with respect to both Zn^{2+} and phosphate binding sites (Experiment 5, Table III), 84% of the Zn^{2+} at pH 6.5 is lost through complexion. A strong zinc affinity (even at the low pH of 4.7) that increases with increasing pH is characteristic of both aqueous bran extract and phytate systems.

As shown by the data in Tables II and III, the water-soluble fraction of whole untreated bran is probably responsible for 39% of the zinc affinity exhibited by whole bran. With acid-treated bran, the contribution of the water-soluble fraction is much greater, probably 60–70%. Acid treatment produces additional amounts of soluble complexing agent at the expense of insoluble complexing agent.

Contribution of Individual Components of Bran to Zinc Binding

Phytate. The effect of pH on complexation of Zn^{2+} with phytate ion in 2.0m*M* $ZnCl_2$ solution is shown in Fig. 4. The concentrations of phytate phosphate groups (binding sites) in the experiments of Series 1 (3.8m*M* in phosphate groups) and Series 2 (1.94m*M* in phosphate groups) are nearly equal to the concentrations of phosphate binding sites that occur naturally in bran extracts obtained by mixing 4 and 2 g of bran, respectively, with 100 ml of water. In Series 2, in which the total number of zinc ions equals that of phytate binding sites, 97% of all the binding sites are occupied by Zn^{2+} at pH 7.0. Presumably, then, had the concentration of phytate

TABLE II
Zinc Binding by Untreated, Acid-Treated, Water-Washed, and Defatted Bran

Type of Bran	Weight (g) of Bran in 100 ml of $ZnCl_2$ Solution	KOH[a] (mmols)	pH	[$ZnCl_2$] Concentration Initial (m*M*)	Concentration Final (m*M*)	Loss Percent	Loss (mmol/g of Bran)
Untreated	0.5	0.055	6.57	1.00	0.13	87	0.174
		0	4.45	2.00	0.76	62	0.248
		0	4.94	2.00	0.77	62	0.246
		0.079	6.38	2.00	0.74	63	0.252
	1.0	0.075	6.41	1.00	0.02	98	0.098
		0.039	5.65	2.00	0.20	90	0.180
Acid-treated[b]	0.5	0	4.50	2.00	0.74	63	0.252
		0	4.60	2.00	0.74	63	0.252
	1.0	0	5.74	1.00	0.05	95	0.095
		0	5.12	2.00	0.13	94	0.187
Water-washed[c]	0.5[d]	0	4.69	2.00	1.19	41	0.162
		0.078	6.45	2.00	1.05	48	0.190
Defatted[e]	0.5[d]	0.021	5.60	2.00	0.69	66	0.262

[a] Added to each 100 ml of solution to adjust pH.
[b] pH ~1.6. Each sample of bran was first agitated with 40 ml of 0.04*N* HCl for 2 hr at 37°C (pH of mixture ~1.6), and the mixture then was neutralized to about pH 6.2 with KOH before appropriate amounts of KCl, $ZnCl_2$, and water were added. Agitation was continued for an additional 2 hr.
[c] Washing was accomplished by agitating the bran for 2 hr with 100 ml of water at 37°C, followed by washing twice with 100-ml portions of water, and finally drying under vacuum at room temperature. The pH was approximately 6.0 during washing. Separation of supernatant from insoluble residue was by centrifugation at 2,000 rpm (810 × *g*). The dry residue was equilibrated at 50% rh (25°C) before being subjected to reaction with Zn^{2+}. A 0.5-g sample of whole bran yielded 0.430 g of water-insoluble residue. Moisture content of residue after equilibration was 12.1%.
[d] Weight of bran before solvent treatment to remove water-soluble material or fat.
[e] Accomplished by extraction with 2:1 (v/v) $CHCl_3$-methanol as described by Rendleman (1982).

TABLE III
Zinc Binding by Aqueous Bran Extract

Type of Bran	Experiment	Weight (g) of Bran in Preparing 100 ml of Extract	KOH[a] (mmol)	pH	$ZnCl_2$ in Extract Concentration Initial (m*M*)	Concentration Final (m*M*)	Loss (%)	Loss (mmol/g of Bran)
Untreated[b]	1	0.5	0	4.6	1.00	0.58	42	0.084
	2		0	4.6	2.00	1.48	26	0.104
	3	1.0	0	4.7	1.00	0.28	72	0.072
	4		0.020	5.5	1.00	0.23	77	0.077
	5		0.049	6.48	1.00	0.16	84	0.084
	6		0.079	7.05	1.00	0.11	89	0.089
	7	2.0	0	5.1	1.00	0.06	94	0.047
Acid-treated[c]	8	0.5	0	4.3	2.00	1.06	47	0.188

[a] Added to each 100 ml of solution to adjust pH.
[b] The concentrations of phosphate group (as determined by total phosphorus analysis) in the extracts obtained by agitating 0.5, 1.0, and 2.0 g of untreated bran in 100-ml portions of water at pH 7 were, respectively, 0.50m*M*, 1.00m*M*, and 2.00m*M*.
[c] pH ~1.6. Bran (1 g) was stirred with 40 ml of 0.04*N* HCl for 2 hr at 37°C, and the mixture was then neutralized with KOH to a pH of about 6.2. Sufficient water was added to make 100 ml of solution, and the resulting mixture was centrifuged. For the binding study, equal volumes of supernatant and 4.0m*M* $ZnCl_2$ were mixed.

in Series 2 been only half as great or less, then essentially all of the binding sites would have been occupied by zinc. In Experiment 2 (Table III), in which the extract from 0.5 g of bran was made 2.0m*M* in Zn^{2+}, the 26% loss of free Zn^{2+} reflects a 100% occupation of available binding sites, even though the pH of this system is quite low (pH 4.6).

The maximum number of zinc-binding sites in 1 g of whole bran at pH 6.2 (0.272 mmol, as determined by zinc-binding experiments summarized in Table I) is only 11% less than the total number of binding sites on phytate (0.305 mmol) in 1 g of bran (based on 3.36% of phytic acid in bran and on the assumption that each phytate ion contains six phosphate groups). When the pH of a bran-$ZnCl_2$ system is raised to 6.8, the maximum number of binding sites appears to increase slightly (Fig. 2), possibly to as much as 0.310 mmol per gram of bran. However, at pH 6.8 and higher, analytical results may not be very meaningful. This is suggested by Fig. 2, which shows a sudden large decrease in concentration of free Zn^{2+} when pH is increased from about 6.8 to 7.3.

Cellulose and Hemicellulose. Table IV summarizes the results of binding studies on cellulose, hemicellulose (isolated from AACC wheat bran), and xylan. The cellulose and xylan were commercial samples.

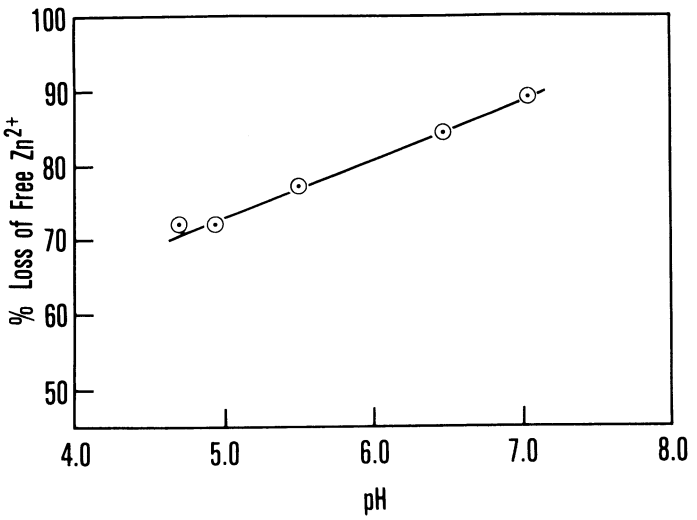

Fig. 3. Influence of pH on binding of Zn^{2+} by bran extract. Concentration of bran solubles is equivalent to that produced by 1 g of bran in 100 ml of water; $[Zn^{2+}]_{initial}$ = 1.00m*M*.

Cellulose and hemicellulose comprise about 9 and 22%, respectively, of AACC wheat bran. Cellulose has essentially no ability to bind Zn^{2+} over the pH range 4–7 and would thus make no significant contribution to the binding power of bran. Hemicellulose and xylan were both found to have a moderate affinity for Zn^{2+}. The contribution of the hemicellulose fraction to the binding ability of bran is probably no more than 6% at pH 6.2–6.8.

Pectic Substances. The citrus pectin (degree of esterification, 61%) used in these studies was assumed to represent naturally occurring pectin in wheat bran. Based on the data in Table V, pectin appears to form a moderately strong complex with zinc, although its ability to do so is considerably less than that of polygalacturonic acid. However, the small percentage of pectin in bran (3.0%) greatly limits the contribution of this component to the binding power of bran. Probably no more than 3% of the zinc-binding ability of bran is caused by pectin.

As with calcium binding, binding of zinc by pectic substances

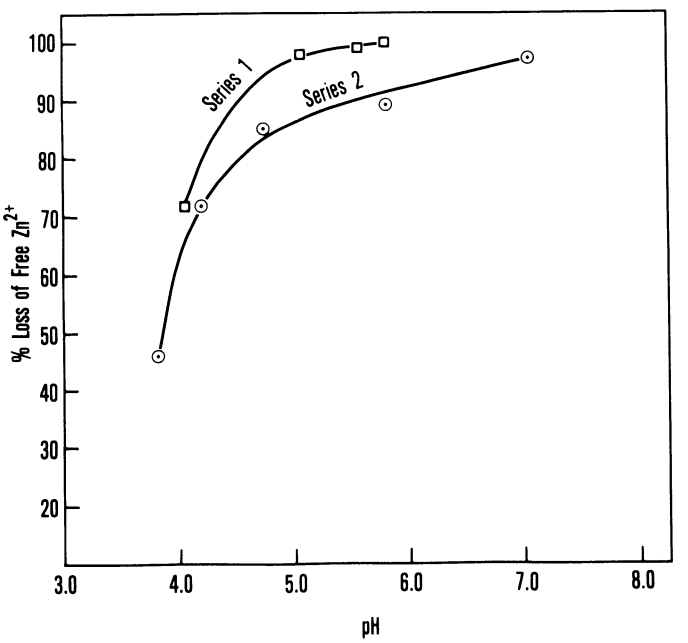

Fig. 4. Influence of pH on binding of Zn^{2+} by phytate ion. Series 1: $[Zn^{2+}]_{initial}$ = 1.90m*M*; $[phytate]_{initial}$ = 0.633m*M* ≙ 3.80m*M* phosphate groups. Series 2: $[Zn^{2+}]_{initial}$ = 1.95m*M*; $[phytate]_{initial}$ = 0.324m*M* ≙ 1.94m*M* phosphate groups.

TABLE IV
Zinc Binding by Cellulose, Hemicellulose, and Xylan

Substrate	Weight (g) of Substrate in 100 ml of $ZnCl_2$ Solution	HCl or KOH[a] (mmol)	pH	$[ZnCl_2]$ Initial (m*M*)	$[ZnCl_2]$ Final (m*M*)	Loss (%)
Cellulose (Avicel)	3.0	0.0036 (HCl)	4.44	2.00	1.98	1
		0.0043 (KOH)	5.89	1.99	1.99	0
		0.0072 (KOH)	6.33	1.99	1.99	0
		0.0120 (KOH)	6.88	2.00	1.94	3
Hemicellulose[b]	1.0	0.040 (KOH)	6.30	2.00	1.38	31
		0.072 (KOH)	7.08	1.99	1.26	37
Xylan[c]						
Acid-treated[d]	1.0	0.32 (KOH)	6.75	1.99	1.58	21
Untreated[e]	1.0	0	5.60	2.00	1.66	17

[a] Added to each 100 ml of solution to adjust pH.
[b] Combined water-soluble and water-insoluble hemicellulose isolated from AACC wheat bran as described by Rendleman (1982). Moisture, 11.8%; Kjeldahl N, 0.32%.
[c] Commercial sample (ICN Pharmaceuticals) isolated from wood gum.
[d] Treated with 0.15*N* HCl to remove mineral matter according to the method described by Rendleman (1982) for removing phytate from impure hemicellulose. Moisture, 12.6%; Kjeldahl nitrogen, 0%.
[e] Moisture, 10.6%.

TABLE V
Zinc Binding by Pectic Substances

Substance	Weight (g) of Substance in 100 ml of $ZnCl_2$ Solution	KOH[a] (mmol)	pH	[$ZnCl_2$] Initial (m*M*)	[$ZnCl_2$] Final (m*M*)	Loss (%)
Pectin[b]	0.136	0.11	5.63	1.99	1.66	17
		0.12	6.26	1.99	1.67	16
	0.272	0.23	5.98	1.99	1.42	29
		0.24	6.39	1.99	1.41	29
Polygalacturonic acid[c]	0.060	0.24	4.36	1.99	1.04	48
		0.27	5.07	1.99	0.92	54
	0.120	0	2.85	2.00	1.56	22
		0.47	4.25	1.98	0.46	77
		0.51	4.65	1.97	0.38	81
		0.55	5.85	1.97	0.31	84
		0.56	6.71	1.97	0.30	85
		0.47	4.4	3.66	1.67	54
		0.54	6.23	3.66	1.38	62

[a] Added to each 100 ml of solution to adjust pH.
[b] Commercial citrus pectin (degree of esterification 61%), which was obtained from Sigma Chemical Co. and had the following composition: 76% galacturonic acid, 7.4% methoxy, and 12% water.
[c] Commercial sample (98% pure) from Sigma Chemical Co. A 0.12-g sample contains 0.68 mmol of carboxyl groups.

TABLE VI
Zinc Binding by Starch[a]

Type of Starch[b]	Weight (g) of Starch in 100 ml of $ZnCl_2$ Solution	KOH[c] (mmol)	pH	[$ZnCl_2$] Initial (m*M*)	[$ZnCl_2$] Final (m*M*)	Loss (%)
Potato						
A (14.9% H_2O)	3	0.005	5.61	2.00	1.99	0.5
		0.029	5.88	2.00	1.83	8.5
		0.077	6.13	1.99	1.55	22
		0.096	6.40	1.99	1.60	20
B (10.0% H_2O)	3	0	5.35	2.00	1.96	2
C (16.8% H_2O)	3	0	5.87	2.00	1.99	0.5
Corn						
Amylose (6.1% H_2O)[d]	3	0	5.50	2.00	2.00	0
		0.024	6.45	2.00	1.96	2
Amylopectin (10.4% H_2O)	3	0	5.12	2.00	1.88	6
		0.034	6.94	2.00	1.61	20
Wheat						
A (11.6% H_2O)	3	0	5.73	2.00	2.00	0
		0.096	6.50	2.00	1.52	24
B (12.2% H_2O)	3	0	5.66	2.00	2.00	0
		0.096	6.60	2.00	1.50	25

[a] None of the starches contained any detectable amount of zinc and none contained more than 0.01% N. The percent moisture in each sample is shown in parentheses.
[b] Three varieties of potato and two of wheat were used.
[c] Added to each 100 ml of solution to adjust pH.
[d] The fraction of amylose in total starch is 0.82.

TABLE VII
Zinc Binding by Proteins

Protein	Weight (g) of Protein in 100 ml of $ZnCl_2$ Solution	KOH[a] (mmol)	pH	[$ZnCl_2$] Initial (m*M*)	[$ZnCl_2$] Final (m*M*)	Loss (%)
Albumin (bovine serum)	0.452	0	5.44	2.00	1.68	16
		0.067	6.62	1.99	1.54	23
		0.110	7.01	1.99	1.38	31
Glutenin (hard red winter wheat)[b]	0.300	0.023	5.5	2.00	1.94	3
		0.039	6.21	2.00	1.86	7
		0.055	6.92	2.00	1.80	10

[a] Added in small increments, with stirring, to each 100 ml of solution (containing protein) to adjust pH.
[b] Contains 10% moisture.

over the pH range 5–7 is not very sensitive to change in pH. Only at relatively high acidity (pH ~3) does polygalacturonic acid lose most of its zinc-binding ability.

Significantly, Davies et al (1977) found that when pectin was added to the diet of rats, it had no discernible effect on the bioavailability of dietary zinc. If the affinity of pectin for zinc had been high instead of moderate, perhaps some effect on bioavailability would have been observed.

Starch. Only at pH ≥6 do potato starch, wheat starch, and corn amylopectin bind zinc significantly (Table VI). Amylose exhibits no significant binding even at pH 6.5. The often stronger affinity of zinc for starch at pH >6 indicates proton removal in the formation of zinc-starch complex. The amount of binding that occurs because of interaction of Zn^{2+} with occasional acid substituents (such as phosphate or carboxyl groups) on the starch chain, and the amount that is caused by interaction with hydroxyl groups that form zinc alcoholates were not determined. Although AACC wheat bran is 17.4% starch, starch can be responsible for no more than 2% of the binding power of bran.

The similarity in zinc binding between amylose and cellulose is striking. Both polysaccharides are very unreactive toward Zn^{2+} and, perhaps significantly, both are highly crystalline. Table VI shows that the starches other than amylose are predominantly amorphous. A high degree of crystallinity possibly reduces the accessibility of metal ions to potential binding sites within the polysaccharide matrix. Another explanation for the higher reactivity of amorphous starches is the possible occurrence in these starches of a greater number of acid-group substituents capable of metal chelation.

Neutral Sugars. AACC wheat bran is 7% sugar. However, because no detectable reaction occurs between D-glucose (0.022*M*) and Zn^{2+} (2.0m*M*) at pH 6.5, the soluble saccharides in bran probably do not contribute significantly to the binding of Zn^{2+} by bran in the human intestine, in which the average pH is 6.8. The apparent gain in concentration of Zn^{2+} at high concentration of D-glucose (0.76*M*) was probably caused by an increase in zinc-ion activity brought about by the lower water content of a high-glucose system.

Lignin. Although lignin comprises 3.2% of AACC wheat bran, it was not studied because it is impossible to remove it from bran in a chemically unaltered state. However, its contribution to the binding power of bran probably would not be greater than that of pectin, which comprises 3.0% of bran and contributes approximately 3% of the zinc-binding ability of bran. The results of a calcium-binding study by Molloy and Richards (1971) are possibly significant; they found that pectin and lignin, both isolated from the grass Yorkshire Fog, have very similar affinities for calcium ion. The lignin was only slightly less reactive than the pectin.

Proteins. Glutenin from hard red winter wheat and bovine serum albumin were chosen to represent typical water-insoluble and water-soluble bran proteins, respectively. (Serum albumin might not be strictly representative, because the amino acid profile of water-soluble bran proteins may differ significantly from that of serum albumin.) Both proteins bind Zn^{2+}, but albumin exhibits the higher reactivity (Table VII). The aggregative nature of glutenin particles may reduce the accessibility of many potential binding sites, thereby lowering the ability of glutenin to bind zinc. The data in Table VII suggests that proteins (which comprise 14.3% of AACC wheat bran) contribute as much as 7% of the total binding by bran.

To avoid obtaining erroneously high values for the binding of zinc by albumin, special care had to be taken in adjusting the pH of albumin-$ZnCl_2$ mixtures with KOH. Small increments of the KOH solution had to be introduced very slowly and with constant stirring.

Zinc Binding by Commercial Breakfast Cereals of High Bran Content

Zinc binding abilities of several commercial breakfast cereals of high bran content were determined in 2.0m*M* $ZnCl_2$ (by the TMM method) and compared with the binding ability of AACC wheat bran (Table VIII). Each breakfast cereal was pulverized in a mortar before use. Whereas the affinity of All-Bran is high and identical to that of AACC bran, Corn Bran has little affinity. A parallel relationship appears to exist between degree of binding and phosphorus content of the cereal. This apparent relationship suggests that phytate content can be a major determinant of relative binding abilities of breakfast cereals.

Contribution of Gastrointestinal Constituents to Binding

Metal-binding components of gastrointestinal secretions may affect the availability of polyvalent cations in the human intestine. Any gastrointestinal substance that forms a soluble, relatively stable metal complex may enhance the bioavailability of that metal. Morris and Ellis (1976), Oberleas et al (1966), and Vohra and Kratzer (1966) established that availability to lower animals of certain metals in highly stable but soluble complex form is greater than that in insoluble form. However, any secretory substance that forms an insoluble metal complex or insoluble metal salt would probably contribute only to a lowering of bioavailability.

Table IX contains data on numerous constituents of human gastrointestinal fluid. At concentrations commonly found in the small intestine (Altman and Dittmer 1968), saliva, D-glucose (representing total sugar), HCO_3^-, and albumin exhibit insignificant binding and probably have little or no influence on zinc bioavailability. However, dietary albumin eaten in large amounts should be able to bind a considerable amount of Zn^{2+} and, thus, influence availability. Mucin is a moderately strong chelating agent that probably has significant influence. Amino acids from gastrointestinal fluid, on the other hand, are not expected to have much influence, because of their low concentration and because of their generally low affinity for Zn^{2+}. L-Histidine, one of many amino acids in gastrointestinal fluid, binds Zn^{2+} strongly to give a soluble complex, but its physiological concentration (~0.1m*M*) is probably too low to affect zinc bioavailability. After a meal, amino acids that arise from digestion of dietary protein might be present in sufficient amount to compete successfully against strong insolubilizing complexing agents (such as phytate) and thus augment zinc availability by forming soluble Zn^{2+} amino acid

TABLE VIII
Zinc Binding by Commercial Breakfast Cereals

Name	Bran Source	Approximate P Content of Cereal[a] (mg/g)	Weight (g) of Cereal in 100 ml of $ZnCl_2$ Solution	pH	[$ZnCl_2$] Initial (m*M*)	[$ZnCl_2$] Final (m*M*)	Loss (%)	Binding Ability Relative to That of AACC Bran
All-Bran (Kellogg Co.)	Wheat	8.5	0.5	4.71	2.00	0.75	63	1.01
40% Bran Flakes (Kellogg Co.)	Wheat	4.2	0.5	4.81	2.00	1.61	20	0.32
Corn Bran (Quaker Oats Co.)	Corn	0.6	0.5	5.94	2.00	1.90	5	0.08
AACC Bran	Wheat	10.4	0.5	4.94	2.00	0.77	62	1.00

[a] Based on manufacturer's claim, which can vary as much as 10–15%.

complexes. Davies (1979) suggested that certain amino acids and peptides, as products of protein digestion, possibly have a solubilizing effect on fiber-bound zinc, thus increasing the chances that this mineral will be absorbed by the intestine at the mucosal surface.

Phosphate ion, which forms a highly insoluble precipitate with Zn^{2+}, may be an important determinant of zinc availability in humans. If all the P in gastrointestinal fluid were in the form of phosphate ion, the average concentration of phosphate in the human small intestine would be about 2.0m*M* (Altman and Dittmer 1968). The fact that inorganic phosphate can interfere with zinc availability in at least some animals was confirmed by Vohra and Kratzer (1966), whose data from weight-gain studies of turkey poults showed that the availability of zinc from $Zn_3(PO_4)_2$ is no greater than that from zinc phytate. Zinc phosphate precipitates from aqueous solution only in the form of the orthophosphate $Zn_3(PO_4)_2 \cdot 4H_2O$, which has a minimum solubility at pH ~7.4 (Jurinak and Inouye 1962). The great zinc-precipitating power of phosphate ion causes some doubt to be cast upon the results of zinc-binding studies by Reinhold et al (1975) and Ismail-Beigi et al

TABLE IX
Zinc Binding by Constituents of Gastrointestinal Fluid

Constituent	Concentration of Constituent (g/100 ml)	Concentration of Constituent (*M*)	pH	[$ZnCl_2$] Initial (m*M*)	[$ZnCl_2$] Final (m*M*)	[$ZnCl_2$] Change (%)
Saliva, human	(2 ml)	...	6.35	1.96	1.94	−1
D-Glucose	13.75	0.763	5.58	1.60	1.76	+10
			6.66	1.60	1.79	+12
	0.396[a]	0.0220	5.83	2.00	1.94	−3
			6.47	2.00	2.03	+2
Albumin, bovine serum	0.0452[a]	...	6.53	2.00	1.98	−1
Mucin, gastric	0.272[a]	...	5.98	2.00	1.67	−17
			6.40	1.99	1.52	−24
			6.72	1.99	1.36	−32
			6.96	1.99	1.28	−36
Glycine	0.038[b]	0.0051	6.11	2.00	1.91	−5
			6.79	1.99	1.90	−5
	0.076	0.0101	6.02	2.00	1.87	−7
	0.760	0.101	6.02	1.99	1.20	−40
L-Histidine	0.0621	0.004	6.03	2.00	0.83	−59
			6.10	2.00	0.71	−65
			6.15	2.00	0.59	−71
			7.95	1.98	0.04	−98
K_2HPO_4[c]	0.035[a]	0.0020	6.37	1.99	0.14	−93
	0.0870	0.0050	4.72	2.00	1.74	−13
			6.02	1.99	0.04	−98
			6.76	1.98	0.01	−99
$KHCO_3$[d]	0.165[a]	0.0165	6.72	2.00	2.00	0
Bile acid salts[e]						
Na glycocholate	0.390	0.0080	5.89	2.00	1.89	−6
Na glycodeoxycholate	0.390	0.0083	5.89	2.00	0.25	−88
			6.30	2.00	0.22	−89
Na glycochenodeoxycholate	0.390	0.0083	6.05	2.00	1.05	−48
Na glycolithocholate	0.390	0.0086	6.04	2.00	0	−100

[a] Amount approximates that present in 100 ml of human gastrointestinal fluid (Altman and Dittmer 1968).
[b] Amount represents the approximately 0.038 g of total amino acid present in 100 ml of human gastrointestinal fluid (Altman and Dittmer 1968).
[c] The order of mixing the components of the reaction mixture is important: first, a weighed sample of K_2HPO_4 is dissolved in 50 ml of water; then an appropriate volume of 0.4*N* HCl is added to lower the pH into the range of 6.0–6.5, and finally, 50 ml of 4.00m*M* $ZnCl_2$ is added. KOH is subsequently introduced to raise the pH to the desired level. In an aqueous solution of K_2HPO_4, the predominant species of phosphate ion are $H_2PO_4^-$ and HPO_4^{-2}, which are in equilibrium with each other. At pH 4.7, 6.0, and 6.8, the molar ratio of $H_2PO_4^-/HPO_4^{-2}$ is 267, 13.3, and 2.1, respectively.
[d] The procedure for preparing these reaction mixtures is similar to that for preparing the K_2HPO_4 reaction mixtures. In an aqueous solution of $KHCO_3$, the species HCO_3^- and H_2CO_3 are the predominant species of carbonate in equilibrium with each other. At pH 6.7 the ratio of HCO_3^-/H_2CO_3 is 2.5.
[e] Reaction mixtures are prepared by first adjusting the pH of aqueous bile acid salt to 6.0–6.5 with dilute HCl and then adding the required amount of $ZnCl_2$. These mixtures are then shaken for 1 hr at 37°C before being centrifuged.

TABLE X
Comparison of Total Soluble Zinc with Concentration of Free Zn^{2+} in Various $ZnCl_2$-Containing Systems

Experiment	Substrate	Concentration of Substrate in 100 ml of Solution	[$CaCl_2$] (m*M*)	pH	[$ZnCl_2$]$_{initial}$ (m*M*)	[Zn^{2+}]$_{final}$ Total Soluble (m*M*)	[Zn^{2+}]$_{final}$ Free (m*M*)
1	AACC bran	2 g	0	5.63	2.00	0.0567	0
2	AACC bran	2 g	0	6.65	0.100	0.0026	0
3	AACC bran	2 g	3.74	6.03	0.100	0.0017	...
4	AACC bran	2 g	0	6.78	0	0	0
5	Na phytate	3.79 m*M* in P	0	5.69	1.90	0.0315	0.01
6	Na phytate	3.81 m*M* in P	0	5.95	0.635	0.0275	0
7	K_2HPO_4	2.01 m*M* in P	0	6.40	1.99	0.11	0.16

(1977), whose reaction mixtures were buffered with 4.0m*M* phosphate (pH 6.0–7.5).

Zinc salts of conjugated bile acids differ considerably in solubility, as shown by data on glycocholate, glycodeoxycholate, glycochenodeoxycholate, and glycolithocholate (Table IX). Of these four acids, only the first three are present in the human small intestine and are of interest in zinc availability. Tauro conjugates of bile acids were not studied. Loss of Zn^{2+} in these $ZnCl_2$-bile acid systems was caused largely, if not entirely, by insolubilization of zinc, as indicated by visual observations of an aqueous mixture of $ZnCl_2$ and sodium glycodeoxycholate. When equal volumes of 4.0m*M* $ZnCl_2$ and 0.017*M* sodium glycodeoxycholate were mixed, a clear, colorless, metastable solution resulted. Subsequent addition of TMM indicator, normally a purple solution, imparted a light yellow-orange color to the solution, as is expected when little or no loss of free Zn^{2+} has occurred. Gentle shaking of the mixture induced the formation of a finely divided zinc glycodeoxycholate precipitate and caused the color of the mixture to change from yellow-orange to pink, indicating a large loss of free Zn^{2+} through formation of an insoluble zinc compound.

Although the solubility of zinc glycodeoxycholate is much lower than that of the corresponding glycochenodeoxycholate and glycocholate, the final concentration of free Zn^{2+} (0.2m*M*) in a $ZnCl_2$-Na glycodeoxycholate system would indicate that bile acids alone may not diminish the bioavailability of zinc in the human small intestine, in which the postprandial concentration of total zinc would normally be less than 0.2m*M*. Nevertheless, by forming a highly insoluble zinc salt, bile acids could conceivably function in a regulatory capacity by reducing to an acceptable level the concentration of soluble zinc in the intestinal lumen of humans who have ingested relatively large, potentially toxic amounts of this metal.

Relationship Between Loss of Free Zn^{2+} and Insolubility of Zinc Complex

Because bioavailability of a metal is limited by its solubility, we determined total soluble zinc (ie, free Zn^{2+} and any soluble complexed zinc) in certain of our reaction mixtures. Several Zn^{2+}-bran, Zn^{2+}-phytate, and Zn^{2+}-phosphate systems were therefore examined by both atomic absorption spectrophotometry (for total soluble zinc) and by the TMM method (for free Zn^{2+}). The results (Table X) show a close similarity between free Zn^{2+} concentration and total zinc concentration in the reaction mixture supernatants and suggest that, in these particular systems, the TMM method is generally adequate for determining unprecipitated zinc at pH 6–7.

Experiment 2 in Table X shows that 2 g of bran insolubilizes virtually all of the zinc in 100 ml of 0.1m*M* $ZnCl_2$. A 0.1m*M* concentration of zinc approximates the concentration of total zinc (soluble and insoluble) in the intestinal lumen after an average meal. Addition of Ca^{2+} to the same reaction mixture results in an even greater insolubilization of zinc (Experiment 3).

Zinc is greatly insolubilized by phytate ion, even when the molar ratio of Zn to phytate is relatively low. For example, in Experiment 6, in which the molar ratio was 1:1, the final concentration of soluble zinc was 0.0275m*M*, reflecting a 96% loss through precipitation. The influence of Ca^{2+} on the solubility of zinc-phytate complex was observed when 7.5m*M* $CaCl_2$ was added to an equal volume of the supernatant from Experiment 6. Soluble zinc concentration dropped from 0.0138m*M* to 0.0050m*M*. A reduction in zinc concentration had been predicted earlier on the basis of a lowering effect that Ca^{2+} has on zinc availability in rats fed diets containing phytate (Oberleas et al 1966).

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LITERATURE CITED

ALTMAN, P. L., and DITTMER, D. S. 1968. Metabolism. Federation of American Societies for Experimental Biology, Bethesda, MD.

CAMIRE, A. L., and CLYDESDALE, F. M. 1981. Effect of pH and heat treatment on the binding of calcium, magnesium, zinc, and iron to wheat bran and fractions of dietary fiber. J. Food Sci. 46:548.

DAVIES, N. T., HRISTIC, V., and FLETT, A. A. 1977. Phytate rather than fiber in bran as the major determinant of zinc availability to rats. Nutr. Rep. Int. 15:207.

DAVIES, N. T. 1979. The effects of dietary fiber on mineral availability. Page 113 in: Proc. 3rd Kellogg Nutr. Symp. Dietary Fiber: Curr. Dev. Importance Health, 1977. K. Heaton, ed. Technomic Publ. Co., Inc., Westport, CT.

ISMAIL-BEIGI, F., FARADJI, B., and REINHOLD, J. G. 1977. Binding of zinc and iron to wheat bread, wheat bran, and their components. Am. J. Clin. Nutr. 30:1721.

JURINAK, J. J., and INOUYE, T. S. 1962. Some aspects of zinc and copper phosphate formation in aqueous systems. Proc. Soil Sci. Soc. Am. 26:144.

LAXEN, D. P. H., and HARRISON, R. M. 1981. Cleaning methods for polythene containers prior to the determination of trace metals in freshwater samples. Anal. Chem. 53:345.

MOLLOY, L. F., and RICHARDS, E. L. 1971. Complexing of calcium and magnesium by the organic constituents of Yorkshire Fog (*Holcus lanatus*). II. Complexing of Ca^{2+} and Mg^{2+} by cell wall fractions and organic acids. J. Sci. Food Agric. 22:397.

MORRIS, E. R., and ELLIS, R. 1976. Isolation of monoferric phytate from wheat bran and its biological value as an iron source to the rat. J. Nutr. 106:753.

OBERLEAS, D., MUHRER, M. E., and O'DELL, B. L. 1966. Dietary metal complexing agents and zinc availability in the rat. J. Nutr. 90:56.

REINHOLD, J. G., ISMAIL-BEIGI, F., and FARADJI, B. 1975. Fiber vs. phytate as determinant of the bioavailability of calcium, zinc, and iron of breadstuffs. Nutr. Rep. Int. 12:75.

RENDLEMAN, J. A. 1982. Cereal Complexes. Binding of calcium by bran and components of bran. Cereal Chem.

VOHRA, P., and KRATZER, F. H. 1966. Influence of various phosphates and other complexing agents on the availability of zinc for turkey poults. J. Nutr. 89:106.

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