Bioavailability of Trace Minerals from Cereals and Legumes[1]

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ABSTRACT

Published research reports point to less mineral bioavailability from foods of plant origin than from foods of animal origin. Endogenous and exogenous factors have been implicated in reduction of mineral absorption from cereals and legumes. Phytic acid and food processing appear to affect the bioavailability of zinc and perhaps iron from cereal and legume-based foods.

Minerals from cereals, legumes, and other plant foods, in contrast to minerals from animal sources, are generally poorly utilized by man and other monogastric animals (O'Dell 1969). Endogenous and exogenous factors have been implicated as causative in reducing the absorption of minerals from plant foods. Phytic acid, dietary fiber components, and certain amino acids and proteins all readily chelate minerals. The effect of these dietary substances upon the bioavailability of minerals depends upon the digestibility of the chelate. Interaction of dietary substances during food processing operations may positively or negatively affect bioavailability of minerals.

Little agreement has been reached as to which dietary components or which food processes physiologically affect mineral

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availability. This article reviews the state of knowledge of mineral availability from cereals and legumes, emphasizing the roles of food components and processing conditions upon mineral utilization. Studies reported involve the trace elements zinc and iron, as well as magnesium and calcium.

BIOAVAILABILITY OF MINERALS FROM CEREALS

Most of the studies of mineral bioavailability from cereal foods have investigated effects of the extent of milling, the type of ingredients, and/or the fermentation of breads on zinc or iron utilization.

Sandström and his coworkers (1978) fed one of four meals to human subjects after a fast of at least 12 hr (Table I). Bread in the form of two rolls per person was prepared from wholemeal wheat flour or from commercial wheat flour (white) of about 72% extraction rate. Before baking, ^{65}Zn and $ZnCl_2$ were added to the dough. Each of the meals was made isocaloric by adding butter to the meals that did not contain milk or cheese. The absorption of ^{65}Zn was calculated by comparing whole-body counter measurements of the 100% value obtained immediately after the meal with the whole-body retention of ^{65}Zn 10–14 days after the meal.

The absorption of zinc from wholemeal bread served with butter and water was poor compared to that from the similar white bread meal (Table I). Some component(s) of wheat that are removed during refining, such as phytic acid and/or dietary fiber, may be responsible for lower zinc absorption. Addition of milk and cheese to wholemeal bread increased the zinc absorption to the level obtained from the meal based on white bread. The authors did not speculate on the component in milk and/or cheese that was responsible for improved zinc uptake.

Oelshlegel and Brewer (1977) investigated the effects of various types of foodstuffs upon the apparent zinc absorption of humans (Table II). Zinc (25 mg as zinc sulfate) was given along with the foodstuff. The change in plasma zinc obtained when zinc was taken along with the foodstuff was divided by the corresponding value in the same individual when zinc was taken without the foodstuff. The relative plasma zinc was generally improved with the amino acids histidine and glutamine and with bacon. Individual amino acids, such as histidine, have previously been shown to chelate zinc and increase the absorption of the metal for the chick (Nielson et al 1967). Other foodstuffs produced less increase in plasma zinc. Milk, which was apparently beneficial in the 1978 study by Sandström (Table I), was poorly effective in this study. The hamburger on bun resulted in poor plasma zinc increase. The authors used the same design to further investigate (results not shown) the effects of the hamburger, the bun, and the combined hamburger and bun on the change in plasma zinc. They found that the meat alone did not appear to inhibit zinc absorption, but the bun with or without the meat caused a reduction in plasma zinc response.

Davies and coworkers (Davies and Nightingale 1975, Davies and Olpin 1979, Davies and Reid 1979, Davies et al 1977) have published a number of articles dealing with the bioavailability of various minerals from high-phytate, high-fiber cereals and legumes. In one such study, Davies et al (1977) looked at the effect of wheat bran, wheat bran fiber, and phytate upon zinc utilization for growth. Rats were fed a zinc deficiency diet for 17 days before being offered one of five experimental diets (Table III). Diets were not isocaloric and isonitrogenous because all dietary supplements were made at the expense of an equal weight of sucrose. After 12 days on these diets, each group was divided into two equal subgroups (N = 4); one of the subgroups was provided drinking water fortified with zinc (2.5 μg/ml) and the other continued to receive distilled water for the last eight days of experimental diets. Test groups received either supplemental wheat bran, extracted bran fiber at a level equivalent to the fiber in the wheat bran, or sodium phytate at a level equivalent to the phytate in the wheat bran. The results (Table III) show that the presence of 15% wheat bran or sodium phytate but not of bran fiber in the diet produced a reduction in growth, which could be reversed by zinc in the drinking water. Caution must be taken, however, when interpreting the effects of feeding highly extracted dietary fiber components and the chemical sodium phytate (Groups 4 and 5). These substances are highly refined and may or may not correspond structurally or chemically with the same substances in their natural state. Nevertheless the results strongly suggest that phytate rather than fiber

TABLE I
Zinc Absorption from Bread Meals by Human Subjects[a]

Meal	Zinc Absorption[b] (mg)
Wholemeal bread, butter, water	0.29 ± 0.02
White bread, butter, water	0.48 ± 0.04
Wholemeal bread, milk, cheese	0.46 ± 0.04
White bread, milk, cheese	0.52 ± 0.05

[a] Adapted from Sandström et al (1978).
[b] Means ± SE of 11 or 12 subjects. Zinc absorption was calculated by comparison of the whole-body counter detection of ^{65}Zn (added to bread doughs before baking) immediately after the meal with measurement 10–14 days later.

TABLE II
Effect of Food Type on Absorption of Zinc from Zinc Sulfate by Human Subjects[a]

Foodstuff	Number of Subjects Tested	Approximate Relative Plasma Zinc Change[b] (%) at Hours After Administration: 1	2	3
Histidine	5	125	···	80
Glutamine	6	105	···	120
Bacon	2	···	120	···
Coffee	3	50	···	40
Milk	2	···	10	···
Hard boiled egg	2	···	15	···
Celery	2	35	···	37
Hamburger on bun	6	20	···	10

[a] Adapted from Oelshlegel and Brewer (1977).
[b] Mean change in plasma zinc after feeding 25 mg of zinc with foodstuff divided by change without foodstuff.

TABLE III
Average Daily Growth Rates of 17-Day Zinc-Deficient Rats Fed Diets for 20 Days[a]

Group	Diet[b]	Subgroup[c]	Average Daily Weight Gain from Day 13 to Day 20 (g/day ± SE)[d]
1	Zinc-deficient	A	1.05 ± 0.36[e]
		B	7.13 ± 0.45[e]
2	Zinc-sufficient	A	5.33 ± 0.27[f]
		B	5.39 ± 0.19[f]
3	Zinc-sufficient + wheat bran	A	1.21 ± 0.32[e]
		B	6.25 ± 0.58[e]
4	Zinc-sufficient + wheat bran fiber	A	5.45 ± 0.15[f]
		B	5.80 ± 0.23[f]
5	Zinc-sufficient + Na phytate	A	1.85 ± .48[e]
		B	6.42 ± 0.31[e]

[a] Adapted from Davies et al (1977).
[b] Diet of group: 1, Basal Zn-deficient diet; 2, basal diet supplemented with Zn (18.5 mg/kg of diet) as $ZnSO_4$; 3, basal diet supplemented with wheat bran (150 g/kg) and containing per kilogram 50 g of fiber, 18.5 mg of Zn, and 5.3 g of phytic acid; 4, basal diet supplemented with extracted bran-fiber (50 g/kg) and Zn (18.5 mg/kg) as $ZnSO_4$; 5, basal diet supplemented with sodium phytate to give (phytic acid 5.3 g/kg) and Zn (18.5 mg/kg) as $ZnSO_4$.
[c] Subgroups: A, on diet for 20 days; B, on diet for 20 days with Zn in water for last eight days.
[d] N = 4.
[e] Difference between related subgroups was significant at $P < 0.001$.
[f] Difference between related subgroups was not significant.

TABLE IV
Hydrolysis of Phytic Acid and Bioavailability of Zinc for Rats Fed Diets[a] Based on Bread or Bread Ingredients[b]

Response[c]	No Phytate Added				Phytate Added			
	No Soy		Soy Added		No Soy		Soy Added	
	Ingredients	Bread	Ingredients	Bread	Ingredients	Bread	Ingredients	Bread
Weight gain (g)	182 ± 12	187 ± 11	186 ± 11	185 ± 8	188 ± 12	185 ± 11	190 ± 11	189 ± 11
Serum zinc (μg/dl)	96 ± 19	96 ± 21	112 ± 34	95 ± 20	105 ± 10	105 ± 10	109 ± 43	174 ± 26
Femur zinc (μg)	60 ± 6	53 ± 4	53 ± 7	57 ± 6	53 ± 6	58 ± 5	50 ± 6	56 ± 7
Apparent zinc absorption (%)	72 ± 6	86 ± 3	70 ± 7	80 ± 5	61 ± 7	82 ± 3	65 ± 6	80 ± 3
Phytic acid hydrolyzed (%)	···	100	···	81.2	···	100	···	92.2

[a] All diets provided 9.5 ppm zinc from the breads or ingredients, 20% egg albumin, 2.2% vitamin mix (extra biotin added), 2% fiber, 2% mineral mix, and corn starch to make 100%. Defatted soy flour added at a rate of 12 g/100 g of bread flour. Na phytate added to level found in 12 g of soy flour.
[b] Adapted from Ranhotra et al (1978).
[c] Means ± SD. (N = 8).

reduced the growth of rats and that phytate is the major determinant of zinc bioavailability in diets fortified with wheat bran.

Mineral bioavailability appears to be low in nonleavened breads made from whole-grain cereals. In work done by Prasad's group (1976), zinc deficiency in rural Iranians was linked to the dietary interference of zinc absorption (Oelshlegel and Brewer 1977). Cases of delayed wound healing, sterility, and growth failure appeared to be caused, at least in part, by consumption of large quantities of high-phytate, high-fiber, unleavened bread by these Iranians.

Preparation of leavened breads with a yeast fermentation step allows yeast and wheat phytases to hydrolyze phytic acid. Ranhotra et al (1974) found that all of the phytate in wheat bread and more than 75% of that in soy-fortified wheat flours (soy 10%, wheat 90%, db) could be hydrolyzed during the process of bread baking. Ranhotra and coworkers (1978) investigated the bioavailability of zinc in bread fortified with zinc (2.2 mg/100 g of flour). Breads were formulated with and without added phytate and with and without added soy flour to produce four test breads. Rats were fed diets based either on breads or the bread ingredients, in all cases at a Zn level of 9.5 ppm for four weeks (Table IV). Diets were not isocaloric or isonitrogenous. Bioavailability was assessed from growth response, apparent absorption, and serum or femur zinc. No significant differences in weight gain between test diets were found. Serum zinc was unexplainably high in the high phytate-soy supplemented group. Among ingredient-based diets, femur zinc was highest in the no-soy-no-phytate group and lowest in the soy-phytate fortified group. A similar result was seen with apparent zinc absorption; the no-soy-no-phytate ingredient-based group had the highest response. When bread rather than the ingredients were used to make diets, no significant difference in femur zinc or apparent zinc absorption was noted whether or not phytate and/or soy was incorporated into the bread formula. These results suggest that the process of bread baking, which hydrolyzes most of the phytate present (Table IV), is responsible for increased uptake of zinc from bread-based diets. The bioavailability of zinc in cookies fortified with one of three soy protein products was compared by Ranhotra et al (1979a) with cookies fortfied with egg albumin. As with the bread study, femur zinc concentrations and apparent zinc absorption was less in soy-fortified cookies. The authors strongly suggest that soy-fortified cereal-based foods should be fortified with zinc as has been previously proposed (NAS/NRC 1974).

Coccodrilli et al (1979) investigated the bioavailability of zinc in ready-to-eat breakfast cereals and wheat bran. After rats were fed a zinc depletion diet for 10 days, they were placed on test diets providing 4, 8, or 16 ppm zinc for 28 days. Slope-ratio analysis (after Momčilović et al 1975) using ZnO as a standard revealed that 1) zinc bioavailability from a corn-based and a pre-sweetened corn-based cereal, both fortified with ZnO during processing, were not significantly different from the standard and 2) a wheat bran-based processed cereal (no added zinc) gave a low relative zinc bioavailability of 44.6 (vs 100 for the ZnO control). Franz (1978) also utilized slope-ratio procedures (without an initial depletion period) and determined the relative bioavailability of zinc from several cereals. Whole corn and brown rice had low relative availabilities of zinc (0.51 or less compared to 1.0 in $ZnSO_4$), and whole wheat flour and unleavened whole wheat bread had medium values (0.73–0.75). Refined cereal products, such as white flour, leavened and unleavened white bread, and white rice had relatively high available zinc (0.87–1.09) as did leavened whole wheat bread (1.05).

Ranhotra et al (1979b) also investigated the bioavailability of iron in commercial variety breads (mixed grain, whole grain, high-fiber diet) and white breads (Table V). Iron availability was determined for breads, using both the AOAC (1975) hemoglobin (Hb) depletion-repletion technique and Hb gain technique (grams of Hb gained per milligrams of Fe consumed) discussed by Miller (1977). Bread-based diets were formulated using appropriate amounts of finely ground bread to replace an equal weight of sucrose in a low iron diet to obtain 15 ppm dietary iron. Diets were fed to groups of rats (N = 8) previously fed an iron depletion basal diet for five weeks. Data in Table V represent the relative iron repletion efficiency of groups of rats fed 15 ppm iron from bread-based diets compared to repletion with iron from $FeSO_4$ after 14 days of feeding. The investigators concluded that ingredients such as wheat bran, soy flour, vegetable flour, but not purified cellulose, interfered with the availability of iron. The unfortified bread diets yielded the lowest relative bioavailability of iron.

The absorption of nonheme iron by human subjects consuming one of nine common Western-type breakfasts was reported by Rossander et al (1979). They found an almost six-fold difference in absorption of extrinsically labelled radioiron with the meals. Tea reduced iron uptake by half, whereas orange juice increased absorption by two and a half times. Egg alone or egg with bacon also improved iron absorption.

Morris and Ellis (1975, 1976) reported that the major portion of iron in wheat bran is monoferric phytate. Iron from this complex appeared to be highly available. They also showed (Ellis and Morris 1977, 1978) improved zinc bioavailability in low-phytate

TABLE V
Relative Bioavailability of Iron from Variety Breads[a,b]

Bread Type[c]	Fortification of Breads	Hemoglobin Gain[d] (g/mg of Fe consumed)	AOAC Method[e] (% of control)
Control diet	$FeSO_4$	100	100
Whole wheat	None	54	48
Patent wheat	$FeSO_4$	78	69
Patent wheat and powdered cellulose	$FeSO_4$	89	81
Variety diet breads (2)	None	38	35
Variety breads (3)	$FeSO_4$	66	59
Variety bread	Reduced iron	58	49

[a] Adapted from Ranhotra et al (1979).
[b] All diets provided 15 ppm iron, primarily from the bread. Powdered bread was added to the basal low-iron diet at the expense of sucrose.
[c] Variety breads contained various ingredients such as soy flour, whole wheat flour, rye flour, vegetable flour, etc. Numbers in parentheses indicate number of breads tested.
[d] Technique of Miller (1977) after 14 days of repletion.
[e] Technique of AOAC (1975), in which experimental diets were compared to a standard curve generated by feeding levels of $FeSO_4$. Calculated after 14 days of repletion.

wheat bran prepared by phytase action or by phytate extraction procedures when low phytate products were compared with whole wheat bran.

The conclusions that can be drawn from these and other reports dealing with the bioavailability of the minerals from cereals and cereal products are 1) the bioavailability of zinc and iron from whole grains and wheat bran is less than that from refined grain products, 2) endogenous zinc and perhaps iron are less available than exogenous minerals, 3) leavening improves the utilization of zinc and iron, and 4) phytate seems to be more detrimental to trace element bioavailability than are components of dietary fiber. Other components of a meal also play a role in the bioavailability of a mineral from a single food. For example, both ascorbic acid and heme iron will improve the bioavailability of nonheme iron (Erdman 1978).

These conclusions must be tempered by two factors. First, whole grains contain higher total quantities of trace elements than do refined grain products, so one must consider both content and percent relative bioavailability to determine the net available mineral. Secondly, many studies comparing high and low phytate-fiber products involve these components being added to or removed from diets. Addition of sodium phytate or a refined dietary fiber may

TABLE VI
Relative Bioavailability of Zinc and Magnesium from Soy Products or of Zinc Carbonate or Calcium Carbonate Added to Soy Products, Evaluated by Growth or Serum or Bone Concentrations in Rats[a]

		Relative Bioavailability (%)[b]						
		Zinc from Soy Product		$ZnCO_3$ Added to Soy Product		Magnesium from Soy Product		Calcium Added to Soy Product
Product	Phytate-Zinc Molar Ratio	Weight Gain	Bone Zn	Weight Gain	Bone Zn	Serum Mg	Bone Mg	Bone Ca
Full-fat soy flour	28	55[c]	34[c]	94	94	96	106	94
Freeze-dried soy beverage	26	63[c]	40[c]	...[d]	...[d]	102	104	106
Spray-dried soy concentrate (neutral form)	52	41[c]	20[c]	77[c]	70[c]	77[c]	80[c]	102

[a] Adapted from Forbes and Parker (1977) and Forbes et al (1979).
[b] Data points represent comparison of slopes of responses of test diets (containing soy) with response of control diets (without soy product) with added mineral as the carbonate (N = 5, 6, or 8).
[c] Significantly different from control diets ($P < 0.05$).
[d] Test not run.

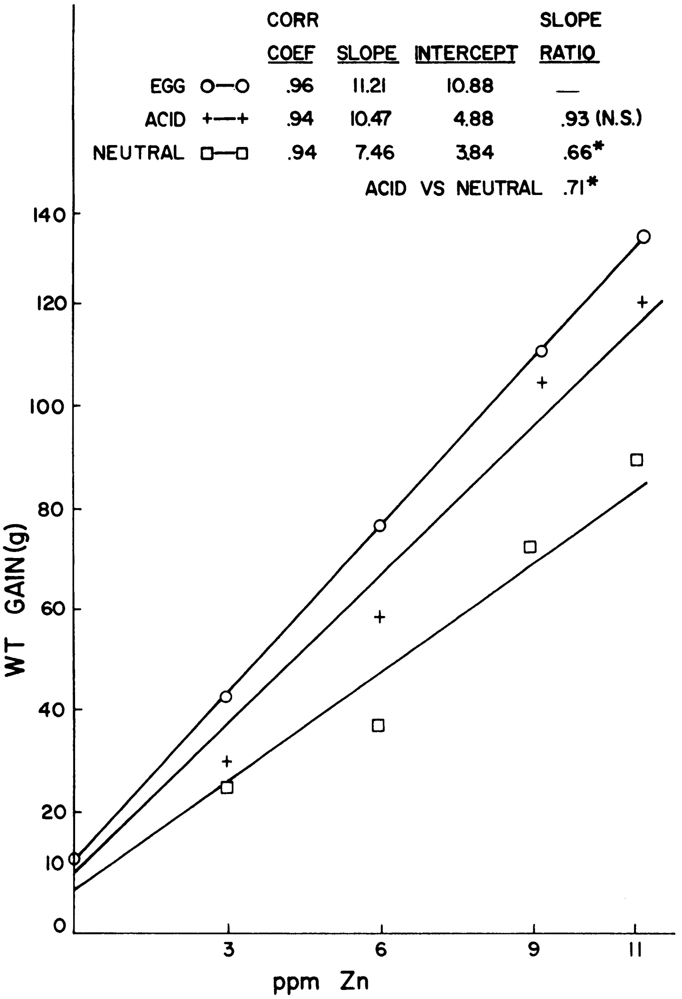

Fig. 1. Regression of weight gain upon dietary zinc. Effect of zinc added as zinc carbonate to egg white protein diet (o) or as acid-precipitated soy concentrate (+) or neutralized soy concentrate (□) to egg white protein on an equal protein basis (N = 6). * = $P < 0.05$, N.S. = not significant.

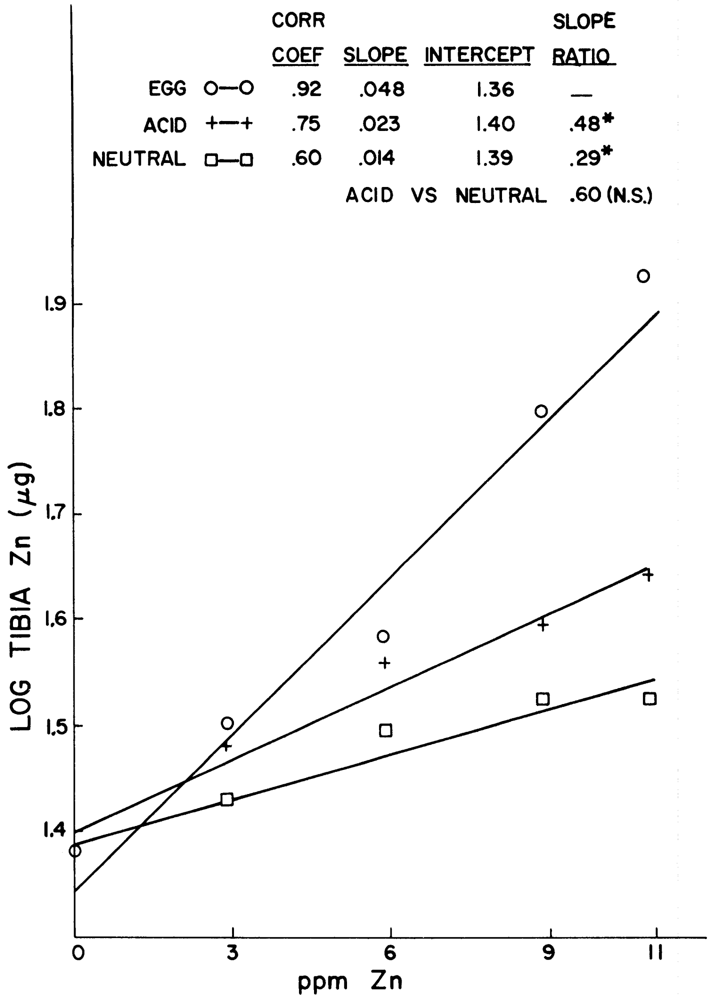

Fig. 2. Regression of log tibia zinc upon dietary zinc. Effect of zinc added as zinc carbonate to egg white protein diet (o) or as acid-precipitated soy concentrate (+) or neutralized soy concentrate (□) to egg white protein on an equal protein basis (N = 6). * = $P < 0.05$, N.S. = not significant.

not reflect the effects of the native phytate or fiber. In addition, leavening or the action of native phytases surely affects dietary components other than phytate.

BIOAVAILIABILITY OF MINERALS FROM LEGUMES

Momčilović et al (1975) developed the slope-ratio assay to study zinc bioavailability, using the rat as a model. They found that total femur zinc response to increasing dietary zinc was a sensitive measure of availability, although growth at low dietary zinc concentrations was also an appropriate test. This group (Momčilović and Shah 1976b) later found that relative zinc bioavailability in infant formula based on cow's milk was 82%, whereas that of soy-based formula was 53% compared to $ZnSO_4$. Johnson and Evans (1978) used the questionable (Jelliffe and Jelliffe 1979) extrinsic ^{65}Zn technique to compare availability of zinc added to ten varieties of milk. They found that soy and milk-based infant formula had reduced zinc bioavailability compared with that of human breast or processed cow's milk.

Forbes and co-workers (Forbes and Parker 1977, Forbes et al 1979) used the procedures of Momčilović et al (1975) to evaluate the bioavailability of several minerals endogenous to soy products and of exogenous minerals added to diets based on one of three soy products. Zinc bioavailability from soy products was poor but magnesium availability was good (Table VI). (O'Dell [1979] also concluded in his review of trace mineral availability from soy protein that only zinc had been clearly shown to have low bioavailability in the presence of soy protein). Calcium added to soy-based diets was highly available, whereas zinc addition was highly available when added to soy flour but only moderately available when added to a soy concentrate-based diet. Zinc utilization from soy products for growth or bone mineralization is obviously variable. This variability may reflect the different processing conditions, or it may be a result of an inverse relationship between phytate-zinc molar ratios and zinc bioavailability (Forbes et al 1979).

Use of the phytate-zinc molar ratio as a prediction of zinc bioavailability from high phytate foods was suggested by Oberleas (1975). Davies and Olpin (1979), in studying zinc availability from various commercial textured vegetable proteins, concluded that the phytate-zinc molar ratio was a valid indicator of zinc bioavailability. Recently, the nutrition community has been warned (Anonymous 1979) to be aware of complications (ie, poor mineral status) that might arise when substituting one protein source for another without careful evaluation of the phytate zinc molar ratio.

One study (Erdman et al 1980) demonstrated that significant differences in zinc bioavailabilities were found in soy products with similar composition and phytate-zinc molar ratios. Soy concentrations (or isolates) were prepared by identical procedures using acid precipitation. Some of the acid-precipitated product was freeze-dried at that pH and some was neutralized before freeze-drying. Growing rats were fed isonitrogenous and isocaloric diets for three weeks based on egg white (with added $ZnCO_3$) or soy product (substituted in egg white diets to provide equivalent zinc). Linear regression analyses relating rat growth and log tibia zinc to dietary zinc for the acid-precipitated and neutralized soy concentrates are found in Figs. 1 and 2. Zinc from the acid concentrate produced excellent growth, whereas significantly less growth was found with the neutralized product. Although with bone mineralization no statistical difference ($P < 0.05$) was noted between the two soy products, a trend toward lower utilization from the neutral product can be noted. Evaluation of soy isolates (not shown) yielded results similar to those shown in the figures. This study suggests that neutralization of soy protein products before drying reduces zinc bioavailability. In addition, this work points to the potentially important influence of unit food processing operations on the bioavailability of minerals; such a concern has been expressed previously by Rackis (Rackis et al 1975, Rackis and Anderson 1977) and Erdman and Forbes (1977).

Some studies have used other legumes to test for zinc availability. Momčilović and Shah (1976a) concluded from work with rapeseed protein concentrates, some of which contained in excess of 7.5% phytic acid (db), that the poor availability of zinc from these products was the major rate-limiting factor for ideal growth and development of young rats. Franz (1978) found that zinc was highly available from lima beans (94–98% of a good inorganic zinc source) whereas small white beans had a medium value (68%). Welch et al (1974) fed ^{65}Zn intrinsically labelled pea seeds to zinc-deficient rats. Zinc bioavailability from immature and mature seeds was good, althrough zinc was less available from the mature seeds. The authors concluded that the phytic acid in mature seeds was not solely responsible for this decrease.

The literature concerned with iron bioavailability from soybean protein was confusing until Rotrock and Luhrsen (1979) pointed out that studies such as their own, testing the availability of endogenous iron without added sodium phytate, always found good bioavailability. However, studies that have shown poor iron bioavailability (eg, Davies and Nightingale 1975) have added purified sodium phytate to diets. Steinke and Hopkins (1978) compared hemoglobin repletion in rats fed one of three soy protein isolates with that in rats fed ferrous sulfate. They found a mean relative iron bioavailability of 61% for endogenous iron. Similar results have been found in our laboratory for three other soy products.[2] Steinke and Hopkins (1978) further reported that inorganic iron added to diets containing isolated soybean protein had approximately the same bioavailability as that of the endogenous soy iron.

Components of dietary fiber may play an important role in the reduction of trace mineral bioavailability from legumes. This topic is reviewed elsewhere in this symposium. However, work from our laboratory (Weingartner et al 1979) demonstrated that inclusion of the soybean hulls (containing approximately 50% of the dietary fiber and less than 5% of the phytic acid of the whole soybean) in soy flour-based diets had no significant effect upon the bioavailability of native zinc or added calcium. This study suggests that soy hull fiber (at reasonable levels in the diet) plays no role in reduction of mineral absorption.

We conclude from this review that 1) mineral availability varies greatly from product to product, 2) of the minerals investigated, zinc availability is limiting in legumes, 3) endogenous zinc (but not iron) was less available than exogenous zinc added to diets containing legumes, and 4) both the phytate-zinc molar ratio and unit food processing operations affect the zinc availability in the final product.

Much more work must be accomplished before we will be able to accurately predict the biological availability of the chemically-present minerals in our food supply. This effort is necessary to permit us to adequately monitor nutritional status of a population or of an individual.

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