quickly. This method requires less equipment, reagents, and time than conventional Kjeldahl procedures.

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In Vitro Assessment of Zinc Binding to Protein Foods as a Potential Index of Zinc Bioavailability. Comparison of in Vitro and in Vivo Data

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An in vitro equilibrium dialysis test for estimating the strength of zinc binding to protein foods was developed for predicting zinc bioavailability. Soy flour, soy concentrate, casein, and dried egg white were labeled with $^{65}ZnCl_2$ before dialysis. The conditions included 24-h dialysis at pH 7.4 against 0.05 M tris(hydroxymethyl)aminomethane buffer (Tris), Tris plus 0.01 M L-histidine hydrochloride (Tris–His), and Tris plus 0.01 M Na₂EDTA (Tris–EDTA). Dialyzate and retentate ^{65}Zn were measured. The protein foods retained ^{65}Zn in the following decreasing order according to treatment: Tris > Tris–His > Tris–EDTA. The bioavailability of residual ^{65}Zn in casein, egg white, soy concentrate, and soy flour after each buffer treatment was determined by giving single doses of the protein foods to young Japanese quail. For these protein foods, the best agreement between in vitro and in vivo data was with Tris–His-dialyzable ^{65}Zn values and the whole-body ^{65}Zn retentions from the labeled casein and egg white (no treatment). The data suggest that this in vitro test could be useful for preliminary assessment of zinc bioavailability of protein foods.

The extent of a nutritional concern with low bioavailability of zinc from plant seed foods is presently not well-defined but would be expected to increase with expanded use of food products made from soybeans and other seeds to extend and/or replace traditional protein foods. O'Dell and Savage (1960) showed that phytic acid in isolated soybean protein contributed to low bioavailability of zinc. Evidence was presented by Oberleas et al. (1966a,b) that insoluble and nonabsorbable zinc–phytate complexes were formed in the gastrointestinal tract of rats, which rendered the zinc unavailable for absorption. Increased calcium accentuated the decreased zinc bioavailability by forming calcium–zinc–phytate complexes that were less soluble than zinc–phytate or calcium–phytate alone. Reinhold and

co-workers (1976), in an in vitro study, found that zinc bound to the fiber of wholemeal breads remained unavailable.

There have been few in vitro studies dealing with the strength of zinc binding to protein foods. Kratzer et al. (1961) shook an isolated soybean protein homogenate with $^{65}ZnCl_2$ solution and then centrifuged it; the radioactivity of the supernatant was assumed to be the amount of ^{65}Zn not bound by the protein. This method was rapid, convenient, and reproducible for estimating the amount of zinc bound by insoluble, isolated soybean protein (Allred et al., 1964). Lease (1967) developed an in vitro digestion technique, which simulated the initial digestive processes of the chick. With this procedure, ^{65}Zn in soybean meal was bound as a water-soluble, dialyzable complex and was shown to be readily absorbed under the conditions present in the intestinal tract of chicks. However, when casein was used with this technique, little zinc dialyzed from the

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casein digest at intestinal pH (Lease, 1968). Seth et al. (1975) developed an in vitro method similar to that of Kratzer et al. (1961) and Allred et al. (1964) and showed that rapeseed meal had greater zinc binding capacity than soybean meal.

In an in vitro study, Reinhold et al. (1974) found that zinc was more available from leavened than unleavened bread prepared with wheat intrinsically labeled with ^{65}Zn. Uptake of ^{65}Zn from suspensions of pulverized bread in saline–glucose by strips of rat jejunum–ileum, a technique similar to that employed by Oberleas et al. (1966 a,b), was greater with leavened bread. Reinhold et al. (1976) demonstrated that starch, protein, and fiber of wholemeal wheat bread bound ^{65}Zn after suspension and rotation of these solids in a saline–glucose–glycine solution containing ^{65}Zn; zinc bound to starch and protein was released in vitro by the action of digestive enzymes. From these studies on zinc solubility, Reinhold et al. (1976) concluded that fiber and not phytate was mainly responsible for the decreased availability of zinc, iron, and possibly calcium in wholemeal wheat bread.

Cousins and Smith (1980) used Sephadex chromatographic techniques to investigate the zinc-binding properties of bovine and human milk in vitro as influenced by the addition of zinc. Chromatography of milk from both species revealed the presence of zinc in low molecular weight fractions. Harzer and Kauer (1982) investigated the in vitro binding of zinc to cow's milk proteins. They found that, after casein was subjected to tryptic and chymotryptic digestion followed by chromatography on Sephadex, the zinc was bound to phosphopeptides.

Most of the early in vitro studies dealt with oilseed meal and cereal grain such as soybean and wheat, respectively. Kratzer et al. (1961), Allred et al. (1964), and Lease (1967, 1968) found no differences in zinc binding to casein and soybean meal. This is contrary to the superior bioavailability of zinc with casein as compared with soy products, which has been shown in numerous animal studies.

In the present studies, an in vitro method involving equilibrium dialysis was designed to investigate the relationship between the strength of zinc binding and zinc bioavailability from soy flour, soy concentrate, casein, and egg white and to assess the effects on the bioavailability of zinc from these treated protein foods. Conditions for physicochemical measurements were defined to provide information on the biological availability of zinc from these manufactured seed and animal protein foods.

MATERIALS AND METHODS

Protein Foods. Fat-extracted soy flour was processed in our laboratory from Lee variety soybeans purchased from P. M. Brooks Mill (Chestertown, MD). The other protein foods were soy protein concentrate (Patti-Pro, Griffith Laboratories, Chicago, IL), vitamin-free casein (Teklad, Madison, WI), and egg white solids (Type-20, Henningsen Food, Inc., White Plains, NY).

^{65}Zn Labeling of Protein Foods. Soy flour (autoclaved dry at 100 °C for 15 min), soy protein concentrate, casein, and egg white (autoclaved dry at 110 °C for 15 min) were individually passed through a stainless-steel sieve with 150-μm openings. A 200-mg subsample of each protein food was extrinsically labeled with 5 mL of carrier-free ^{65}ZnCl$_2$ (New England Nuclear, Boston, MA) solution to wet the entire subsample and to supply approximately 4.6 μCi of ^{65}Zn. These protein foods were subjected to equilibrium dialysis assay. The protein foods (2 g each) prepared for in vivo bioavailability studies were similarly labeled with ^{65}ZnCl$_2$ solution to supply 0.05 μCi of ^{65}Zn for 80 mg of final material. The ^{65}Zn-labeled protein foods

were frozen and lyophilized. The dried, labeled foods were ground in a mortar until they were very fine and uniform.

Equilibrium Dialysis Assay. Dialysis tubing (6000–8000 molecular weight cutoff and 31.8-mm diameter) was cut into 15.2-cm segments, treated to remove sulfides and contaminant metals by a procedure recommended by Spectrum Medical Industries, Inc. (Los Angeles, CA), and stored in deionized distilled water at 6 °C with a small amount of chloroform added to deter bacterial growth.

The chelating buffers used were as follows: 0.05 M tris(hydroxymethyl)aminomethane (Tris), 0.01 M disodium ethylenediaminetetraacetate in 0.05 M Tris (Tris–EDTA), 0.01 M L-histidine hydrochloride in 0.05 M Tris (Tris–His), 0.01 M glycine (aminoacetic acid) in 0.05 M Tris (Tris–Gly). These four buffers were prepared daily (pH 8.4). The pH was adjusted to 7.4 and 3.2 with 6 M HCl.

A 200-mg subsample of each labeled and corresponding unlabeled food was used. The dialysis tubing was closed on one end, and successive 2.45-mL aliquots of chelating buffer were used to quantitatively transfer the protein food into the bag to give a final volume of 9.8 mL of buffer. The bags were closed in a manner to minimize trapped air. The final length of the dialysis bag was approximately 10 cm. The bag was wetted with deionized water and gently shaken so that the protein food was suspended in the buffer. The filled bag was placed in a 250-mL square polyethylene bottle that contained 100 mL of the same buffer. Bags were handled with nylon forceps.

The bottles were placed in an Eberbach shaker (A. H. Thomas Co., Philadelphia, PA; 54 cycles/min; 3.8 cm stroke/cycle) in a chamber maintained at 6 °C. The buffer was changed at 24-h intervals from 1 to 6 days. To examine the dialysis assay at shorter time intervals (2, 4, 6, 8, 24 h), the dialysis bag was similarly changed to a fresh bottle of buffer.

Dialysis of each protein food against the buffers was carried out in at least two experiments, each including one radioactive sample and one nonradioactive sample. A 5-mL dialyzate aliquot from the ^{65}Zn-labeled protein food was taken with each change of buffer to measure the amount of ^{65}Zn removed from the protein food at each time interval. The radioactivity of all dialyzates and the final retentates, which accounted for virtually all radioactivity in each case, was measured at the end of the experiment as described below.

Zinc Determination. Duplicate subsamples (0.500-g each) of the nonlabeled, untreated protein foods, the unlabeled soy flour and casein retentates after dialysis against each buffer, and the buffer components (Tris, histidine, EDTA, glycine) were acid digested with a 3:1 (v/v) mixture of concentrated nitric acid–70% perchloric acid. (Nitric acid–perchloric acid digestions require special equipment and great care to avoid explosive conditions.) The digested samples were transferred to 25-mL volumetric flasks containing 10% gylcerol, diluted to volume with deionized distilled water, and transferred to small Teflon bottles. Zinc was determined by conventional flame atomic absorption spectrophotometry (AAS) (Model 503; Perkin-Elmer, Norwalk, CT). The buffer components contained negligible amounts of zinc (not quantifiable, <0.9 μg of Zn/g of subsample). To verify analytical procedures, duplicate samples of National Bureau of Standards Standard Reference Material bovine liver were digested and analyzed as described above.

Protein Determination. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as a protein standard. Duplicate 1-mL aliquots were taken from each exchange of the 100-mL dialyzate

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of the unlabeled protein foods. The protein assay was performed for each chelating buffer solution to determine the absorbance of the buffer blanks. To compensate for EDTA complexing with copper ion in the alkaline copper reaction, additional copper sulfate was added (e.g., equal to the equivalent of EDTA) to the dialyzate with Tris–EDTA and to the Tris–EDTA buffer blank (Okubo et al., 1975).

No protein was lost from casein or egg white during dialysis. The loss was <5% for the soy protein foods.

Preparation of Test Foods for in Vivo Bioassay. Subsamples of radioactive and nonradioactive protein foods were dialyzed for 24 h against the Tris, Tris–His, and Tris–EDTA buffers at pH 7.4. After 24-h dialysis, the suspensions were dialyzed against deionized distilled water for four 2-h and one overnight exchanges to dialyze out the residual buffer that might affect the bioavailability of zinc. The protein foods were quantitatively transferred from the dialysis bags into plastic-lined stainless-steel trays, frozen, and lyophilized. All dried retentates were ground uniformly fine in a mortar. These preparations were carried out in small and large batches for both labeled and unlabeled foods. The amounts for each step were as follows: 200 mg and 2 g of food, 31.8- and 63.7-mm diameter dialysis tubing, 9.8 and 98 mL of buffer, 100 and 1000 mL of deionized distilled water, respectively. Polyethylene bottles and double polyethylene bags (25.4 × 30.5 cm) were used to hold buffer or water.

Preparation of Labeled Food Tablets for in Vivo Bioassay. The ^{65}Zn-labeled protein foods and retentates to be used in the bioassay were uniformly ground in a mortar. Stearic acid was added to a concentration of 0.1% as a lubricant to allow release of the tablet from the dies, and each material was pressed manually into tablets (Stokes' tablet machine, Model 900-511-6, Warminster, PA). Each tablet was 4.76-mm in diameter and approximately 7-mm long and weighed 80 mg.

Bioassay of Retentate ^{65}Zn and Untreated Protein Food. From days 0 to 7, Japanese quail (*Coturnix coturnix japonica*) were housed, 12 birds per group, in suspended stainless-steel small-mesh cages with ample lighting and heat. The racks were covered with nonwoven cloth and polyethylene film to prevent environmental contamination, as previously described (Jacobs et al., 1969). From hatching to day 7, the birds were fed a soy isolate diet containing 30 ppm zinc (Table I). On day 6, the birds were wing banded. On day 7, the birds were weighed and redistributed by body weight into groups of 10 birds each. From 7 to 14 days, each group was fed a casein–gelatin diet containing 60 ppm zinc (Table I). All diets and deionized distilled water were supplied ad libitum.

On day 9, the birds were given untreated, nonlabeled protein food or nonlabeled retentate for 4 h. After 2 h, the corresponding radioactive food or retentate tablet was administered. The tablet was placed in a disposable plastic powder transfer pipet. Each bird was restrained in one hand, the beak of the bird was held open with the end of the pipet, the pipet was quickly placed in the upper esophagus, and the tablet was released. Any loss of the tablet was noted. All empty vials were counted for residual radioactivity, if any, after dosing and corrections were made. Following the final 2-h feeding of unlabeled retentate, the casein–gelatin diet was continued to day 14. On day 14, the birds were killed by asphyxiation with carbon dioxide, placed in small tared self-sealing plastic bags, and weighed.

Measurement of Radioactivity. Five-milliliter aliquots of each dialyzate were taken at the time of each 100-mL buffer exchange and placed in disposable plastic

Table I. Composition of Diets[a]

component	diet, g/kg	
	casein–gelatin	soy isolate
casein[b]	280	
gelatin[c]	70	
soy isolate[d]		350
glycine[e]	5	5
DL-methionine[e]	6	6
L-arginine hydrochloride[e]	6	
choline dihydrogen citrate[e]	6.5	5.3
mineral mixture[f]	58.7	58.1
corn oil	40	40
glucose monohydrate	527.8	535.6

[a] Component sources, except as noted, and levels of vitamins as reported by Jacobs et al. (1978). [b] Vitamin-free; Teklad, Madison, WI. [c] Wilson, 2× gelatin; Wilson Food Corp., Calumet City, IL. [d] Purina Assay Protein RP-100; Ralston Purina Co., St. Louis, MO. [e] NRC grade; Teklad, Madison, WI. [f] Supplied per kilogram of diet: CaHPO$_4$, 28.4 g; CaCO$_3$, 10 g; Na$_2$HPO$_4$, 7 g; NaCl, 4 g; KCl, 7 g; KIO$_3$, 10 mg; Na$_2$SeO$_3$, 0.44 mg (reagent grade chemicals; J. T. Baker Chemical Co., Phillipsburg, NJ). For the casein–gelatin diet, the following amounts of the specified elements were added to a total concentration of 60, 127, 12, 5, and 300 mg/kg of diet zinc (as ZnCO$_3$); iron (as ferric citrate·xH$_2$O); manganese (as MnSO$_4$·H$_2$O); copper (as CuSO$_4$); magnesium (as MgSO$_4$). For the soy isolate diet, the following amounts of the specified elements (same sources given above) were added to a total concentration of 30, 110, 12, 5, and 300 mg/kg of diet: zinc; iron; manganese; copper; magnesium. To provide precise diet formulation, each elemental salt mixture in glucose and dietary protein was analyzed for metal content by AAS.

culture tubes (16 × 125 mm). Radioactivity in each sample was measured twice in an Auto-Gamma scintillation spectrometer (Model 5285; Packard Instruments Co., Inc., Downers Grove, IL) equipped with an NaI (Tl) crystal. An integral window was employed to maximize counting efficiency. Radioactivity was measured in all tablets used for the bioassays.

The radioactivity in the retentates (from both large and small treated batches) and whole birds used in the biological assessment of retentates and untreated protein foods was measured in a whole-body scintillation spectrometer (Model 446 ARMAC; Packard Instrument Co., Inc., Downers Grove, IL). To minimize counting errors due to geometry, clear plastic tubes of various sizes were used to position the retentates (set in plastic weighing dishes) and the birds (in self-sealing plastic bags) in the center of the detector chamber during the measurement of radioactivity. Five tablets were prepared to serve as radioactive reference standards for use in both the Auto-Gamma and whole-body scintillation spectrometers, so that a correction for different counting efficiencies between the two spectrometers could be made.

Statistics. Data in Table II were evaluated by analysis of variance and Duncan's multiple-range test (SAS Institute, 1979).

RESULTS AND DISCUSSION

Suitable Conditions for Equilibrium Dialysis Assay. A pH of 7.4 was determined to be the most appropriate throughout the 6-day dialysis. Our preliminary dialysis assay studies at pH 8.4 and pH 3.2 showed that there was increased solubility of proteins at the alkaline pH, and at the acidic pH the effect of the chelating buffers was negligible. In both cases, most of the ^{65}Zn was removed. Ismail-Beigi and co-workers (1977) observed maximum zinc binding at pH 6.5–7.5 when using their in vitro method of incubation of powdered wheat meal bread in 4.0 mM phosphate bufer. In zinc binding in vitro studies, Harzer and Kauer (1982) also observed that pH 7.4 was satisfactory. Our chelating buffers had the fol

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Table II. Percentage of ^{65}Zn Retained by the Protein Foods Dialyzed against the Chelating Buffers[a]

				^{65}Zn retentate		
protein food	no. of assays	Tris	no. of assays	Tris–His	no. of assays	Tris–EDTA
casein	3	98 ± 1.1[b]	2	52 ± 11[b]	3	6 ± 2.5[b,c]
egg white	3	100 ± 0[b]	3	78 ± 0.8[c]	3	12 ± 1.7[b]
soy flour	11	34 ± 1.3[c]	9	26 ± 1.1[d]	9	3 ± 0.3[c]
soy concentrate	3	91 ± 0.3[d]	3	38 ± 0.4[e]	3	6 ± 0.4[b,c]

[a] The mean percentage ±S.E. of ^{65}Zn retained by each protein food after 24-h dialysis against Tris, Tris–His, and Tris–EDTA buffers. [b-e] Values within columns with different superscripts are significantly different ($p < 0.01$).
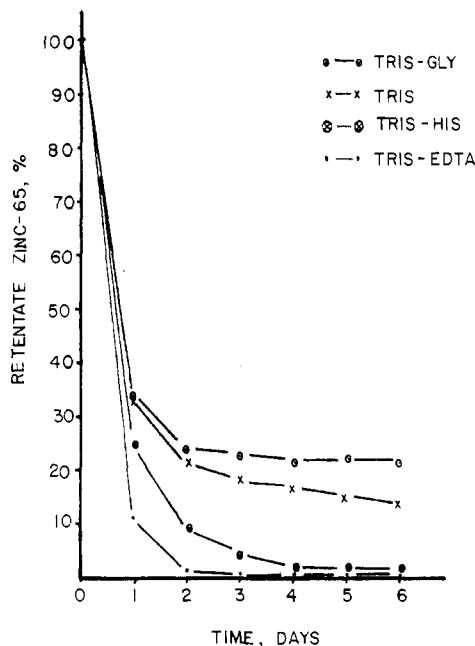

Figure 1. Retention of ^{65}Zn after 6-day equilibrium dialysis of soy flour at pH 7.4 against the chelating buffers Tris, Tris–EDTA, Tris–His, and Tris–Gly.

Figure 2. Retention of ^{65}Zn after equilibrium dialysis of soy flour at pH 7.4 against Tris, Tris–EDTA, Tris–His, and Tris–Gly at shorter time intervals within 24 h.

lowing decreasing order of ability to extract ^{65}Zn from soy flour; Tris–EDTA, Tris–His, Tris, Tris–Gly (Figure 1).

The maximal removal of zinc from soy flour with each buffer had occurred by 3 days, and most of this occurred during the first 24 h. For a useful, practical method, a short time period is desirable, and we thought that times less than 24 h (2, 4, 6, 8 h) might be suitable. The amounts of ^{65}Zn retained at 2 h from the soy flour were identical except for greater removal with Tris–EDTA (Figure 2). The differences in ^{65}Zn removal from soy flour were greater by 8 h; however, 24 h was the most suitable time interval because during this interval even greater amounts of ^{65}Zn were removed between the buffers. Even though each buffer had been changed four times during the 24 h, the total ^{65}Zn that was retained by each buffer was only slightly greater than when no buffer was changed during this time (Figure 1).

Conditions for assessing the amount of zinc binding to soy flour and other protein foods by the equilibrium dialysis assay included the following: temperature, 6 °C; pH 7.4; time, 24 h; chelating buffers, Tris, Tris–His, and Tris–EDTA. The Tris–Gly buffer duplicated the effect of the Tris buffer; therefore, Tris–Gly buffer was not used further.

Strength of Zinc Binding to Protein Foods. The equilibrium dialysis assay was tested on three additional protein foods: casein, egg white, soy concentrate. The amount of ^{65}Zn retained by these protein foods in at least two experiments each and nine experiments with soy flour are shown in Table II. The various buffer treatments resulted in major differences with each food. All foods retained ^{65}Zn in the following decreasing order according
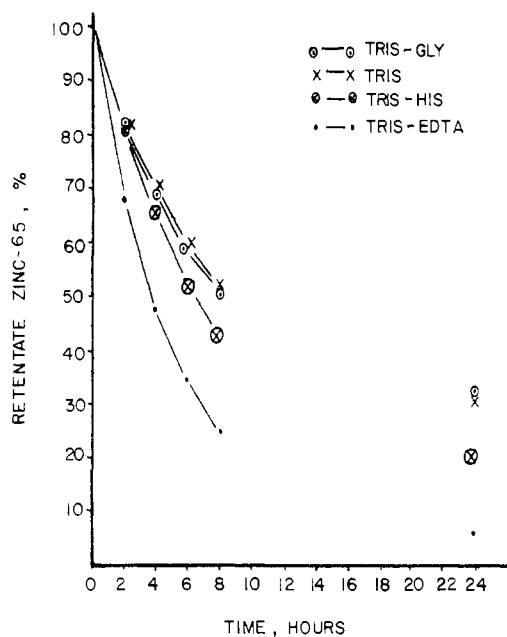
to treatment: Tris > Tris–His > Tris–EDTA. Casein and egg white retained significantly more ($p < 0.01$) ^{65}Zn than was retained by soy flour and soy concentrate after Tris treatment. Moreover, the soy concentrate retained more ^{65}Zn after Tris dialysis treatment than the soy flour. After Tris–His treatment, each protein food retained significantly different amounts of ^{65}Zn ($p < 0.01$); casein and egg white retained more ^{65}Zn than did soy flour and soy concentrate. With the Tris–EDTA treatment, only egg white and soy flour were significantly different ($p < 0.01$) from each other in the amount of ^{65}Zn retained. For casein, zinc retention after Tris and Tris–EDTA treatments was similar to that obtained with the in vitro method of Harzer and Kauer (1982).

Soy flour was the only food that lost significant ^{65}Zn during treatment with Tris. Soy flour was the only food that contained large amounts of carbohydrate; consequently, it contained the lowest proportion of protein, approximately 50%. This compares with approximately 70% protein for soy protein concentrate and essentially 100% protein in the other foods. The nonprotein components of soy flour may have been responsible for the difference in ^{65}Zn retention. For the soy products, the lowest ^{65}Zn retention with all buffers was found with the flour, the less refined of the two products (Table II). This agreed with the in vitro results of Erdman et al. (1983), which showed that concentrates have higher zinc retention than flour.

To ascertain whether the ^{65}Zn removal was similar to the removal of the native zinc content in the protein foods, the zinc concentrations from the retentates of dialyzed casein and soy flour were determined by conventional

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Table III. Zinc Concentration in Casein and Soy Flour after Dialysis Treatment with the Chelating Buffers[a]

protein food	buffer treatment	concn of zinc, μg/g	retentn of Zn, %
casein	none	42	
	Tris	42	100
	Tris–His	29	69
	Tris–EDTA	4	10
soy flour	none	48	
	Tris	40	83
	Tris–His	33	69
	Tris–EDTA	6	12

[a] Casein and soy flour were dialyzed for 24 h against Tris, Tris–His, and Tris–EDTA buffers. After dialysis, these protein foods were washed and freeze-dried. Duplicate subsamples of the treated and nontreated protein foods were taken for zinc determination by AAS.

flame AAS (Table III). The same sequences of relative zinc removal from these foods were observed as those from the [65]Zn-labeling study. For casein dialyzed against Tris, Tris–His, and Tris–EDTA, the proportion of retained zinc represented as the percentage of zinc retention based on the zinc concentrations (Table III) was similar to the amounts of [65]Zn retained with each buffer; however, greater amounts of the intrinsic zinc of soy flour were retained with Tris and Tris–His than extrinsic [65]Zn (Table II). This difference in the amount of zinc retained in the soy flour may be due to a phytate–protein–mineral complex formed during growth of the seed that was resistant to dialysis. Another possible reason for the differences in zinc retention may be that the intrinsic zinc was tightly bound to the soy flour protein and thus not enough similar sites were available for the extrinsic [65]Zn label. In previous studies, animals fed intrinsically labeled [65]Zn soy flour and extrinsically labeled [65]Zn soy flour showed a similar exchange of [65]Zn (Lee et al., 1975); thus, it was not anticipated that this would be a problem due to the use of carrier-free [65]Zn and the negligible amount of radioactive zinc to be bound.

Assessment of in Vivo Measurements of [65]Zn Bioavailability and in Vitro Estimates. The bioavailability of zinc from [65]Zn-labeled foods (casein, egg white, soy flour, soy concentrate) and retentates of each food with each buffer system was measured in young Japanese quail (Table IV). The whole-body (WB) [65]Zn retention values for the Tris-treated retentates from casein, egg white, and soy protein concentrate were similar to those not treated. This was most likely due to the fact that Tris removed little (if any) [65]Zn except from soy flour. Sixty percent of the [65]Zn was removed from the soy flour after Tris treatment; therefore, only approximately 10% of the [65]Zn originally present in the flour was retained by the birds after dosing (Table IV). For the Tris–His retentates, the WB [65]Zn retention from all the protein foods was similar except for egg white, which was higher. Since after the treatment the [65]Zn would be tightly bound, based on the percentage of [65]Zn left in the Tris–His retentate food, more WB [65]Zn was retained from both casein and egg

white than from either soy protein product (Table IV). The [65]Zn bioavailability for all the Tris–EDTA-treated protein foods was low.

In our study, the [65]Zn bioavailability from in vivo measurements of Tris treatment and no treatment was greater from egg white and casein than from soy flour and soy concentrate. This agrees with other bioassays in that the bioavailability of zinc from animal protein sources was generally higher than from plant sources (O'Dell et al., 1958; Morrison and Sarett, 1958). The results from using this tablet dose technique for [65]Zn bioassay of nontreated soy flour, soy concentrate, and egg white are in agreement with those obtained by the zinc bioassays of Forbes and co-workers (1977, 1979) and Davies and Reid (1979). Bioassays of [65]Zn-labeled foods have shown that an extrinsic labeling technique was an accurate measure of zinc bioavailability and that no difference was observed between absorption of intrinsic and extrinsic [65]Zn, which indicated that endogenous and exogenous [65]Zn entered a common pool before being absorbed from the rat's intestine (Evans and Johnson, 1977; Meyer et al., 1983). According to the work of Evans and Johnson (1977), the accuracy of this type of bioassay technique to determine zinc availability from different protein foods was dependent on the zinc content of the food tested and the zinc status of the animal. In our study, the birds were fed a casein–gelatin diet containing 60 ppm Zn before and after [65]Zn-labeled food dosing, and the zinc content (ppm) of these labeled foods (casein, 42; egg white, 0.8; soy flour, 48; soy protein concentrate, 37) was lower than that in the diet. In a separate study (Jones and Fox, 1984), following identical procedures, the whole-body [65]Zn retention was approximately twofold greater with 30 ppm than with 60 ppm zinc in the casein–gelatin diet, thus showing an influence of dietary zinc content on the WB retention from a [65]Zn-labeled test food.

The Tris–EDTA in vivo and in vitro retention data were similar for the protein foods (Table IV). The [65]Zn retention for both in vivo and in vitro assays was low. This low WB [65]Zn retention of the Tris–EDTA-treated foods may have been due to the presence of such a small quantity of [65]Zn label relative to the amount of zinc present in the diet. If the zinc entered a common pool under these conditions (a high ratio of zinc to [65]Zn), less [65]Zn would be expected to be absorbed. Another possible reason for this occurrence was that the residual [65]Zn was all very tightly bound to the EDTA-treated protein foods, leaving less [65]Zn to be absorbed.

With the Tris–His treatment, similar zinc bioavailability was predicted from the in vitro assay (dialyzable [65]Zn) as was found by the in vivo bioassay of untreated casein and egg white, 38 vs. 47% (±6) and 22 vs. 38% (±6), respectively (Table IV). The Tris–His treatment of soy proteins did not result in [65]Zn availability similar to that of the WB [65]Zn bioassay of untreated labeled soy protein probably because a large amount of [65]Zn was initially extracted from these soy proteins (Table IV). This may be due to the

Table IV. [65]Zn Retention in Vitro and in Vivo by Japanese Quail (Whole Body, WB) Receiving Untreated Food or Retentate

protein food	in vitro,[a] [65]Zn retent, %			in vivo[b] [65]Zn WB retentn, %			
	Tris	Tris–His	Tris–EDTA	none	Tris retent	Tris–His retent	Tris–EDTA retent
casein	100	62	11	47 ± 6	58 ± 2 (58)	22 ± 3 (14)	6 ± 0.2 (0.7)
egg white	100	78	9	38 ± 6	32 ± 4 (32)	37 ± 6 (29)	8 ± 0.8 (0.7)
soy flour	40	34	11	24 ± 3	25 ± 4 (10)	20 ± 3 (7)	9 ± 0.7 (1.0)
soy concentrate	90	38	6	25 ± 3	28 ± 3 (25)	22 ± 2 (8)	8 ± 1.2 (0.5)

[a] The [65]Zn retention of each buffer treatment was determined on a single batch of approximately 2 g of the respective protein foods. [b] The mean percentage ±S.E. of [65]Zn retained by the birds after dosing with the respective treated retentate and untreated food of each protein food are shown (nine to 10 birds per dose of protein food). Values in parentheses are calculated percentages based on the amount of [65]Zn in the retentate multiplied by the respective percentage of [65]Zn WB retention.

difference in the intrinsic zinc and extrinsic ^{65}Zn exchanges of soy flour previously mentioned (Table III).

The in vitro ^{65}Zn values from the Tris–His treatment may be useful for predictive ranking of values for zinc bioavailability from other protein foods. Further investigation is needed to validate the procedure fully for a wider range of foods.

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Effect of Phytate Removal Treatments upon the Molecular Weight and Subunit Composition of Major Soy Protein Fractions

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Soy protein extracts were prepared from commercially defatted flakes and treated by alkaline pH or ion exchange phytate removal processes. Control and phytate-reduced soy protein extracts were characterized for phosphorus content and for changes in molecular weight profile and subunit composition of the major protein fractions by Sepharose 6B gel filtration chromatography and SDS gel electrophoresis. The alkaline pH phytate removal treatment only removed 62% of the total phosphorus content of soy extract but caused major amounts of aggregation of its glycinin and β-conglycinin-containing Sepharose protein fractions. In contrast, the ion exchange phytate removal treatment removed 86% of the total phosphorus content of soy extract and resulted in only minor alteration of the molecular weight profile and subunit composition of the Sepharose protein fractions. This experimental approach could be used to evaluate the effects of different processing treatments upon the physicochemical properties of the major soy protein components.

INTRODUCTION

The high quality and protein content of soy protein products make them attractive as ingredients in formulated food products (Erdman, 1979). As soy proteins become more extensively utilized in our food supply, modification and improvement of their nutritional and functional properties will become more important (Hartman, 1979).

Phytate, the hexaphosphate salt of myoinositol, is the chief storage form of phosphorus in the soybean (Okubo et al., 1975; Cosgrove, 1966). Phytate is coextracted and recovered with the proteins during soy protein isolate manufacture, resulting in phytate contents in the range of 1.5–2.5% (Brooks and Morr, 1982; Hartman, 1979).

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