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Techniques for Intrinsically Labeling Wheat with ^{65}Zn

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Several techniques of intrinsically labeling wheat with ^{65}Zn were compared: stem injection of ^{65}Zn, stem injection of $^{65}Zn + ZnSO_4$, foliar application of ^{65}Zn, and the addition of ^{65}Zn to a hydroponic solution. Incorporation levels of ^{65}Zn into the grain were 62.6% stem injected, 45.2% stem-injected $^{65}Zn + ZnSO_4$, 57.5% foliar application, and 2.3% hydroponic solution. Four protein fractions were extracted from fat-free whole wheat flour. Distribution of ^{65}Zn into the protein fractions for all treatments, was 8.5–20.3% in albumins and globulins, 47.4–60.3% in glutenins, 1–2.6% in gliadins, and 9.8–28.3% in the remaining proteins. Separation of the fractions by gel chromatography showed that protein and Zn distributions were similar among the treatments and when compared to the controls. Zinc-65 distribution was similar to the natural Zn distribution. These data illustrate that stem-injected ^{65}Zn is incorporated in the same manner and ratios as Zn naturally utilized by wheat.

INTRODUCTION

The increased emphasis on determining the bioavailibility of trace elements from major food stuffs of the world as outlined in the USDA/ARS 6-year implementation plan 1984–1990 has placed new emphasis on developing a simple, cost-effective, rapid method of intrinsically labeling foods with isotopes.

Traditionally, hydroponic systems have been used to label plants intrinsically with radioisotopes. Bergh (1950) labeled sweet peas with radiozinc by adding 0.2 mCi of ^{65}Zn to a Hoagland's solution. After 24 h, the activity in plant material was determined. Bergh noted that most of the radiozinc remained in the roots.

In recent years several investigators have used hydroponics to label plants intrinsically with ^{65}Zn (Meyer et al., 1983; Levine et al., 1982; Ketelsen et al., 1984; Schmitt and Weaver, 1984). This technique, although the most physiologically natural, requires relatively large amounts of isotopes and produces large volumes of radioactive liquid waste. Also, the limited upward mobility of Zn and Fe in plants produced a low ratio of applied to incorporated isotopes (Noggle and Fritz, 1976). Garcia et al. (1977) endogenously labeled corn plants with ^{65}Zn in a hydroponic system. After tassel emergence the plants were placed in nutrient solution containing ^{65}Zn. After harvesting, only 27.6% of the ^{65}Zn was incorporated into the corn kernels. Similar incorporation, 21.3–27.6% of ^{65}Zn administered hydroponically to soybean plants, was reported by Janghorbani et al. (1983). In addition, the absorption via the root system is dependent upon a multiplicity of factors, pH, ionic concentrations, age of plant, ion chelation, etc., and these may vary for each plant species.

One method of fertilizing plants, foliar application, may be applicable to intrinsic labeling. Foliar application of phosphates, iron, and zinc has been used in agricultural systems to correct mineral deficiencies and to avoid possible soil interactions that would render the applied nutrient unavailable to the plant. The use of foliar application for labeling plants has been very limited and, to our knowledge, our use of this method is currently unique.

A third technique is stem injection. Roach (1938) used a modification of this technique to determine mineral deficiencies in fruit trees. Since this method was used on trees, for which hydroponic culturing is not applicable, most advantages listed by Levy (1939) are not applicable. Only the fact that one could be sure the particular substance supplied would actually enter the plant system is pertinent to this study.

The only previous work concerning the absorption and translocation of stem-injected mineral isotopes is that of Zeind (1967). He concluded that in stem injection, the isotope distribution within corn seeds approached equilibration and that it was the most efficient method for intrinsically labeling plants.

We have been using a modification of Zeind's method (1967) to intrinsically label wheat, soy, and oats for the past 3 years. Criticism has been raised that although this technique provides good incorporation of the injected isotope into the seed, the "unnatural" method may result in deposition of Zn at higher than natural levels or in different forms than the physiological natural form.

This study was initiated to compare hydroponic, foliar, and stem-injection applications of ^{65}Zn to wheat and to characterize the proteins and their Zn content to determine which method produced the best incorporation without altering the seeds' natural protein content or Zn distribution.

MATERIALS AND METHODS

Wheat, *Triticum aestivum* var. Waldron, was grown in a greenhouse with supplemental lighting provided by 400-W high-pressure sodium lamps (Energy Technics, York, PA) to produce a 16-h light:8-h dark cycle. Plants were grown in 8-in. plastic pots, 7 plants per pot in soil with 30% perlite, and watered as needed. All plants except the ones grown in the hydroponic system were grown in the soil mixture.

The hydroponic system was a "closed aggregate" system with 70% perlite and 30% vermiculite. Modified Hoagland's solution plus the A–Z micronutrient solution were used (Hoagland and Arnon, 1950). Iron was added as

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Figure 1. Stem injection process.

Sequestrene (Fe-EDTA, Ciba-Geigy Corp., Summit, NJ).

All applications of ^{65}Zn as carrier free $^{65}ZnCl_2$ (New England Nuclear) were done at anthesis following pollen formation at a rate of 1 μCi per plant.

Foliar application of ^{65}Zn was done in a fume hood as follows: ^{65}Zn as $^{65}ZnCl_2$ was added to a citrate–phosphate buffer (0.1 M citric acid, 0.1 M sodium citrate), pH 5.5, (McIlvaine, 1921) plus 1% sodium lauryl sulfate (wetting agent). Twenty microliters of the solution were drawn into a microsyringe and then dropped onto the surface of a healthy growing leaf. After the solution dried, the plants were returned to the greenhouse.

Hydroponic application was done as follows: individual wheat plants, at anthesis, were transferred from the "closed aggregate" hydroponic system to PVC tubes containing the same nutrient solution. Care was taken to remove the plants carefully with minimal root damage. All of the perlite and vermiculite was washed from roots using nutrient solution. The ^{65}Zn as $^{65}ZnCl_2$ was diluted in H_2O to an activity of 1 μCi/mL of solution. This solution was added to each tube. Individual plants were placed into the tubes (1 plant per tube) and each tube was aerated. After maturity the plants were removed from the tubes and the grain harvested. In addition, wheat grown hydroponically and intrinsically labeled with ^{65}Zn was obtained from C. M. Weaver, Purdue University, Lafayette, IN. The growing conditions are given in Weaver (1984).

Three treatments were applied by stem injection to soil-grown plants: (1) Citrate–phosphate buffer pH 5.5. (2) ^{65}Zn as $^{65}ZnCl_2$ plus buffer. (3) ^{65}Zn as $^{65}ZnCl_2$ plus 0.3 mg of Zn as $ZnSO_4$ plus buffer. All three treatments were administered as follows: The second stem internode of each plant was chosen. A 1.0-mL tuberculin syringe was used. One hole was made at the bottom part of the internode with the syringe needle. Care was taken to puncture the stem until the needle entered only the cavity in the pith. This hole reduced the turgor pressure within the cavity and enabled the injection of liquid into the plant. The syringe was then filled with 0.7 mL of the injection solution. A second hole was made in the upper portion of the internode and the solution was slowly injected into the cavity in the pith (Figure 1). If the solution were administered too rapidly, the fluid would flow out of the lower hole. With practice, this method can be performed very rapidly, approximately 30 plants per hour.

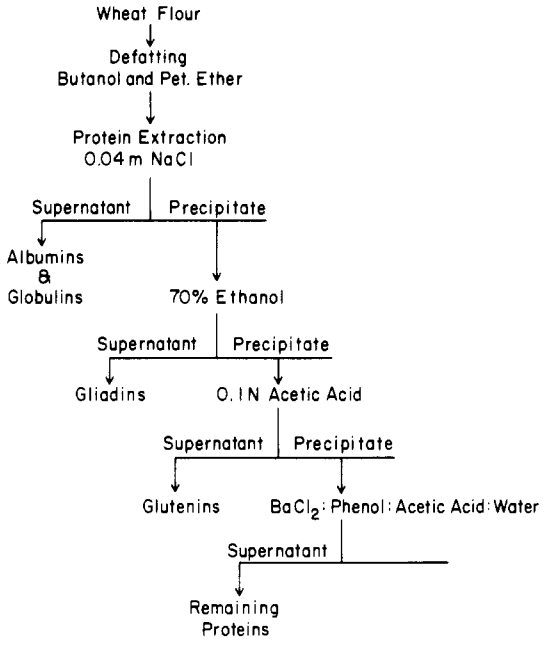

Figure 2. Flow diagram of wheat protein extraction process.

The citrate–phosphate buffer was used as a sham. This was done to observe whether the actual injection process caused any physiological changes in the plants.

The injection of ^{65}Zn + 0.3 mg of Zn as $ZnSO_4$ was done to simulate injection of stable isotopes. This was done to examine what effects a large dose, 0.3 mg, would have on the plant and to test the feasibility of using stem injection for intrinsically labeling plants with stable isotopes.

Control plants were grown in the same soil mixture and under the same conditions as plants given the other treatments.

All plants were harvested after the grain was dry. Seeds were separated from the chaff by hand. Chaff and seeds were counted for radioactivity in a small animal whole body counter and the percent incorporation of the ^{65}Zn in each treatment was calculated.

After separation and γ counting, the wheat fruits were ground in a coffee and spice mill. The resulting flours were defatted by triple extraction with butylalcohol (1 g of flour:3 mL of butyl alcohol), 30 min stirring per extraction, followed by one similar extraction with petroleum ether (35.8–37.0 °C), and air-dried (Bietz and Wall, 1975).

A sequential extraction of flour proteins similar to that of Bietz and Wall (1975), with modifications, was used. For each extraction, 8 g of defatted flour was placed in a 250-mL beaker and 60 mL of extractant was added. The mixture was stirred continuously for 30 min and then centrifuged 30 min at 2000*g*. The supernatant was decanted and saved and the step repeated. After the second extraction with the same extractant, the precipitate was mixed with the next extracting solution. In this manner, the flours were sequentially extracted with each of the following solutions: 0.04 M NaCl, 70% ethanol (EtOH), 0.1 N acetic acid (HOAc) (Bietz and Wall, 1975), and a 1:1:1:1 mixture of H_2O, liquid phenol, glacial acetic acid, and 0.2 M $BaCl_2$ (extract D) (Gallus and Jennings, 1968) (Figure 2).

The resulting supernatants were lyopholized, except the 70% EtOH extract, which had the EtOH distilled off under a vacuum and then was lyopholized.

Thirty milligrams of each of the lyopholized extracts was redissolved in 1 mL of buffer and placed on Sephadex columns. The eluted fractions were passed through a UV monitor at 280 nm to determine protein content. One-milliliter fractions were collected, analyzed by atomic absorption spectrometry for Zn content, and placed in a γ well counter to determine the distribution of ^{65}Zn in the various protein fractions.

The 0.04 M NaCl and 70% EtOH extracts were chromatographed on a G-75 column, 2.0 cm × 90.0 cm with a phosphate buffer, pH 7.0. The 0.1 N HOAc extracts were chromatographed on a G-50 column, 2.0 cm × 45.0 cm with an acetate buffer, pH 4.65, and the D extracts were chromatographed on a G-75 column, 2.0 cm × 90.0 cm with an acetate buffer, pH 4.65. Two portions of wheat from each treatment were extracted and each extract was eluted twice on the proper column. Thus, values reported are means of four runs for each extract.

All flow rates were 1 mL eluted per 4 min. After the void volume, 160 mL of eluted substances was collected for each run.

RESULTS AND DISCUSSION

The question may be raised as to the necessity of intrinsically labeling food for mineral bioavailability studies. Extrinsic labeling with iron is a common practice (Consul and Lee, 1983), and extrinsic labeling with zinc has also been employed on several occasions (Sandström et al., 1980; Sandström and Cederblad, 1980; Johnson, 1984). To date, only a few publications have reported comparisons of intrinsic and extrinsic labeling with Zn and its subsequent absorption by rats or humans. Neathery et al. (1975) found that intrinsic ^{65}Zn from corn forage or ryegrass was better absorbed than extrinsically added $^{65}ZnCl_2$. Evans and Johnson (1977) found a mean ratio of 1.06 ± 0.11 for the ratio of extrinsic ^{65}Zn/intrinsic ^{65}Zn absorption from corn and liver by rats. Flanagan et al. (1985) reported ratios of 1.06 ± 0.27 and 1.16 ± 0.33 for extrinsic/intrinsic ^{65}Zn absorption from turkey meat by humans. While the mean values for intrinsic and extrinsic Zn absorption were not significantly different, their observed extrinsic/intrinsic ratios ranged from 0.70 to 1.74. Using stable isotopes of Zn, Janghorbani et al. (1982) found that absorption of intrinsic Zn by humans was much greater than absorption of extrinsic Zn from chicken meat. Even though absorption of intrinsic and extrinsic Zn may be highly correlated (Janghorbani et al., 1982), they are not identical, and there are insufficient data to say that absorption of either intrinsic or extrinsic zinc is consistently greater. Even in the area of extrinsic iron tagging, problems remain. These include conditions under which complete exchange of intrinsic and extrinsic mineral may fail, and the effects of different foods and food preparation or processing on the exchange of the extrinsic tag and minerals intrinsic to the food (Consul and Lee, 1983). Similarly, the use of extrinsic tagging in zinc bioavailability research is an area in which problems remain to be studied. In the case of zinc, considerable evidence is needed to establish the validity of extrinsic tagging and the conditions necessary for its validity. Thus, it is important to develop methods of producing intrinsically labeled foods as efficiently as possible.

Since the object of intrinsically labeling edible plants with radioisotopes was to incorporate the greatest possible percentage of the isotope into the edible portion of the plant, we chose anthesis (flowering) as the best development stage for isotope application. This stage corresponded to stage 6 of Waldren and Flowerday's (1979) description of winter wheat growth stages. The anthers were emerging from the middle section of the inflorescence of the main culm. From flowering (stage 6) until anthesis is complete and grain filling begins usually requires 3 weeks. During anthesis and the resulting seed formation, vegetative growth in wheat has been found to stop and the majority of the plant's energy is used to develop grain (Noggle and Fritz, 1976). A definite translocation of dry matter from the leaves occurs during grain development (Waldren and Flowerday, 1979).

Table I. Total Zn Content of Whole Wheat Flour

treatment	Zn, $\mu g/g^a$
control	41.7 ± 2.2
citrate–phosphate buffer stem injected	28.9 ± 0.7
^{65}Zn stem injected	28.9 ± 0.7
^{65}Zn + $ZnSO_4$ stem injected	59.9 ± 1.2
^{65}Zn foliar	30.1 ± 1.1
^{65}Zn hydroponics	20.1 ± 1.7

[a] Mean ± S.D.; $N = 3$.

Generally, the capacity of salt accumulation decreases as the plant matures, but the translocation pattern within plants shows that at the time of seed development there is a general migration of materials from all parts of the plant toward the developing seed. Minerals deposited in the leaves may be withdrawn prior to abscission and translocated to reproductive areas of the plant (Noggle and Fritz, 1976). Therefore the majority of ^{65}Zn introduced into the wheat by foliar application, hydroponics, or stem injection would be transported to the forming and ripening grain.

The pH of xylem fluids has been shown to range from 5 to 6. At this pH the relatively low stability constant of zinc–citrate suggests extensive dissociation of Zn (Tiffin, 1972). The stability constant for the Zn–citrate complex $ZnHL^-$ (where citric acid is HL_3) is $10^{4.85}$ (Sillen and Martell, 1964). The conditional stability constant for formation of this complex at pH 5.5 can be calculated; log $K' = 1.25$. Thus a high proportion of the Zn in the buffer, about 6%, was in the free ionic state. Earlier work with tomatoes showed that zinc was transported predominantly as an inorganic cation in stem exudates (Tiffin, 1967). Weaver (1984) has stated that "until the mineral complexes which are transported in the vascular system are clearly defined, the technique (stem injection) may not result in deposition of isotopes which are characteristic of field grown crops". We purport that the medium used in stem injection was similar to the zinc complex transported in the xylem and in fact much of the zinc is transported as a free cation as would be found in the buffer system employed in this study.

In this experiment, the three stem-injection treatments had no deleterious effect on grain size, yield, and overall plant growth. Total zinc content of whole wheat flour was affected by the method employed in labeling (Table I). The values for the hydroponically grown and labeled wheat were lower (20.1 $\mu g/g$) compared to the remaining treatments. In contrast, the higher level of zinc in the ^{65}Zn + $ZnSO_4$ stem-injected plants (59.9 $\mu g/g$) was due to translocation to the seed of a portion of the Zn injected into the plant stem. Although these levels were higher than the minute levels of Zn as ^{65}Zn (1.5 ng) in the ^{65}Zn stem-injected wheat (28.9), they were less than twice the control values (41.7 $\mu g/g$), (Table I).

Incorporation of ^{65}Zn into the seed varied among the treatments (Table II). The ^{65}Zn stem injected and the foliar-applied ^{65}Zn were incorporated at comparable levels. The lower incorporation for the stem-injected ^{65}Zn + $ZnSO_4$ was probably due to competition between the ^{65}Zn and the Zn from the $ZnSO_4$ or to isotope dilution. This is corroborated by the greater Zn content of wheat flour

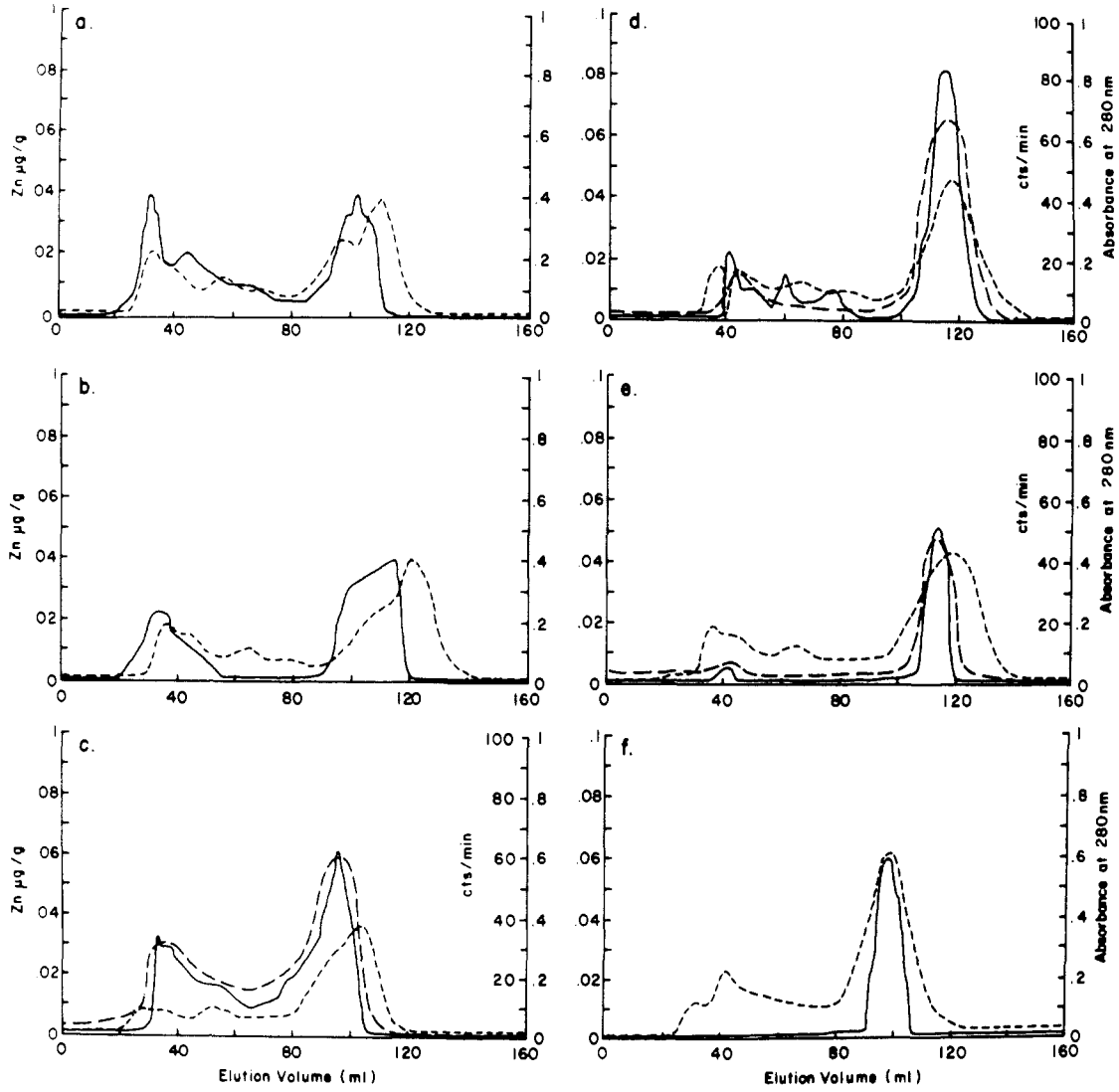

Figure 3. (a) Control. Elution curve and Zn content. Globulins and albumins extract. (—) Protein; (– –)^{65}Zn; (- -) Zn. (b) Citrate–phosphate buffer stem injected. Elution curve and Zn content. Globulins and albumins extract. (c) ^{65}Zn stem injected. Elution curve, Zn and ^{65}Zn content. Globulins and albumins extract. (d) ^{65}Zn + $ZnSO_4$ stem injected. Elution curve, Zn and ^{65}Zn content. Globulins and albumins extract. (e) ^{65}Zn foliar. Elution curve, Zn and ^{65}Zn content. Globulins and albumins extract. (f) ^{65}Zn hydroponics. Elution curve and Zn content. Globulins and albumins extract.

Table II. Percent Incorporation of ^{65}Zn into Wheat Plants[a]

treatment	whole grain	chaff	remaining plant
stem injection	62.62 ± 2.14	9.28 ± 0.27	28.10 ± 0.32
stem injection + $ZnSO_4$	45.20 ± 3.17	22.25 ± 1.01	32.55 ± 2.68
foliar	57.50 ± 3.24	10.92 ± 0.48	31.58 ± 1.97
hydroponic	2.30	<0.01	not determined

[a] Mean ± S.D.; N = 4.

from the ^{65}Zn + $ZnSO_4$ stem-injected plants (Table I). Garcia et al. (1977) published ^{65}Zn incorporation levels of over 27% for corn grown in a hydroponic system. Weaver (1984) reported a 25.6% incorporation of applied dose of ^{65}Zn and ^{75}Se in wheat grain grown hydroponically and in a similar study reported a 45% incorporation of ^{65}Zn into wheat grain grown hydroponically (Weaver, 1984).

The low incorporation values reported in this study for the hydroponically grown and labeled wheat were due to several factors (Table II). The time of exposure to the isotope was much less than normally used in hydroponic labeling (Weaver et al., 1984; Schmitt and Weaver, 1984; Hussain et al., 1965; Layrisse et al., 1968). The time from anthesis until the grain was ripened and dry was selected for the period of exposure to the isotope to coincide with the other treatments so that the techniques could be easily compared. In addition, low levels of ^{65}Zn (1 µCi/plant) were used in comparison to the concentrations used by other investigators (1.0 µCi–5 mCi) and due to competition with the Zn^{2+} in the growth medium a lesser amount of ^{65}Zn was taken up by the plants (Janghorbani et al., 1983; Garcia et al., 1977). In addition, roots tended to concentrate the $^{65}Zn^{2+}$ in the hydroponic treatment and this concentration concurred with the findings of Wallace and Romney (1977). Due to the low levels of ^{65}Zn in this treatment, hydroponically grown and intrinsically labeled wheat was obtained from C. M. Weaver and analyzed (Table III).

Autoradiographs of the various ^{65}Zn treatments demonstrated the transport of the isotope to various plant parts and confirmed the incorporation levels among the treatments (Table II).

The percent incorporation of ^{65}Zn into the various protein fractions of the whole wheat flour, including the wheat obtained from C. M. Weaver, was similar among treatments (Table III).

The lower levels of ^{65}Zn in the albumin and globulin fraction and the higher concentration in the butyl alcohol

Table III. Percent Incorporation of ^{65}Zn in Various Wheat Extracts

	fat extracts		protein extracts				
treatment	butyl alcohol	petroleum ether	albumins and globulins	glutenins	gliadins	remaining proteins	residue
^{65}Zn stem injected[a]	8.7	3.2	20.3	51.9	1.1	12.5	2.0
^{65}Zn stem injected + $ZnSO_4$[b]	17.2	7.3	8.5	49.1	1.5	14.7	1.5
^{65}Zn foliar[b]	3.0	5.2	16.9	60.3	1.9	9.8	2.7
^{65}Zn hydroponic[b]	c						
^{65}Zn hydroponic[d]	2.0	1.0	15.1	47.4	2.6	28.3	7.5

[a] Pool of 80 samples. [b] Pool of 40 samples. [c] ^{65}Zn in all samples of hydroponically labeled wheat was below detectable limits. [d] ^{65}Zn hydroponically grown wheat obtained from Dr. C. M. Weaver, Purdue University, Lafayette, IA (Weaver, 1984).

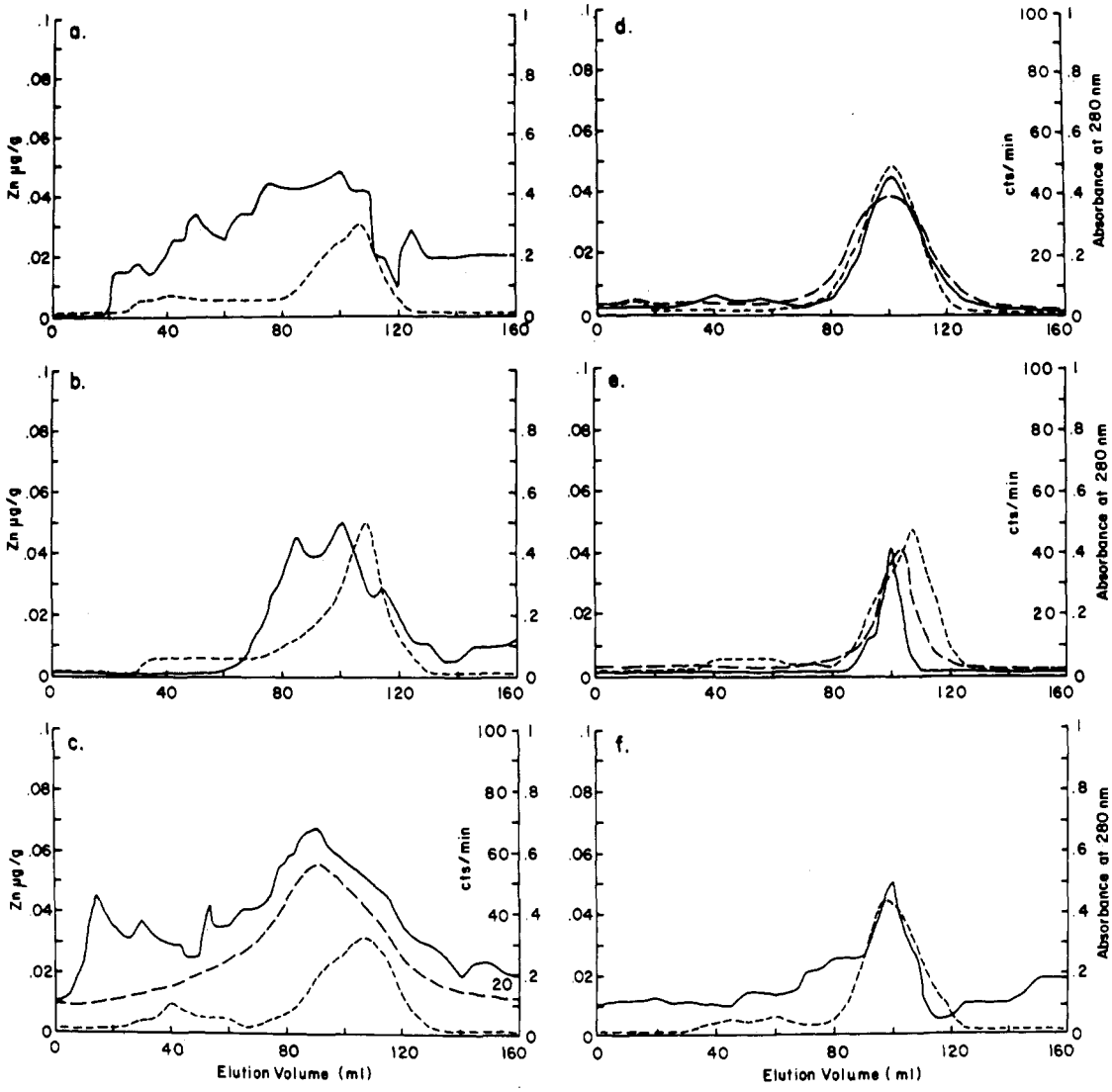

Figure 4. (a) Control. Elution curve and Zn content. Gliadin extract. (—) Protein; (- -) Zn; (– –)^{65}Zn. (b) Citrate–phosphate buffer stem injected. Elution curve and Zn content. Gliadin extract. (c) ^{65}Zn stem injected. Elution curve, Zn and ^{65}Zn content. Gliadin extract. (d) ^{65}Zn + $ZnSO_4$ stem injected. Elution curve, Zn and ^{65}Zn content. Gliadin extract. (e) ^{65}Zn foliar. Elution curve, Zn and ^{65}Zn content. Gliadin extract. (f) ^{65}Zn hydroponics. Elution curve and Zn content. Gliadin extract.

extraction in the ^{65}Zn + $ZnSO_4$ stem-injected treatment were probably due to competition between the ^{65}Zn and Zn from the $ZnSO_4$. Some of the Zn was bound to sites that in the ^{65}Zn stem injected and the ^{65}Zn foliar application were occupied by ^{65}Zn foliar application were occupied by ^{65}Zn. This would account for the lower levels in the albumin, globulin fraction and the higher level in the butyl alcohol extraction (Table III).

The majority of the ^{65}Zn was incorporated into the protein with the glutenin fraction having the greatest percentage of the isotope (Table III). Although gliadins normally account for 40–50% of the protein in wheat (Larkins, 1981) only a very small amount of the ^{65}Zn was incorporated into this fraction. Glutenins had the greatest level of ^{65}Zn incorporation. These proteins normally make up 30–40% of the total protein fraction in wheat (Larkins, 1981) (Table III).

After the fat and protein sequential extractions, less than 3% of the ^{65}Zn remained in the residue. This demonstrated that the majority of the ^{65}Zn, and therefore the Zn, was incorporated in the protein fractions. This incorporation was confirmed by chromatography and Zn determinations of eluted protein fractions and will be discussed later.

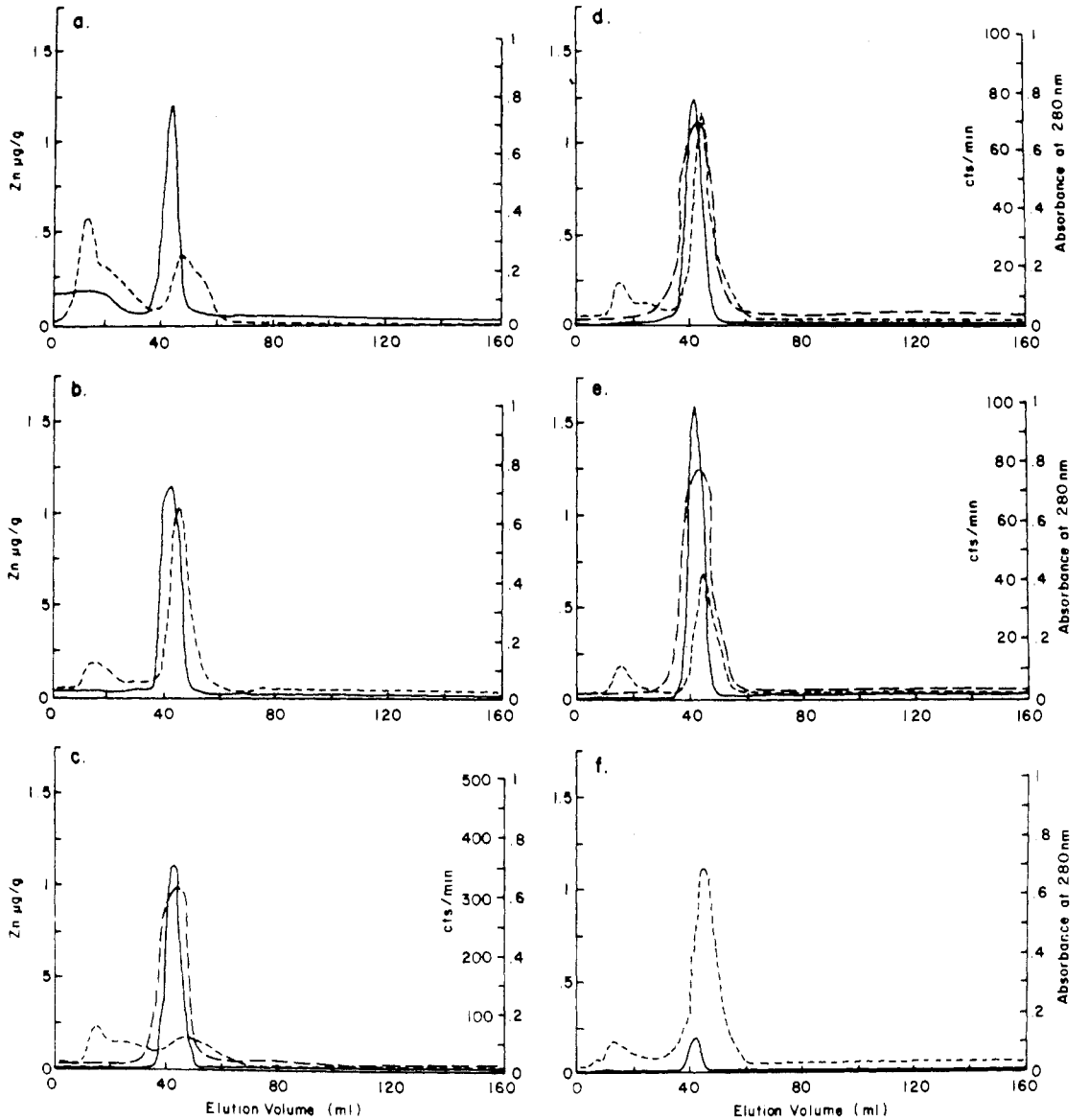

Figure 5. (a) Control. Elution curve and Zn content. Glutenin extract. (—) Protein; (- -) Zn; (--) ^{65}Zn. (b) Citrate–phosphate buffer stem injected. Elution curve and Zn content. Glutenin extract. (c) ^{65}Zn stem injected. Elution curve, Zn and ^{65}Zn content. Glutenin extract. (d) ^{65}Zn + $ZnSO_4$ stem injected. Elution curve, Zn and ^{65}Zn content. Glutenin extract. (e) ^{65}Zn foliar. Elution curve, Zn and ^{65}Zn content. Glutenin extract. (f) ^{65}Zn hydroponics. Elution curve and Zn content. Glutenin extract.

The high rate of incorporation via foliar application was expected as this method has been used successfully in agriculture to correct Zn and Fe deficiencies. In another study done at this laboratory stable ^{65}Cu was applied by a foliar method employing a misting of the leaves on plants grown outdoors. Stable ^{65}Cu content of the grain was increased substantially and the enrichment was high enough to be used in bioavailability studies (Lykken, 1984).

To determine if the site of ^{65}Zn incorporation was identical for various techniques of Zn administration, protein fractions were chromatographed on Sephadex and the eluted volumes analyzed for Zn content.

The chromatograms of the 0.04 M NaCl extracts containing the albumin and globulins showed no major differences due to the various treatments (Figure 3 parts a–f). The protein fractions were very similar, with the only differences found in the second peak. These differences were minimal with only the hydroponically grown wheat having a higher second peak, but the elution volume of this peak was the same as for the other treatments (Figure 3f).

The Zn content of the eluted fractions was similar and followed the protein, proving that the Zn in this fraction was associated with the protein. The higher levels of Zn in the ^{65}Zn + $ZnSO_4$ stem-injected treatment were explained earlier. Although the levels varied among the treatments, the albumin and globulin fraction contained a small amount of the total Zn in the wheat flour with a peak range of 0.04–0.08 µg/g of Zn of eluted fraction (Figure 3 parts a–f). The stem-injection technique did not alter the protein content or distribution or the Zn distribution as compared to the other treatments.

The chromatograms of the gliadin extracts were similar for all treatments (Figure 4 parts a–f). The Zn content did vary but the total amount of Zn in this fraction was minimal with the greatest value (^{65}Zn stem injected) at 0.07 µg/g. These variations in the Zn content were on the order of 0.01–0.03 µg/g. As with the 0.01 M NaCl extract, the Zn and ^{65}Zn coeluted with the protein.

The chromatograms of the glutenin extracts varied but only in the intensity of the second protein peak. The first protein peak was very similar among the treatments (Figure 5 parts a–f). Although the second peak varied, this variation was only in the intensity. The Zn content was similar for all treatments except the hydroponically grown wheat. The lower levels in the glutenin extract of the hydroponically grown wheat reflect the lower concen-

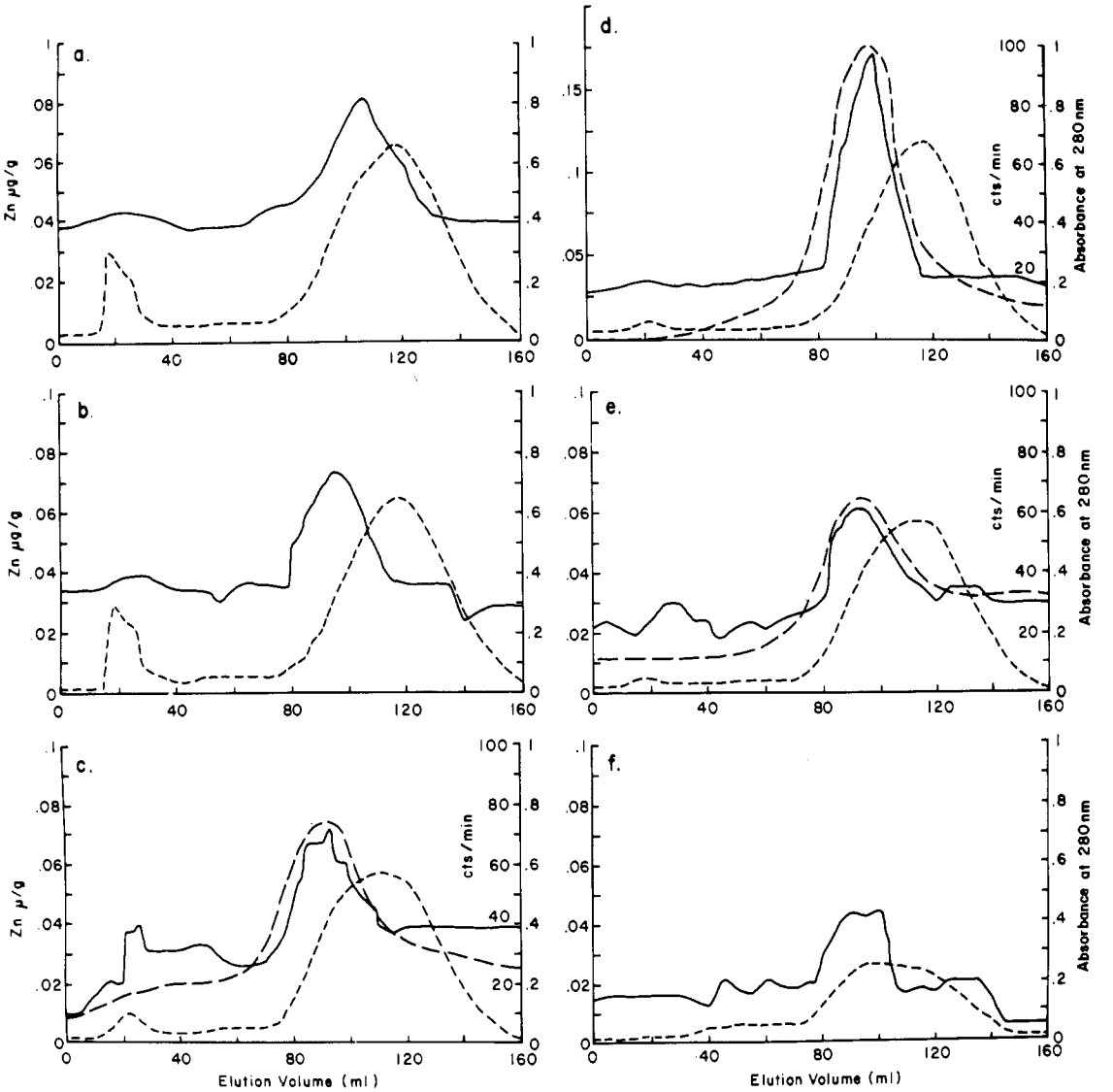

Figure 6. (a) Control. Elution curve and Zn content. Remaining proteins. (—) Protein; (- -) Zn; (– –) ^{65}Zn. (b) Citrate–phosphate buffer stem injected. Elution curve and Zn content. Remaining proteins. (c) ^{65}Zn stem injected. Elution curve, Zn and ^{65}Zn content. Remaining proteins. (d) $^{65}Zn + ZnSO_4$ stem injected. Elution curve, Zn and ^{65}Zn content. Remaining proteins. (e) ^{65}Zn foliar. Elution curve, Zn and ^{65}Zn content. Remaining proteins. (f) ^{65}Zn hydroponics. Elution curve and Zn content. Remaining proteins.

trations for the total Zn in the whole wheat flour (Table I, Figure 5f). The glutenin fraction, although the second most abundant protein in wheat (Larkins, 1981), contained the largest amount of Zn. The levels of Zn in the glutenin extract were at least one order of magnitude greater than in the other three extracts (Figure 5 parts a–f).

The protein chromatograms for the extract D (remaining proteins) were similar, with only the intensity of the first protein peak varying among the treatments. The lack of a first peak and the low intensity of the second peak for the hydroponically grown wheat were difficult to interpret, and we can offer no plausible explanation at this time. The levels of Zn and the elution volume in relation to the protein were similar for all treatments. The extract from $^{65}Zn + ZnSO_4$ treatment had greater levels of Zn, but this was expected (vide supra). Although Zn levels were higher in this fraction, they were still an order of magnitude lower than the Zn levels in the glutenin extracts (Figure 6 parts a–f).

The distribution of the ^{65}Zn for the radioisotopic treatments followed the same pattern as the Zn (Figures 3c,d,e, 4c,d,e, 5c,d,e, and 6c,d,e). Even though the correlation between ^{65}Zn and Zn was not perfect, deviations were small. This suggested that after foliar application or stem injections (levels in the hydroponically grown wheat were below detectable limits), the isotope did not randomly diffuse throughout the plant, but instead followed the normal physiological path for zinc at anthesis and was deposited in the wheat seed.

Using corn stem injected with ^{59}Fe-citrate Zeind (1967) found that the ^{59}Fe was distributed in a similar fashion to the ^{65}Zn in this study. Electrophoretic studies of kernel extracts showed that the stem-injected ^{59}Fe was metabolized into physiological ^{59}Fe-bound compounds in the corn kernels. These findings were confirmed by ultrafiltration, dialysis, and subcellular fractionation (Zeind, 1967).

The results of this experiment demonstrated that foliar application and stem injection are valid and reliable means of intrinsically labeling wheat. It is concluded that stem injection is superior to foliar application. Foliar application is limited in the number of isotopes that could be used. Several elements are not readily absorbed through the leaf surface. Among these are the trace elements boron, selenium, molybdenum, and manganese (Devlin, 1975). Foliar application of radioisotopes was more difficult than stem injection and the danger of contamination and isotope loss was greater due to possible runoff from the leaf. However, based on findings in this laboratory, the use of foliar ap-

plication of stable ^{65}Cu may be the method of choice (Lykken, 1984).

Stem injection of ^{65}Zn did not appear to alter the yield, grain size, total Zn content, protein type, or Zn distribution in the wheat flour as compared to the controls. Stem injection was easily performed, produced no measurable damage to the plant, had the highest rate of incorporation of ^{65}Zn into the edible plant part, and used significantly less isotope per plant than hydroponics. In addition, the danger of contamination or spillage was minimal.

Stem injection has been used successfully on other plant species. Recently, in our laboratory, soybeans [*Glycine max.* (L.) Merr. var. "Century"] were stem injected with ^{59}Fe by using the same injection medium described in this paper. Over 59% of the ^{59}Fe was incorporated into the seeds. This value is more than double of any of the reported values for intrinsically labeling with hydroponics (Starks and Lykken, 1984).

The major goal of intrinsically labeling edible plants is to label the edible portion of the plant with the greatest amount of the isotope without altering the natural state of the plant. Stem injection accomplishes this goal with a greater percent incorporation.

Registry No. ^{65}Zn, 13982-39-3.

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