to those of mature plants (ca. 2 m high). The use of different growing locations decreases the slight possibility that the compounds could be associated with the soil attached to the roots. The authors had also previously studied the volatiles of the soil in the general area where the corn was grown in Berkeley and had not detected any sesquiterpenes.

For the isolation the authors cut the roots to allow them to fit through the neck of the flask. This, of courses, causes damage to some cells and could possibly produce some volatiles (e.g. hexanal) not present in the intact plant. However, in the authors' previous experience with the roots of other plants (e.g., Kamm and Buttery, 1984) damage to root cells produces very little volatiles in contrast to damage to leaves or fruit where cell damage volatiles can be 100 times those present in the intact plant material.

Known Attractants. Ladd et al. (1983) had found that eugenol is an attractant for the adult northern corn root worm (*Diabrotica barberi* Smith and Laurence). Eugenol was actively searched for in the corn roots but could not be detected. It also had not been detected in other parts of the corn plant (cf. Buttery and Ling, 1984). It is interesting that one of the authors had recently identified methyleugenol in the roots of another major crop, red clover (Kamm and Buttery, 1984).

ACKNOWLEDGMENT

We thank Merle L. Weaver for corn root samples (Bonanza variety).

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Received for review February 11, 1985. Accepted May 6, 1985.

Growth and Intrinsic Labeling of Peanuts with ^{65}Cu for Use in Human Bioavailability Studies

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Peanuts (*Arachis hypogaea* L.) were grown under greenhouse conditions. After pegging was extensive, each plant was stem-injected with 1.5 mg of ^{65}Cu (a stable isotope). After harvesting, the enrichment was 91 atom % of ^{65}Cu. These peanuts will be used in a copper bioavailability study with human subjects.

INTRODUCTION

Copper, an indispensable trace metal in human metabolism, is widely distributed in foodstuffs. The richest food sources of copper are shellfish, nuts, organ meats, and legumes (Pennington and Calloway, 1973). A daily coppper intake of 2–3 mg is recommended for adults (National Academy of Sciences, 1980), but dietary surveys show that actual intake is sometimes much lower, even below 1 mg/day (Klevay, 1975). Thus, the availability of copper for absorption from food is an important factor in the nutritional adequacy of the diet. Information on the bioavailability of copper from foods is very limited. Recently Lo et al. (1984) demonstrated that copper was equally available to rats from isolated soy protein and copper carbonate. Absorption of copper as $CuCl_2$ from purified diets (Turnlund, 1984; Turnlund et al., 1982; King et al., 1978) and in the fasting state (Johnson, 1984) has been studied in humans, but the availability of copper to humans from foodstuffs is virtually unknown. Copper has only two short-lived radioisotopes, ^{64}Cu, $t_{1/2}$ = 12 h, and ^{67}Cu, $t_{1/2}$ = 61.9 h. Thus only the stable isotope, ^{65}Cu, is of use for studies of copper absorption by humans. In addition, the ethical considerations which apply to radioisotope use in humans are not a concern when stable copper is used.

Peanuts (*Arachis hypogaea* L.) are relatively high in copper (7–8 ppm) and could constitute a good source of dietary copper. The phytate in legumes inhibits absorption of some trace minerals (O'Dell and Savage, 1960; Atwal et al., 1980). Spanish peanuts contain 1.88% phytic acid (Graf and Dintzis, 1982), a fairly high concentration. However, a study using extrinsically labeled peanuts fed to rats showed fairly high (41%) Cu absorption (Johnson et al., 1985), compared to 46% absorption of $CuSO_4$.

It is of special interest to determine whether copper intrinsic to a foodstuff is absorbed in the same manner as copper added extrinsically to a meal as a salt. It is well-known that extrinsic and intrinsic nonheme iron form a common pool in the gastrointestinal tract and are absorbed in approximately equal amounts (Consul and Lee, 1983). Extrinsic labeling for Zn, Cd, and Mg also appears to be a valid procedure (Evans and Johnson, 1977; Janghorbani et al., 1982; Welch and House, 1980; Schwartz et al., 1981). However, extrinsic labeling is not a valid technique for all elements (e.g., Se) (Siewicki and Balthrop, 1983). The validity of this procedure must be tested for each mineral. For these reasons, a study was initiated to determine the feasibility of intrinsically labeling peanuts with a stable isotope of copper, ^{65}Cu.

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MATERIALS AND METHODS

"Pronto" variety Spanish peanuts (*Archis hypogaea* L.) were grown in a greenhouse with supplemental lighting provided by 400-W high-pressure sodium lamps (Energy Technics, York, PA) to produce a 12 h L:12 h D cycle. On May 10, 1984, peanut seeds were planted three per pot in 12-in. standard clay pots in sterilized clay loam soil containing 50% perlite and 5% peat moss. The seeds were treated with Captan fungicide (Chevron Chemical Co., San Francisco, CA) and inoculated with *Rhizobium* sp. In addition, 100 g of gypsum (calcium sulfate, 19.9%) were added to each pot. After germination, plants were thinned to one plant per pot. Plants were watered as needed with tap water and fertilized with a solution of Hydro-sol (5-11-26) (Peter's Fertilizer, W. R. Grace and Co., Fogelsville, PA) approximately every two weeks. Spider mites were controlled, as required, by spraying plants with Pentac Aquasol (Zoecon, Corp., Palo Alto, CA).

Flowering began during the fifth week of growth. Plants had reached the R3, reproductive stage (Boote, 1982) by the tenth week. During the twelfth week pegging was extensive and we decided to label the peanuts with ^{65}Cu. Each peanut plant was labeled with 1.5 mg of ^{65}Cu as $^{65}CuCl_2$ in a citrate–phosphate buffer, pH 5.5 (McIlvaine, 1921). The labeling process was done during the twelve, thirteenth, and fourteenth weeks of growth. Injections were performed as follows by using a 25-μL syringe. The needle was inserted into a stem of the plant near the "pegs". Care was taken to puncture the stem until the needle entered only the xylem. The first hole was made to reduce the turgor pressure. The needle was then removed and the syringe filled with 25-μL of the injection solution (citrate–phosphate buffer plus $^{65}CuCl_2$). A second hole was made approximately 2 cm from the first hole, and the solution was slowly injected into the xylem. The process was repeated on different stems and the main stem until 100 μL of the solution had been injected into each plant. Stem injection of the peanuts was easily done due to the soft tissue in the stems. This is in contrast to stem injecting soy plants, in which we have encountered difficulty because of the hard stem tissue and limited amount of liquid (20 μL) that can be injected.

Peanut pod maturity was checked weekly by the method of Williams and Dexler (1981).

RESULTS AND DISCUSSION

After 27 weeks of growth, the peanuts were harvested and the yield for each plant determined ($\bar{X}$ = 72.2 ± 24.5 g/plant). The peanuts were shelled and the shelled weight determined. The shelled yield for 33 plants was 1524 g or 46.2 g/plant.

The peanuts were pooled, dry roasted, and random samples taken to determine the percent enrichment of ^{65}Cu and the total copper content. The total copper content was determined on three samples by using standard atomic absorption spectrophotometric methods after ashing with nitric acid and hydrogen peroxide (Bock, 1979). The enriched peanuts contained 7.6 ± 0.1 ppm of copper. The normal copper content of fresh peanuts is 6.2 ppm and peanut butter is 5.7 ppm (Pennington and Calloway, 1973).

Another aliquot of the dry roasted peanuts was used for determining the percent enrichment of ^{65}Cu by mass spectrometry (Johnson, 1982). The enrichment was 91 atom % of ^{65}Cu. The natural abundance of ^{65}Cu is 30.91 atom %. Human copper absorption studies require the feeding of 1–3 mg ^{65}Cu in excess of its natural abundance in food (Johnson, 1984, Turnlund, 1984). Three hundred grams (11 oz) of the ^{65}Cu-labeled peanuts described herein would provide 1.40 mg of ^{65}Cu in excess of the natural abundance (2.07 mg of total ^{65}Cu, 2.28 mg of total Cu).

Labeling of wheat with ^{65}Cu by the stem injection method performed in this laboratory resulted in an increase of copper content from 8 ppm to 22 ppm, but did not alter the protein content or profile of the glutenin and gliadin fractions as determined by column chromatography (Starks and Johnson, 1985). A low molecular weight protein (M_r 12 000–13 000 in the globulin and albumin fraction was found in a higher concentration. Tukendorf et al. (1984) found that in the presence of excess copper, spinach formed copper complexes due to copper binding by preexisting proteins and their synthesis was stimulated by the excess copper. These results suggest that the protein content of the stem injected peanuts would be similar to "normal" peanuts and the bioavailability of copper would be unaffected by the labeling method. Stem injection is a simple, effective method of intrinsically labeling peanut plants without significantly altering the total copper content of the peanut.

Registry No. ^{65}Cu, 14119-06-3.

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Received for review Janurary 22, 1985. Accepted May 20, 1985. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.