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INTRINSIC RADIOLABELING OF NUTRIENTS FOR HUMAN NUTRITION STUDIES USING ACCELERATOR MASS SPECTROMETRY

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¹⁴C labeling of plant nutrients coupled with detection of label by Accelerator Mass Spectrometry (AMS) represents an attractive means for investigating the metabolism of nutrients that are an intrinsic part of a food or diet [1,2]. AMS quantitates attomolar concentrations of ¹⁴C-labeled substrates from biologic tissues and fluids at ethically acceptable levels of radiation exposure, eliminating the need for compounds of high isotopic concentration. Accordingly, the generic photosynthetic labeling of plants with ¹⁴CO₂ can be used to provide an inventory of difficult to synthesize phytochemicals that can be isolated and tested in their natural form. To assess the intrinsic bioavailability of nutrients as they are consumed within the diet requires labeling strategies that deliver specific, committed precursors in a biochemical pathway. These precursors can be delivered by hydroponic culture or through their leaves and stems by surface application or injection into the xylem flow. We have used some of these labeling strategies with spinach and kale, two commonly consumed leafy vegetables, to produce ¹⁴C-labeled caroteneoids and folates, respectively.

PHOTOSYNTHETIC RADIOLABELING (BETA-CAROTENE AND LUTEIN): The carotenoid (plant pigments that serve as vitamin A precursors and biological antioxidants) content of spinach (*Spinacia oleracea*) is distributed between β-carotene and lutein. ¹⁴C-β-carotene and ¹⁴C-lutein was prepared by growing spinach in an atmospherically sealed labeling chamber (LC) pulsed with ¹⁴CO₂. (Fig 1). Photosynthetic labeling using ¹⁴CO₂ results in randomly labeled biomolecules and other applications have been described [3,4,5]. Several plants were grown on a 12-h light cycle under metal halide lights supplemented with tungsten illumination in a plant growth box. At 30 days, they were placed in the

LC. Plants were allowed to equilibrate for one day prior to the administration of $^{14}\text{CO}_2$. A total of 50 mCi of $^{14}\text{CO}_2$ that was provided at the rate of 10 mCi/d for 5 days. Exposure was initiated on the first day by adding 10 mCi as a solution of $^{14}\text{C-NaHCO}_3$ to 18N H_2SO_4 over a 4 hr period releasing $^{14}\text{CO}_2$. This level of exposure was repeated for the following 4 days. Following the final exposure, the plants were maintained for 72 h in LC prior to harvesting. The aerial parts of the plant were harvested and were extracted for carotenes. Final radiometric and chemical purity was determined to be greater than 98% by analytical RP-HPLC. The specific activities were 1.45 and 0.35 mCi/mmol for lutein and β -carotene, corresponding to 0.023 and 0.0056 ^{14}C atoms per molecule, respectively. One subject has been exposed to a 200 nCi (306 ug, 444,000 dpm) dose of purified β -carotene (see Dueker et al. In this compendium). Other compounds of nutritional interest are also being purified for human testing.

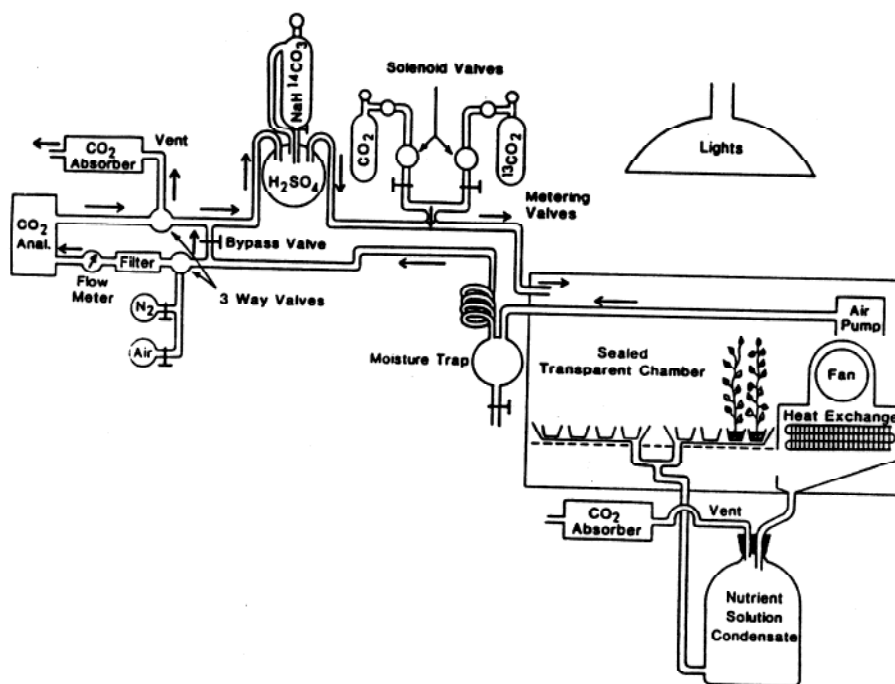


Fig 1. Illustration of Plant Labeling Box (3)

COMMITTED PRECURSORS BY COTTON-WICK METHOD (FOLIC ACID):

Folic acid (folate) is vitamin needed for one-carbon transfer reactions [6,7]. Kale is a rich source of folic acid but its bioavailability from plant sources is an unsettled issue and this has led to its use as a supplement. To assess the intrinsic bioavailability of folates a kale plant (*Brassica oleracea acephala*) was grown in an open plant box as described above. At 52-d post germination the plant was administered para-aminobenzoic acid (pABA)(see fig. 2) by the cotton-wick method which entailed the insertion of a cotton thread through the stock of the plant below the first set of leaves, with both ends being inserted into a vial containing ^{14}C -pABA (40 μCi ; 58mCi/mmol); labeling began at 11:30 AM and 500 μL of material was taken up completely after 6 hours, at which time the vial was washed with 800 μL of water to remove residual material in the vessel and the wick. The plant was then allowed to grow for one week. Aerial parts of the plant were harvested and the bulk of the radioactivity was found to be distributed within the fastest growing shoots and the tips of the larger leaves, verifying that the dose translocation from the site of administrations through the plant vascular system. A 2-gr sample was ground by mortar and pestle in ice-cold HPLC mobile phase, filtered and a small aliquot (containing ~ 10 DPM of activity) was injected onto an RP-HPLC system characterized for folate separation. Fractions were analyzed for ^{14}C by AMS. The metabolite profile showed that less than $\sim 5\%$ of the label was associated with known folates. 95% of the activity eluted in the solvent front portion of the chromatogram, which corresponded to either free pABA or polar metabolites. Given the low incorporation of label into folate, the material was not considered appropriate for human experimentation. Further studies are underway to optimize this approach.

CONCLUSION: Photosynthetic labeling of plants with $^{14}\text{CO}_2$ yields a wide inventory of low specific activity biomolecules that, given appropriate extraction methods can be purified for use in human studies using AMS. Because only a fraction of the radioactivity is associated with the target molecule(s), the plant cannot be consumed whole to assess the intrinsic bioavailability of the target compounds. Assessment of nutrient bioavailability from intrinsically labeled plants requires the use of committed biosynthetic precursors. This method demands considerable knowledge of plant biochemistry, and experimentation with a variety of labeling techniques and growth conditions to achieve useful results. It is

expected that with further experimentation, intrinsic labeling of nutrients by this method with subsequent AMS detection will facilitate investigations into the role of plant matrix on nutrient bioavailability.

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