Chelating Agents and Plant Nutrition*

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A NUMBER of aminopolycarboxylic acids that form water-soluble metal chelates have come into use for correction of iron-deficiency chlorosis under field conditions. The best known of these materials is the iron salt of ethylenediaminetetraacetic acid (Fe-EDTA). It forms 5-atom, strain-free chelate rings, and there are 6 atoms, two of nitrogen and four of oxygen, that can donate electrons to metals. EDTA can grip an atom of iron with five chelate rings. Martell and Calvin (*1*) have recently published an extensive account of the chemistry of the metal chelate compounds.

Citric, malic, lactic, and tartaric acids and certain amino acids are naturally occurring chelating agents (*1*), but they are not as powerful as EDTA. If the root hairs of plants excrete chelating agents (*2*), this accounts for the way in which iron is made available from such slightly soluble compounds as ferric oxide or calcium from calcium carbonate. Chelating materials present in humus also aid in keeping iron available to plants (*3*). Therefore, many of these chelating materials (*3–5*), as well as the metaphosphates (*6*), have been used to keep iron available to plants under conditions of high phosphate, high *p*H, and under highly aerobic conditions in solution and in sand cultures.

Living organisms contain a number of chelate compounds that possess catalytic activity among which are hemoglobin, chlorophyll, the cytochromes, and the enzymes catalase, peroxidase, cytochrome oxidase, polyphenol oxidase, and ascorbic acid oxidase (*1*).

In selecting a chelating agent for use in plant nutrition, a number of factors should be taken into consideration. The most important of these are the stability constant of the metal chelate and the *p*H of the medium in which it is to be used. The higher the stability constant, the less tendency there is for the complex to dissociate and yield metal ions. The stability constant for the Fe^{+++} complex of EDTA is greater than that of EDTA chelates of other metal elements known to be essential in the nutrition of higher plants (*1*), and this holds true over a fairly wide *p*H range. The stability constant of Fe^{+++}-EDTA is followed in the order of decreasing values by those of the divalent cations Cu^{++}, Zn^{++}, Fe^{++}, Mn^{++}, Ca^{++}, and Mg^{++}. Therefore, it has been our experience (*7*) that soil applications of Mn-EDTA to manganese-deficient plants may result in a replacement of manganese in the chelate by iron in the soil.

Investigations by Jacobson (*8*) indicated that Fe-EDTA was a satisfactory nutrient source of iron for plants growing in solution culture. Work in our laboratory (*9*) has shown that Fe-EDTA is an excellent source of iron for sunflower plants growing in solution cultures adjusted to *p*H 7.0, a *p*H at which iron as ferrous sulfate is not readily utilized.

Because of the strong affinity of EDTA for iron, field applications of Fe-EDTA have been made primarily for the purpose of curing iron-deficiency chlorosis in acid soils. Stewart and Leonard (*10*) made some of the first applications for the control of iron-deficiency chlorosis of citrus. Successful applications have also been made to avocado (*11, 12*), azalea, gardenia, roses, corn, sweet gum, hydrangea (*11*), inkberry, pin oak, pieris, blueberry (*13*), banana (*14*), and many other plants. Use of Fe-EDTA on neutral or alkaline soils has met with little success, and new chelating agents, such as diethylenetriaminepentaacetic acid (DTPA), N-hydroxyethylethylenediaminetriacetic acid (HEEDTA), and N, N′-dihydroxyethylethylenediaminediacetic acid (HEEDDA), are being tested (*12, 15*).

Iron-deficiency chlorosis induced by accumulations of heavy metals is a serious problem in many parts of the world. Many soils of Florida are naturally low in iron, and the accumulation of heavy metals, especially of copper, is believed by Reuther and Smith (*16, 17*) to be the primary cause of iron chlorosis in citrus growing on certain acid soils. Westgate (*18*) suggests that a similar condition exists in old celery fields that have received heavy applications of copper sprays. He has successfully treated a copper-induced iron chlorosis in a variety of vegetable crops growing in these soils by applying physical mixes of $FeSO_4$ and Na_3EDTA. Smith and Specht (*19, 20*) obtained favorable responses from chelated iron when it was applied to citrus seedlings growing in solution cultures containing high concentrations of manganese, copper, and zinc. Solution-culture experiments in our laboratory (*9*) with sunflower plants have indicated that addition of 0.5 ppm of iron as Fe-EDTA to culture solutions containing 10.0 ppm of manganese as $MnSO_4$ resulted in excellent plant growth. Plants supplied with 0.5 ppm of iron as $FeSO_4$ and 10.0 ppm of manganese as $MnSO_4$ exhibited severe symptoms of iron-deficiency chlorosis (Fig. 1).

Because Fe-EDTA is very effective in correcting or preventing iron-deficiency chlorosis, the question naturally arises whether the chelator keeps the iron soluble

* Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Departments of Plant Physiology and Soils, New Brunswick. Paper was presented at symposium cosponsored by sections C, G, and O at AAAS meeting, Boston, Mass., 30 Dec. 1953.

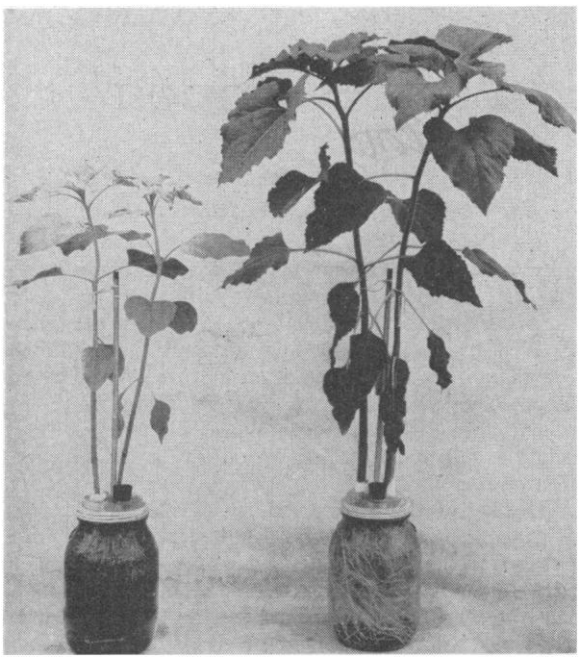

Fig. 1. Sunflower plants growing in culture solutions supplied with 10.0 ppm of manganese as $MnSO_4$ and 0.5 ppm of iron as $FeSO_4$ (left) or Fe-EDTA (right).

until it can be absorbed by the plants, or whether the chelate actually enters the plant carrying the iron along with it to the leaves. Wallace and North (21) have concluded that EDTA is absorbed by plants by using Fe-EDTA that contained isotopically labeled nitrogen. Their conclusion that this complex was metabolized by plants was drawn from data obtained by analyses for isotopic nitrogen in various soluble and insoluble nitrogen fractions of corn leaf blade tissues.

In order to gain further information concerning the absorption and translocation of EDTA, sunflower plants have been grown in solution cultures with the divided or split-root system technique (*22*). The same basic nutrient solution adjusted to *p*H 7.0 was used in all treatments with the exception of $FeSO_4$ or EDTA as indicated. One-half of the root system of one treatment (Fig. 2) was grown with $FeSO_4$, whereas the other half of the root system was grown with Na_2EDTA. The roots with EDTA but with no iron developed mild symptoms of iron deficiency. However, the roots with $FeSO_4$ and without EDTA were white and well developed, and the leaves of these plants were green. For comparison, in another split-root culture, one-half of the root system received $FeSO_4$, whereas the other half received neither $FeSO_4$ nor Na_2EDTA. The roots in each half of this split-root culture were light brown, and the leaves were yellow. Both conditions are characteristic of iron deficiency in this species. The total fresh weight of plants in these cultures amounts to only 15 percent of the weight of plants from cultures supplied with EDTA.

It appears from this experiment that EDTA is absorbed by the roots of sunflowers and that iron is, in some way, made available to the plants. At *p*H 7.0, iron from $FeSO_4$ is absorbed, but after it enters the plant it apparently is not readily available for incorporation into iron enzymes. When EDTA was supplied through one portion of the root system, it apparently migrated to another part of the root system and to the top, chelating the iron and keeping it in a form available for metabolic use throughout the plant. Whether the iron chelate is transported without change to the leaves or whether the iron moves with some metabolized product of EDTA is not evident. It is possible that some of the iron may be released for enzyme use only after EDTA has been partially metabolized. Because of the high stability constant of Fe-EDTA, it would not be expected that this compound would readily give up its iron while still unchanged.

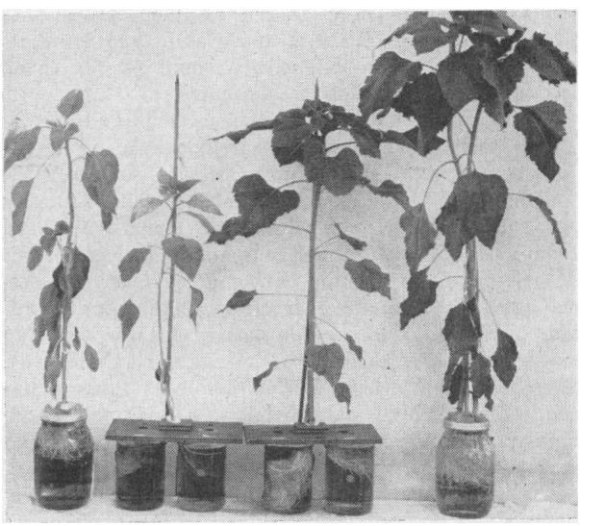

Fig. 2. Sunflower plants grown in split-root and complete-root solution cultures at *p*H 7.0 (Left) Supplied with 0.5 ppm of iron, no Na_2EDTA. (Left center) Left compartment supplied with 0.5 ppm of iron, right compartment with no iron and no Na_2EDTA. (Right center) Left compartment supplied with 0.5 ppm of iron, right compartment with 5.0 ppm of Na_2EDTA. (Right) Supplied with 0.5 ppm of iron and 5.0 ppm of Na_2EDTA.

Reports from a number of investigators have indicated that EDTA and its metal salts are toxic to plants in high concentrations. The physiology of this toxic effect has not been carefully studied. However, certain conclusions can be drawn from results of enzyme studies. Gross (*23*) has noted a stimulation of adenosine triphosphatase from mouse heart by use of low concentrations of EDTA, but a significant inhibition of activity resulted at higher concentrations. Swanson (*24*) reported a similar response with Mg^{++}-activated pyrophosphatase. Inhibition is presumably due to removal of enzyme-activating ions by EDTA. *In vitro* studies (*25*) at this station have shown a slight inhibition of polyphenol oxidase activity of sunflower leaf tissue but no inhibition of cytochrome oxidase and catalase. However, in studies with growing

plants, it was found that, with a nutrient level of 200 ppm of Na_2EDTA supplied to plants in nutrient solutions, the respiration rate of sunflower leaf tissue was only about 60 percent, the catalase activity was about 30 percent, and total fresh weight yield was about 54 percent of the respective values of leaf tissues from plants supplied with 5 ppm of Na_2EDTA. These results suggest that there is a competition between EDTA and the enzymes in the plant for metals essential for enzyme activity.

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Enzymatic Transfer of Alpha-Amino Groups*

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THE first step in the metabolism of many amino acids in animals, plants, and microorganisms is separation of the α-amino group from the amino acid carbon chain, resulting in the formation of an α-keto acid. Reversal of this reaction—that is, conversion of a keto acid to an amino acid—is frequently the final step in the biosynthesis of an amino acid.

With the demonstration of enzymatic transamination by Braunstein and Kritsmann (*1*), it became apparent that interconversion between certain amino and keto acids took place by an intermolecular transfer of an amino group from an amino acid to a keto acid by a mechanism not involving the intermediate participation of ammonia. Although the enzymatic transamination reaction was first described in 1937, it has been only recently that the extent and significance of amino group transfer reactions in the anabolism and catabolism of the amino acids has been recognized.

Several years ago, it was generally believed that three amino acids (alanine, aspartic acid, and glutamic acid) were the major, if not the sole participants in transamination (*2, 3*). However, the rapid incorporation of administered amino acid nitrogen into almost all the amino acids in animals (*4*) and the ability of the α-keto analogs of certain essential amino acids to substitute for these in supporting the growth of animals and microorganisms made highly probable the occurrence of reactions involving the exchange of amino groups of many other amino acids.

The major changes that have taken place in our understanding of the role of transamination in the biosythesis and degradation of the amino acids have been due to large measure to the development of new techniques of amino acid and keto acid preparation, identification, and quantitation. It now appears that virtually all the naturally occurring amino acids participate in transamination reactions, and that these reactions are catalyzed by a number of separate transaminase enzyme systems. Finally, as a result of recent work, the function of two phosphorylated derivatives of vitamin B_6, pyridoxal phosphate and pyridoxamine phosphate, as coenzymes for transaminase has become more firmly established.

This article considers some of the results obtained in the course of studies on transamination carried out in our laboratory (*5*). It was recognized at the start of these investigations that a comprehensive study would require pure preparations of the optical isomers and α-keto analogs of a wide variety of amino acids. The enzymatic methods of resolution of racemic amino acids developed by Greenstein and collaborators (*6*) have made possible the preparation of the D- and L-isomers of all the naturally occurring (and a number of other) amino acids in good yield and in a state of high optical purity (*7*). I was fortunate in being associated with some of these studies and to have been the recipient of generous amounts of these optically pure amino acids, which were essential for the present

* Paul-Lewis award address in enzyme chemistry, presented before the 125th national meeting of the American Chemical Society, 26 Mar. 1954, Kansas City, Mo.