SYMPOSIUM ON CHELATION—MECHANISM AND RELATION TO NUTRITION

CHELATION IN NUTRITION

Chelation As a Basic Biological Mechanism

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The reaction of metal ions and organic ligands provides a buffer system for concentration of metal ion in the medium. Modification of the ligand by intermediary metabolism or metabolic alterations in pH may selectively vary the metal ion concentration. Application of these principles to the problems of iron absorption, cobalt metabolism, and the growth of microorganisms is described.

METAL CHELATES are defined as cyclic structures of metal atom and organic ligand formed by donation of electrons from the ligand donor atoms, usually oxygen, nitrogen, or sulfur, to the electron acceptor metal ion (*19*). The chemical bond formed in this manner may vary in characteristics from the covalent to the almost completely ionic type. The typically five- or six-membered ring structure of the chelate may be a labile one with the metal ion in a loose and tenuous equilibrium with the organic ligand or may be of the rigid geometry and tight metal-binding characteristic of the metal porphyrins. Structural variations and the subtle integration of the electron donor characteristics of the ligand atoms with the orbital electron acceptor properties of the recipient metal ions make possible a high order of selectivity in the combination of the two components of metal chelate formation. As a result of these interactions, the organism has available the means to select and accumulate specific biologically required metal ions from the environment. In addition to the selection processes for metal ions available through chelation, certain aspects of the chemistry of metal chelates are especially noteworthy in the applicability of these materials to problems involved in the life process. These characteristics of chelation chemistry pertinent to life processes are implicit in the generalized formulation by Chaberek and Martell (*4*) of metal chelate equilibria (equation 1).

$$pM = \log K_{MA} + \log \frac{A^{-m}}{MA^{n-m}} \qquad (1)$$

where pM = the negative log of the free metal ion concentration in the system

K_{MA} = stability constant of the metal chelate

A^{-m} = concentration of the chelating ligand

MA^{n-m} = concentration of the metal chelate

In form, equation 1 is similar to the familiar Henderson-Hasselbach equation. In the case of chelate metal ion buffers, the pM value is set by the log K_{MA}, and the arithmetic value log of the ratio of the concentrations of the metal chelate and ligand.

In response to shifts in the metal ion concentration of the medium, systems of this type will tend to maintain constancy of metal ion concentration by either dissociation or formation of metal chelate. The chelate buffer effect, as will be evident in subsequent discussion, provides a valuable means of maintaining a reservoir of available metal ions at a nontoxic concentration level.

Effects of Ligand Concentration and pH Changes

Two endogenous means could be utilized by the organism to control metal ion levels set by chelate buffers. The metabolic removal or production of the chelating ligand will alter the value of the last term of equation 1 and thus modify the metal ion levels in the system. In this manner, the free metal ion level in vivo may be responsive to the controlling factors of intermediary metabolism. In addition to changing ligand concentration by metabolic processes, the organism may alter free metal ion levels by replacement of one ligand by another. By this process, areas of selective ion accumulation could be anticipated in the heterogeneity of the compartmented systems of multicellular forms.

Since the free ligand in the last term of equation 1 is a compound able to combine with hydrogen ion to form the undissociated acid form of the ligand, HA, it is also clear that local shifts in pH will markedly influence the concentration of A^{-m} and the value of the log term. The ability of the animal to

Table I. Distribution of Oral Iron 48 Hours after Administration to Normal and Anemic Rats

	$FeCl_3$	Fe EDTA	Fe DTPA
NORMAL RATS			
Number of animals	12	4	4
	Average % of Iron Recovered		
Feces	61.5	52.0	43.7
Gastrointestinal tract	22.2	30.5	44.1
Blood	4.5	4.1	2.7
Total tissues	5.4	3.2	2.2
Urine	0.5	7.5	6.1
Total non-absorbed (feces & GI tract)	83.7	82.5	87.8
ANEMIC RATS			
Number of animals	11	4	10
	Average % of Iron Recovered		
Feces	12.6	39.9	24.9
Gastrointestinal tract	54.7	25.0	42.3
Blood	27.1	14.1	13.4
Total tissues	2.0	4.8	6.1
Urine	0.6	6.5	2.0
Total non-absorbed (feces & GI tract)	67.3	64.9	67.2

Table II. Urinary Iron Excretion in Normal Rabbits after Oral Iron Administration

Compound	Number of Animals	Average Percentage in Urine
Fe EDTA	4	8.5
Fe HOEDTA	7	6.8
Fe NTA	2	0
Fe di-(betahydroxy-ethyl)glycine	3	0

exert a very precise control over hydrogen ion production, in turn, can become a sharp tool to control free metal ion levels in chelate buffers. Removal of A^{-m} by combination with hydrogen ion to form HA will result in further dissociation of MA to yield free metal ion and A^{-m}. The net result of acidification will be an increase in free metal ion concentration.

Specific examples of the above considerations may be illustrated by the application of the widely used synthetic chelating agent, ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), and betahydroxyethylethylenediaminetriacetic acid (HOEDTA), to some problems of mineral nutrition of animals and microorganisms.

Chelation and Iron Metabolism

Iron deficiency anemia as a nutritional problem develops in some species at periods when a low dietary intake is unable to meet increased physiologic demand for the metal. The human infant from the third to ninth month and the human female starting with the second trimester of pregnancy are prone to develop anemia on a marginal dietary iron intake. The suckling pig is another example of an animal whose normally rapid growth rate may be limited by the dietary availability of iron. In this paper, the authors will report on the effect of EDTA chelation on the metabolism of oral iron in normal and anemic rats and rabbits.

Materials and Methods. Rats used in this study were of the Osborne-Mendel strain. They were housed under metabolic conditions in all glass-lined cages (Figure 1). For the development of iron deficiency anemia, the animals were maintained on a diet of powdered milk after weaning. Hematocrit and hemoglobin levels were measured at weekly intervals. The experimental group of anemic animals were drawn from those whose hemoglobin levels had fallen to at least 6.5 grams per 100 ml. For study of tissue distribution of administered radioactive iron, animals were anesthetized by intraperitoneal injection of 5 mg. of Nembutal. The heart was exposed by midsagittal incision and injected with 0.2 ml. of heparin containing 1000 units. After 5 minutes, as much blood as possible was removed from the heart and the animal then perfused with physiological salt solution containing 0.4% sodium citrate.

After introduction of about 1 liter of fluid, the perfusion was usually complete as indicated by the tissue blanching. Following complete dissection, the tissues and excreta were wet ashed with nitric and sulfuric acids and brought to volume quantitatively. The iron-59 was counted in a well-type scintillation counter. The recorded data are drawn only from experiments in which the total recovery of radioactive iron was in the range of 90 to 100% of the administered dosage.

The total dosage of oral iron in these studies was standardized at 6.0 mg. per kg. Ferric EDTA was prepared by mixing stoichiometric quantities of ferric chloride and EDTA disodium salt in water. Ferric DTPA and ferric HOEDTA were prepared by dissolving stoichiometric quantities of the free acids in sodium carbonate and mixing with ferric chloride. A tracer amount of radioactive ferric-59 chloride was added (approximately 0.25 μc. per ml.). The pH was adjusted to about 6.5 by addition of sodium carbonate solution and made up to volume. Aliquots of the stock solution were used for counting standards or for administration to the animals.

Normal adult albino rabbits between 1.2 and 2.3 kg. were utilized to study oral absorption of various iron chelates. Rabbits were individually housed in metabolic cages prepared for the purpose by a coat of spar varnish and a paraffin glaze. This preparation of the cages prevented iron contamination of metabolic collections. The animals were maintained under metabolic conditions on a daily diet of 400 grams of raw cabbage with an iron content, by analysis, of 1.89 mg. The iron analysis on ashed samples was carried out by the method of Dean and Lady (*9*) using the 720 mμ absorption of the green complex between ferrous iron and nitroso-R salt. Animals used in the study were preconditioned for 1 week on the cabbage diet and were then transferred to the metabolic cages. After an additional 3-day control period, the animals were intubated with 10 to 20 mg. per kg. of iron chelate under study.

Results. Distribution of Oral Iron in Normal and Anemic Rats. In normal rats, the oral administration of 6 mg. per kg. of iron varying in form from the highly dissociated ferric chloride to the tightly chelated ferric diethylenetriaminepentaacetic acid shows a characteristic pattern of uptake and tissue distribution (Table I). In these normal animals, the absorption of oral iron is low regardless of the nature of the iron compounds. The slightly enhanced iron absorption of the chelated forms is more than compensated by their increased urinary iron output. The net result is that lesser quantities of oral chelated

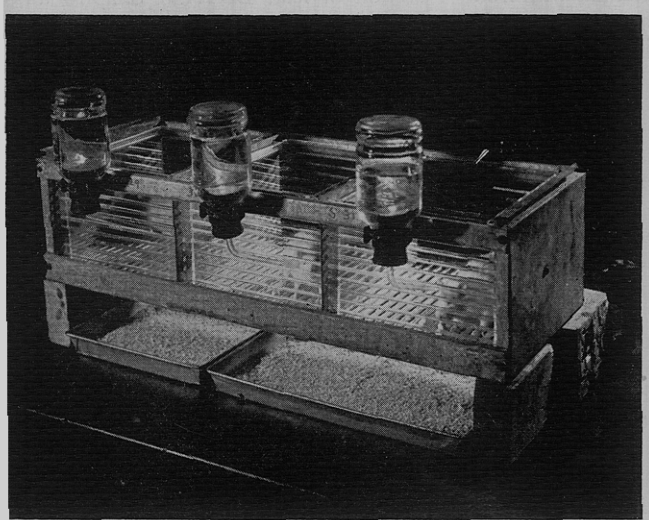

Figure 1. Glass-lined cages used in iron metabolism studies

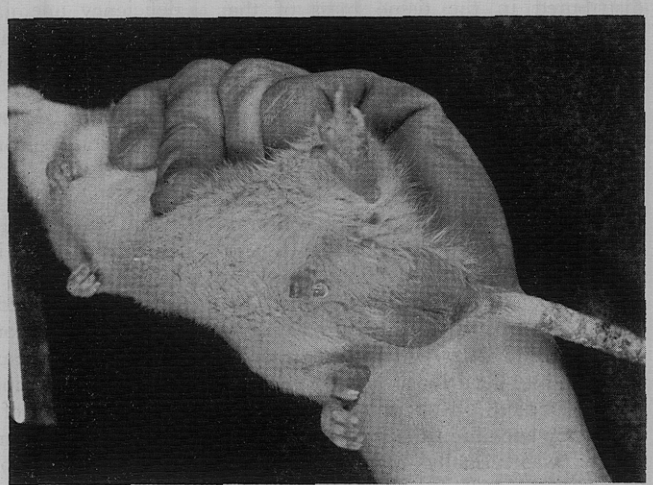

Figure 2. Testicular inflammation and enlargement in iron deficiency anemia

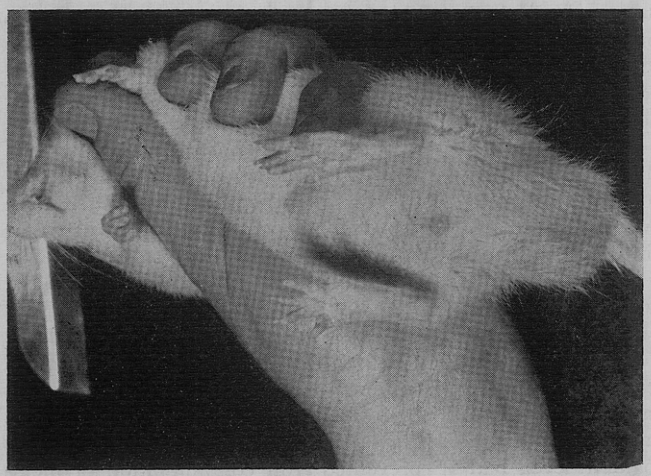

Figure 3. Testicular response after effective iron therapy

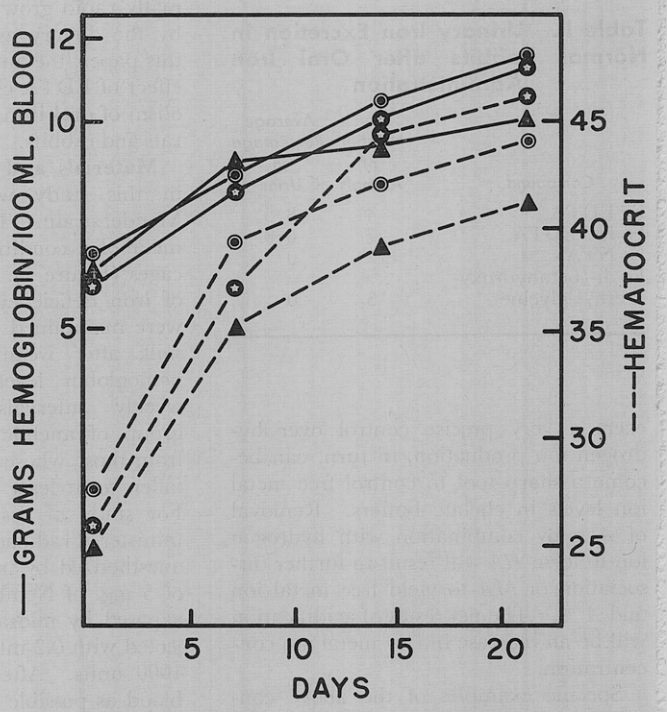

Figure 4. Response to oral iron administration

(◉ ferrous sulfate; ☆ ferric EDTA; ▲ Ferric HOEDTA; —— hgb level; – – – hct level)

iron is retained by these normal animals than oral ferric chloride. Almost all of the nonabsorbed iron has been accounted for in either the fecal content or the material remaining in the intestinal lumen. Variability in the ratio of nonabsorbed iron between the intestinal contents and feces is not significant.

Absorption, tissue distribution, and excretion of oral iron in iron deficient rats are also presented in Table I. While there is a marked variability in the distribution of the iron between the fecal output and the material remaining in the gastrointestinal tract at the end of 48 hours, the total amount of nonabsorbed iron is about the same regardless of the nature of the iron carrier. For the fraction of iron absorbed across the gastrointestinal tract, however, one may note a marked variability in iron distribution depending on the nature of the iron carrier. The tightly bound iron chelates, such as Fe EDTA and Fe DTPA, show a significant increase in urinary iron output compared to oral ferric chloride. Less of the orally administered chelated iron of these forms is available for hemoglobin formation at the end of 48 hours, and more has been distributed in the tissue mass of the animals compared to the results with oral ferric chloride.

The results of metabolic study of the urinary iron output in rabbits following oral intubation of various iron preparations in the range of 6 to 8 mg. per kg. is presented in Table II. Fe EDTA and Fe HOEDTA provide a form of oral iron which results in significant quantities of urinary iron excretion. In contrast, the ferric nitrilotriacetic acid (Fe NTA), and ferric di-(beta-hydroxyethyl)-glycine are chelates which do not carry oral iron into the urinary output.

Hemoglobin Regeneration. Iron deficiency anemia in the male rats of the colony was visually manifest by inflammation and enlargement of the testes (Figure 2). On treatment with oral iron, the condition was rapidly alleviated (Figure 3). The hemoglobin and hematocrit levels following daily intubation of 0.5 mg. of ferrous sulfate, Fe EDTA, and Fe HOEDTA are presented in Figure 4. All compounds were effective in the rapid restoration of normal hemoglobin levels.

After a period of treatment, the therapeutic administration of iron was stopped. The subsequent decrease in the hemoglobin and hematocrit levels (Figure 5) affirms the validity of the initial hemoglobin regeneration effect as due to the iron administration in this experiment.

Discussion. Will and Vilter (*36*) studied the absorption and hemoglobin incorporation of Fe^{59} EDTA following oral administration to humans with iron deficiency anemia. They were unable to demonstrate any significant differences in the chelated iron metabolism compared to ferrous sulfate. Seeberg *et al.* (*25*) reported on the hemoglobin regeneration in iron deficient rats following Fe EDTA. These authors found the chelated iron to be an effective oral iron source. Herridge utilized Fe EDTA orally in iron deficiency anemia of children (*11*). It was his conclusion that the chelated iron was better tolerated and a more efficient iron source for the human than ferrous sulfate.

The studies reported here amplify and clarify the role of chelation in modification of the absorption, tissue distribution, and excretion of oral iron. It has not been clear from previous studies whether chelated iron may be absorbed as such from the gastrointestinal tract or must first be dissociated from the carrier. Seeberg *et al.* (*25*) suggested that dissociation of the iron chelate was a preliminary step to absorption. The evidence of the present study, however, establishes that part, at least, of the oral chelated iron appears in the urine in both normal and anemic rats and rabbits. This finding is in contrast to the normally minimal urinary iron excretion under any conditions. Chelated iron, on the other hand, is rapidly cleared by the kidney when introduced into the body. The conclusion that chelated iron may be partly absorbed from the gastrointestinal tract is in agreement with a similar report for the oral absorption and urinary excretion of lead EDTA. Shapiro and Papa (*26*) pointed out that this chelated lead compound was rapidly absorbed from the intestinal tract and thence transported to the kidney and excreted in the urine.

That some absorption of Fe EDTA occurs as such is also evident from the distribution data of Table I. The sharply decreased hemoglobin incorporation of the chelated oral iron compared to the ferric chloride is inexplicable if the iron were dissociated in the gastrointestinal tract to then follow the same pattern of absorption and distribution as inorganic iron. Supporting this conclusion is the evidence of a more widespread tissue distribution of the iron administered in the chelated form compared to the ferric chloride. The wide distribution of chelated metals in the tissues has been pointed out by Foreman

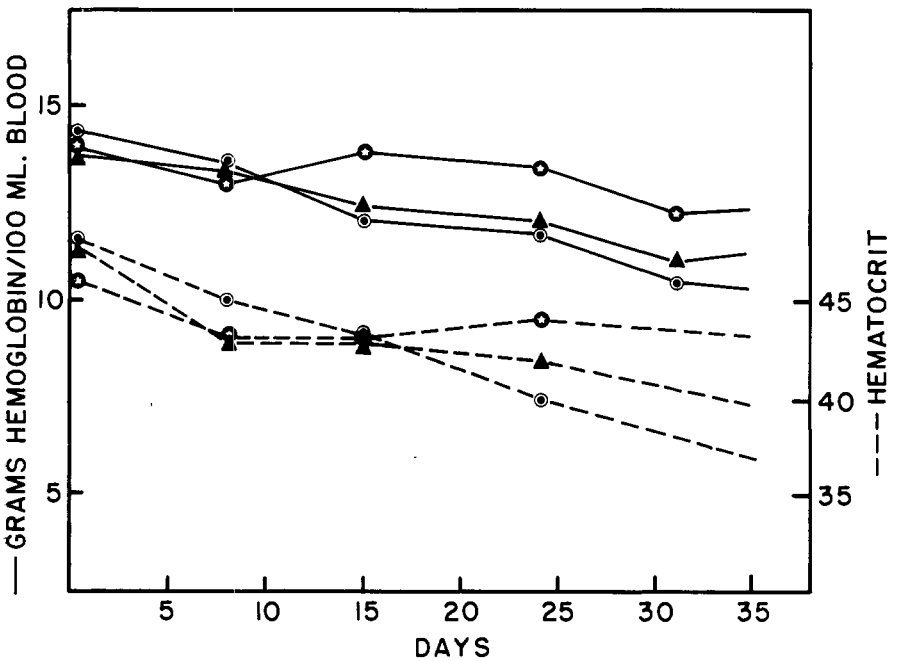

Figure 5. Effect of cessation of oral iron administration

(▲ ferrous sulfate; ☆ ferric EDTA; ◉ ferric HOEDTA; —— hgb level; – – – hct level)

et al. (*10*) in a study of the distribution of carbon-tagged EDTA.

Comparison of the absorption of all forms of iron in the normal and the anemic rat (Table I) affirms the fact that the anemic animal has a selective ability to extract iron from the diet. That this ability extends to iron presented in a chelated form as well as in the ionized form of ferric chloride is an unexpected finding which has led us to undertake a more detailed examination of the cellular transport of various iron forms.

Chelation and Cobalt Metabolism

The buffer action of chelation on the absorption of a mineral component of the diet has been well illustrated by studies on the effect of EDTA on cobalt absorption in rats. High levels of oral cobalt in rats result in a characteristic polycythemia. This hematologic effect of toxic levels of cobalt is inhibited by administration of oral EDTA as reported by Child (*5*) and Child and Leonard (*6*). That cobalt EDTA itself does not yield toxic levels of cobalt ion in vivo has been affirmed by Sullivan (*29*) who fed a diet containing 5% cobalt EDTA to weanling rats without untoward effect. The question may be raised as to biologic availability of EDTA-chelated cobalt. While an early patent (*37*) described the nontoxicity of cobalt EDTA and its utility for formation of red cells, the verification of these claims by controlled studies was not reported until later. Mascitelli-Coriandoli and Maiocchi reported (*20*) that oral cobalt EDTA in conjunction with iron salts produced a marked erythropoiesis in rat anemia induced by aminoadipic acid. In a more extended study, Murdock and Klotz (*21*) concluded that cobalt EDTA was the least toxic and the most useful oral cobalt compound available for oral cobalt therapy. The conclusion was based on the hematologic responses in anemic rats, the measurement of the absorption of cobalt in rats and humans, and the low toxicity of the compound compared to other forms of cobalt.

The buffer action of EDTA in setting tolerable ionic levels of cobalt for fatty acid synthesis by yeast homogenates was also evident in the study of Klein (*16*). A toxic effect of high levels of cobalt ion could be reversed by addition of EDTA to the medium. Conversely, the presence of cobalt EDTA was without detrimental effect on the lipide synthetic ability of yeast homogenates. The effect of chelation on the absorbability of the cobalt containing vitamin B_{12} is a matter of some significance for ruminants. In an extensive study of cyano cobalamin (*8*), calcium EDTA and manganous EDTA permitted the typical enhancement in absorption of vitamin B_{12} in the rat in the manner usually associated with the concomitant administration of gastric juice. Further, the free chelating agent had no effect on the dialyzability of the vitamin whether free or bound to rat stomach extract. These studies demonstrate that the chelation of cobalt by EDTA provides cobalt ion at a nontoxic, biologically available level.

Chelation and Microbiological Growth

That the presence of many cations in high concentration results in decreased growth of microorganisms is, of course, well known. The general effects of metals as protoplasmic poisons undoubtedly are involved in this toxic effect. Interaction of metals and proteins, a facile reaction, is considered a significant factor in the toxic effects of metals on microorganisms.

It has been demonstrable only with more difficulty that the absence of some metals can have an equally detrimental effect on microbiological growth. The problems involved in the recognition of low levels of metals as the limiting factor in growth of microorganisms have been reviewed by Hutner *et al.* (*13*). Technical difficulties, such as the contamination of substrates with the low levels of metals required for growth, the minimal effects of metal addition compared to control experiments, and the severe difficulties in elimination of contamination from water, glassware, and chemical reagents, all served until recently to limit our understanding of the essential inorganic requirements for microorganisms. If too much metal ion provides a hostile environment for the organism, and if too little metal ion is not sufficient to satisfy the requirements for optimal growth, the question may be asked as to what cation levels may be considered as desirable. In 1950, Hutner and his colleagues (*13*) pointed out that EDTA had unique advantages as a means of stabilizing metal ion concentrations in the medium at a level in the range required for optimal growth. Thus, these authors found that certain marine organisms were especially amenable to use of EDTA containing media for control of the ionic environment. Among these were a chlamydomonas, a flagellate—*Duneliella salina*, the diatom—*Nitzechia closterium*, and a variety of other marine organisms.

The studies of Hutner *et al.* (*13*) were a forerunner of extension of the same type of observation of the beneficial effects of chelation in the growth of organisms in other systems. Shipe and Fields (*27*) found that *E. coli* grown in suspension in an aqueous medium and then plated for cell count gave a marked increase in count when grown in a medium containing EDTA in comparison with a parallel experiment to one without the chelating agent. In extension of this study, they found that the copper and the zinc EDTA chelates served as excellent sources of the metal, but that increase in the free metal ion concentration above the level of EDTA chelation rapidly brought about the decreased cell counts associated with metal ion toxicity. They concluded that not only was EDTA nontoxic to the organisms, but that it was of marked value in supporting their viability. Hutner, Provasoli, and Filfus (*12*) reported that conditions for optimal culture of organisms which included *Ochromonas malhamensis* and *Poteriocheomonas stipitata* were attained with a

basal medium which included EDTA and metals. The use of EDTA to inhibit precipitation of metals, especially ferric phosphate, has been used by Carlucci and Pramer (*3*) in the culture of bacteria in sea water. Use of EDTA in the same way for the mass culture of various species of mesophilis and thermophilis has been equally rewarding. Thus, *B. licheniformis, B. circulans* of the mesophilic group, and *B. licheniformis* var. *B. circulans*, and var. *B. Stearothermophilis* have grown well on an EDTA metal-buffered medium as reported by Baker *et al.* (*1*). The same use of EDTA to ensure availability and to ensure against precipitation of critical metals was used in a basal medium supporting growth of a variety of pseudomonads utilized in metabolic studies of nicotinic acid (*14, 15*).

Since the copper chelate of 8-hydroxy quinoline (Oxine, "Copper 8") is one of the most widely used fungicides, it has been a logical consequence that the relation of fungistatic activity and copper chelation properties should have been explored. Byrde and Woodcock (*2*) pointed out that the fungistatic or fungicidal activity of a chelating agent was not dependent simply on the chelate binding capacity. Penetrability of the toxic species through the lipoid barriers of the cell membrane was as significant a factor in the effect on the organism as the intrinsic ability to bind the metal atom involved in the biological action. Thus, they demonstrated that the conversion of "Copper 8" to the lipide-nonpermeable copper EDTA removed the fungicidal properties of the agent and permitted free growth of the *A. niger*. In a previous, more extensive study, Manowitz (*18*) had similarly reported that, at a concentration of 250 p.p.m., EDTA was without effect on the growth of *A. niger*. Manowitz, in an extended investigation of the correlation of structure and antifungal activities, also pointed out that the biological activity was better related to lipide solubility than to inherent chelation characteristics. The reversibility of the toxic action of copper 8-hydroxyquinoline to the organism *Myrothecium verrucaria* (QM 460) by the stoichiometric addition of EDTA was also clearly demonstrated. In an effort to determine the upper limit of permissible EDTA levels, Manowitz examined the effects of high concentrations of EDTA in the medium. At levels of 750 p.p.m., it was possible to obtain some growth inhibition. This is in contrast to an active compound which was fully effective at 1 p.p.m. In further study of the system, Manowitz proved that the copper EDTA species was formed and that this was nontoxic to the organism.

The utility of naturally occurring organic acid chelating agents, such as citric acid, to solubilize iron and thus support the growth of algae in nutrient culture, had been pointed out by many investigators starting with Uspenski in 1927 (*31*). Some limitations in the utility of this naturally occurring chelating agent caused Waris (*34*) to re-examine the question. Waris utilized ferric EDTA as the iron source in the culture of myriad organisms. He concluded that the beneficial effects of the chelate could be attributed not only to the maintenance of a high level of soluble iron, but also possibly to the suppression of the accumulation of toxic levels of heavy metals or of other cations which would adversely affect the colloidal state of the cytoplasm of the organism. Krauss (*17*) made a systematic study of the factors involved in the growth of algae in mass culture. For Chorella and Scendesmus, Krauss worked out the experimental variables which would permit maximal growth limited only by the requirement for continuous repletion of inorganic micronutrients. Under these conditions, it was possible to study the effects of the additions of metals in various forms. Clear evidence was obtained to support the thesis that the EDTA-chelated forms of iron, calcium, manganese, zinc, copper, and cobalt provided the most satisfactory system for the supply of the mineral micronutrient needs of the optimally growing algae.

The other possible approach to the problem of the determination of the micronutrient requirements for algae, namely, by establishing growth requirements in the absence of minerals or in the presence of limiting quantities of the elements, has been explored by Walker (*32, 33*) for the algae *Chorella*. By rigid control of the medium, Walker was able to show that *Chorella pyrenoidosa* could satisfy its specific mineral needs for calcium, strontium, copper, molybdenum, iron, manganese, and zinc when grown in a medium containing EDTA. In related studies, Provasoli and Pintner (*23*) examined the growth requirements of 37 varieties of algal chlorophyta, chrysophyta, Euglenophyta, and Pynaphta. They reported that the mineral requirements were amply provided by the EDTA medium containing magnesium, calcium, iron, zinc, manganese, molybdenum, cobalt, and copper. Implicit in these studies is the assumption that the chelated metal is a nontoxic species. The direct and dramatic test of this assumption is provided by the study of Palmer and Maloney (*22*) who grew algae of the genera of Cylindrospermum, Microciptis, Scendesmus, Chlorella, Comphonema, and Nitzchia in a medium containing copper EDTA as well as in the control cultures without the chelating agent.

An interesting study on the effect of free EDTA in the medium on the germination of ascospores, serves to clarify certain points regarding the action of the chelate on some phases of microbiological growth patterns. Sussman (*30*) showed that, although the dormant spores were insensitive to the free chelating agent, there was a point during the activation process at which the free chelating agent inhibited the growth of the organisms. This growth inhibition was reversed by the addition of copper or calcium ions to the extent that when the chelate was in equivalent or less concentration than the cation, germination was normal in up to ten times the concentration of free EDTA which was otherwise inhibitory. Sussman also reported, as had Reischer (*24*), that EDTA was unable to penetrate into the cell at any stage of its growth.

In addition to the effects on growth of various organisms, it is of some interest to consider possible effects of chelating agents, such as EDTA, on the morphology of bacteria. Factors influencing bacterial variation may be of some significance in the utilization of feeds. In a paper by Cole (*7*), the effect of EDTA on the suppression of normal variation in *Brucella abortus* was explored. The free EDTA in a chemically defined medium was able to suppress the formation of nonsmooth variants of the organism. This morphologic alteration was reversed selectively by the addition of manganese. The manganese EDTA chelate provided normal growth for the organism.

In a recent review, Weinberg (*35*) has pointed out that many antibacterial compounds have an ability to bind metal ions and that their function is intimately related to the metal ion level in the system. For many such systems, the presence of reactive metals in concentrations sufficient to combine with the antibiotic, inhibits its antimicrobial properties. The addition of EDTA permits the normal function of the antibiotic. This type of chelate effect has been utilized by Stephan *et al.* (*28*) to study the relation of dental caries in the rat to the nature of the oral flora. In control experiments, these authors showed that a diet containing EDTA permitted normal oral flora development in rats coincidently with a high caries incidence. The addition of various antibiotics, such as penicillin and terramycin, altered the oral flora but maintained the caries incidence. The pertinent finding for present consideration was that the inclusion of EDTA in the diet of the animals did not alter the normal oral flora. Lactobacilli, streptococci, and coli grew normally in the presence of EDTA.

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CHELATION IN NUTRITION

Review of Chelation in Plant Nutrition

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The use of synthetic chelating agents as a means of supplying Fe and to a lesser extent Zn and Mn to plants is increasing. The manner in which chelating agents function is not as yet fully understood. Chelating agents and their metal components can be absorbed by plant roots and transported to leaves, but there is evidence that chelating agents do not penetrate plant cells. Especially under low pH conditions the chelating agent either remains outside the plant root or is excreted by the plant root with only the metal component being accumulated. At high pH, there is a greater tendency for accumulation of both components. Some chelating agents increase yields independently of their effect on supplying deficient micronutrients. This effect continues to be investigated and may be of practical importance.

Although a biological phenomenon of great importance, chelation of metals was not taken advantage of as a practical means of supplying micronutrients to agricultural crops until about 9 years ago. As yet, economics does limit the use of synthetic chelating agents in fertilizers. They are used, however, as a means of supplying iron and, to a lesser extent, Zn and Mn to high value crops. Chelated Fe is exceptionally valuable for a wide variety of ornamental and fruit trees, vines, and shrubs.

Many woody plants are very susceptible to iron deficiency, and for these species and especially for certain ornamental species the fertilizers should routinely contain chelated iron. Specialty fertilizers for use with plants such as roses, azaleas, rhododendrons, gardenias, camellias, hydrangeas, and other acid-loving plants should contain chelated iron.

Chelated iron is especially valuable for growing plants in soil-less culture (hydroponics) both experimentally and commercially. The synthetic agent, ethylenediaminedi(*o* - hydroxyphenylacetate) (EDDHA) has proved most adaptable for this use.

The method of application most widely used in agriculture is on the soil. For this reason, the chelated metals can be mixed with complete fertilizers with which they are very compatible. Since very low application rates of chelated metals are required, their use in mixtures ensures proper distribution. This is particularly so for band placement of fertilizers.

Foliage application of chelated metals to fruit trees would be more economical than soil application. The results, however, from foliar application with the polyaminopolycarboxylic acid chelating agents have not been as successful as those from soil application. Some other chelating agents prepared from waste forestry and agricultural products as foilage sprays have given better results but in general not good enough.

The use of chelating agents in practical agriculture is increasing slowly. The amount is measured in the hundreds of thousand pounds. As the use increases, it is increasingly important to understand the total picture of what