

Ion chamber (center foreground) is used to monitor a water solution of radiozinc entering the trench around an apricot tree, the subject of a zinc nutrition study

Micronutrients in Crop Vigor

In the last 50 years, six elements have been added to the roster of those essential for plant life, and all of them have been micronutrients

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MUCH IS HEARD of micronutrient elements these days because—as with many other discoveries of modern science—we have learned to attach importance to something that previously had no meaning. There are seven micronutrients presently recognized as required for the growth and development of higher plants. Listed in the order in which scientific evidence caused them to become generally accepted as essential, they are: iron, manganese, boron, zinc, copper, molybdenum, and chlorine.

From time to time suggestions or claims have been made that other trace elements are involved in the nutrition of higher plants. However, unassailable proof that such suggestions

have real and understandable meaning is often quite another thing.

Of the 100-plus different chemical elements, either naturally occurring or synthesized, which make up the mass of the universe, considerably less than one fourth are needed for the structures and activities of living things. Our experience tells us immediately that any element found in the structure of protein will be essential for life forms, so we understand readily the requirement for carbon, hydrogen, oxygen, phosphorus, nitrogen, and sulfur. Similar reasoning tells us that iron as a component of hemoglobin, the magnesium of the chlorophyll molecule, and the calcium of the bony skeleton give three more chemical elements

which we can accept as essential without having to prove our observations indisputably correct. From this point on, however, questions as to life functions requiring further chemical elements become harder to answer unequivocally.

Potassium serves to illustrate the latter point. Potassium has been accepted as an essential element in plant nutrition since the earliest days of the science of plant physiology, although it is only within the past few years that an equal degree of confidence has been associated with the idea of a potassium requirement for animal nutrition. This acceptance of potassium as a required nutrient has come about through many times repeated experiments demonstrating that plants do not survive if potassium is omitted from their growth medium. But we are still without knowledge of an organic potassium compound in living organisms comparable with compounds containing hydrogen, phosphorus, or sulfur, nor is there information within the realm of biochemistry that suggests the indispensability of potassium in any specific link within the complex of biochemical processes of living organisms.

From a chemist's point of view it would seem reasonable to try substituting other monovalent light metals for potassium. However, all such efforts have failed. It is true that in many instances sodium or rubidium can substitute partially for potassium, but never completely, and the fact remains that plants must have some potassium to survive. The explanation as to why this should be so still awaits clarification.

The 10 different chemical elements we have touched upon so far were for many years thought to be all that were needed for the growth and development of higher plants. This was the situation in 1860 when Julius Sachs and W. Knop showed conclusively that higher plants had no fundamental need for soil or organic matter. Independently these two men showed that plants could grow quite satisfactorily with their roots immersed only in water, provided the latter was fortified with soluble salts containing nitrogen, potassium, calcium, magnesium, sulfur, and iron. Water with suitable concentrations of these elements dissolved in it became known as a culture solution. The atmosphere provided carbon (from its carbon dioxide) and some oxygen, and the water of the culture solution provided both hydrogen and oxygen. Sachs added three to six drops of "ferric chloride medicinal solution" to each liter of his cultures, so that, within this historical list of 10 elements, iron should be regarded as the first micronutrient. This

state of knowledge of the chemical essence of life remained as it was for the next 45 years.

Slowly, during these last 50 years, six more chemical elements have become recognized and accepted as essential elements for the growth and development of higher plants. All of them are micronutrient elements!

First Addition Was Manganese

The first addition to the modern list was manganese, proposed as an essential element by G. Bertrand in 1905. The last was chlorine, proved conclusively by T. C. Broyer and associates in 1954. Between these dates boron, zinc, copper, and molybdenum had acquired acceptable proof of essentiality.

Students and others have often raised the question as to why the discovery and acceptance of these micronutrient elements has been delayed for so long. Answers must be given in several parts. The most confusing situation is provided by plants' natural habit of absorbing every kind of soluble inorganic material. The probability is very high that every chemical element occurring in the earth's crust would be found in plants if analytical chemical techniques were only adequate to detect and measure them in the amounts which are involved. Also, plants may contain relatively large fractions of extraneous substance which may be inconsequential for their welfare. One of the most impressive occurrences in this respect is that of the silicon and aluminum always found in soil-grown plants—sometimes in the order of half their total ash constituents.

Most of the bulk of mineral soils consists of aluminum silicate minerals, and soil moisture always contains silicon and aluminum in solution. Moreover, soil-grown plants always absorb these two elements. The presence of silicon may be observed very easily in some plants, especially cereals. For example, if one burns rice hulls carefully, with little physical disturbance, a complete siliceous skeleton in the form of the original hull may be left behind. Chemical analysis would show quantitatively the amount of silicon. However, the presence of silicon in the plant is not evidence that it is needed. To test the possibility, one grows the plant not in soil, but in a culture solution, using his best skills to exclude silicon from the growth medium. When plants are grown on this kind of solution, the hulls or other plant parts do not have easily detectable amounts of silicon in them.

Thus, one addresses the question directly to the plant: How do you respond to a growth medium from which we have attempted to remove

all the silicon? So far with silicon only inconclusive answers have been given, but we do know that plants do not need the amounts of silicon normally found in them when grown in soils.

Because of the impossibility of guaranteeing complete absence of any chemical element from a given system, we can never prove that any element is *not* essential. However, we can prove that an element *is* essential if we are successful in completely preventing reproduction by depriving the plant of enough of the element in question.

Creation of a wider consciousness about the micronutrient elements should be credited to P. Mazé of France, who in 1914 published results of water culture experiments in which he verified Bertrand's 1905 claim that manganese was an essential element, and also reported that zinc was necessary for the growth of the maize plant. Mazé continued this kind of work and a few years later claimed further that aluminum, boron, chlorine, and silicon also were essential.

Mazé's work was in some respects meritorious, although some of his suggestions are still viewed with reservation. As a case in point, it is doubtful whether any plant physiologist today feels that adequate evidence has been produced to place either silicon or aluminum in the class of essential elements. Manganese, on the other hand, was quite well accepted as a required micronutrient in 1915, since many investigators had confirmed Bertrand's findings of 10 years earlier. About 20 different species of crop plants have now been shown to require manganese, both in culture solutions and under field conditions.

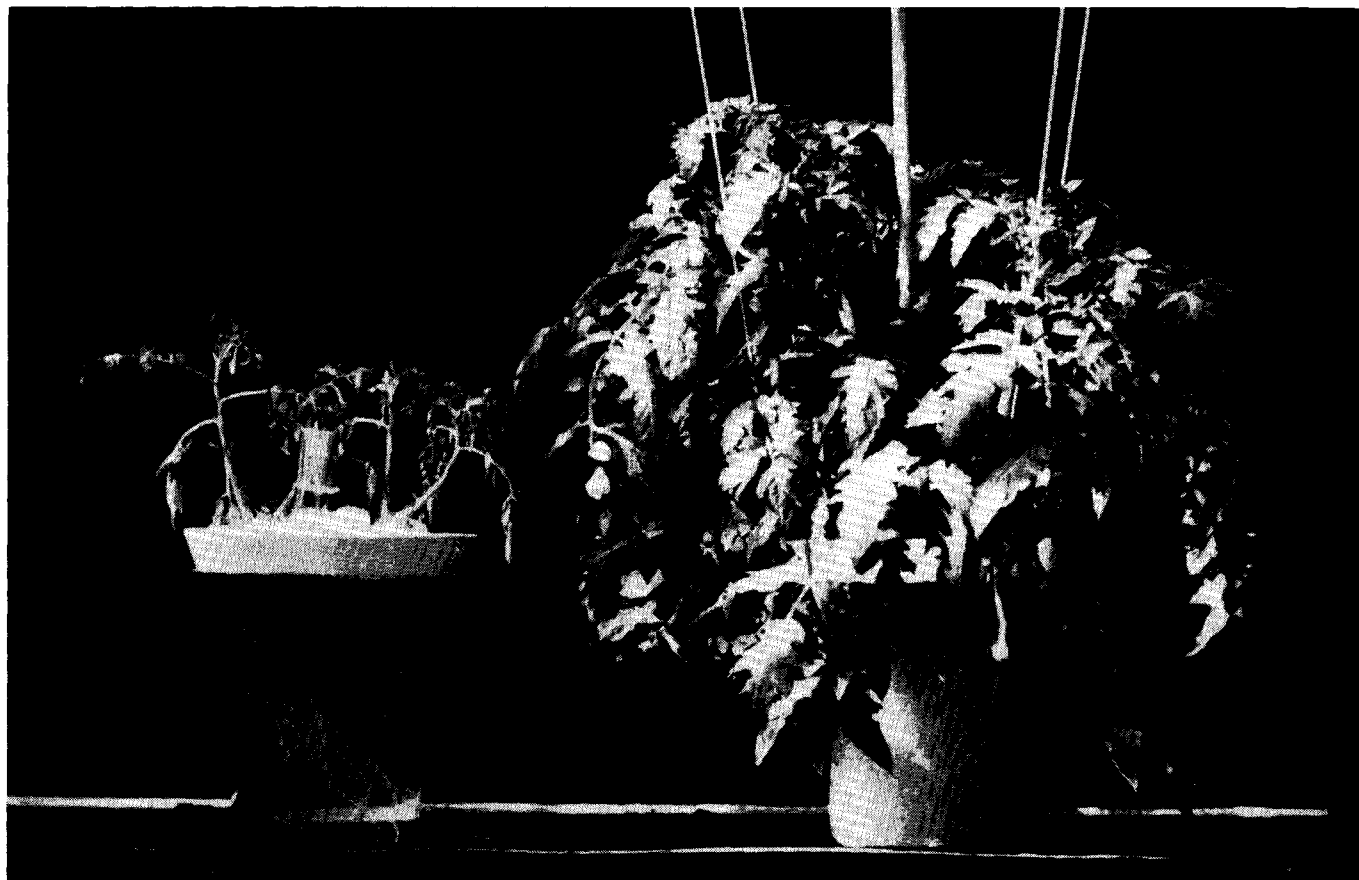
Mazé's claims for boron, zinc, and chlorine have been proved eventually beyond doubt, but the process has been slow.

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Effect of chlorine on growth of tomato plants. Only difference between two culture solutions was that one on right was supplied with 3 p.p.m. of chlorine as potassium chloride at the beginning of the experiment. To get responses such as is shown here, great care must be taken to exclude as much chlorine as possible from the control plant

The work of Catherine Warrington in England between 1923 and 1927 provided firm knowledge of the boron requirement for clovers, lucerne, and several varieties of beans, whereupon the importance of boron as an agricultural chemical grew very rapidly.

Recognition of zinc as an essential element began to take on further meaning in 1928 following the laboratory confirmation, by Sommer and Lipman in California, of a zinc requirement for buckwheat, bean, and barley plants. In 1932, also in California, Chandler, Hoagland, and Hibbard discovered that a long recognized "little leaf" disease of fruit trees was in reality a zinc deficiency. Simultaneously, Alben, Cole, and Lewis concluded that "pecan rosette" in the southeastern states was also a zinc deficiency disease. Since that time the expansion of zinc as an agricultural fertilizer has been truly remarkable.

Copper came into prominence in the early 1930's. When in 1932 copper was shown by Lipman and Mackinney to be an essential element for plant growth, there was already an accumulated body of information from the field suggesting that copper might be involved basically in plant nutrition. In the light of present day information, exanthema of fruit trees is

recognized as a copper deficiency disease. This disease was recognized as early as 1875 in Florida, where in 1917 P. F. Floyd reported that copper sulfate treatments of exanthema-affected citrus were beneficial. These findings were confirmed by G. W. Wickens in experiments with citrus in Western Australia, and further amplified by R. E. Smith and H. E. Thomas of California, who in 1928 reported successful treatment of exanthema of prunes, apples, pears, and olives with copper sulfate. The combination of laboratory demonstration of the essentiality of copper and correction of a recognized field disease by the provision of copper compounds served to popularize copper as a plant nutrient of agricultural significance beyond that attached to its usual role as a fungicide.

At this point, one may wonder with reason why the unfolding of knowledge of micronutrients has been so slow. At first glance it would seem that such problems could be approached simply and directly by preparing chemically pure salts and making culture solutions from them. Indeed, this is the way in which plant physiologists have always proceeded with their investigations, but unfortunately salts of ultimate chemical

purity have never been prepared by anyone, nor is it likely that they ever will be. In the manufacture of chemical compounds there are always contaminants carried along from the original raw materials, besides others introduced during manufacture. Chemical compounds used in the laboratory and in industry demand a degree of purity in keeping with costs. There are terms for the more refined grades such as "chemically pure" or "reagent grade." Nearly always the labels of these more expensive grades are provided with a chemical analysis showing the percentage of measurable impurities. Very often impurities are present in quantities too small for accurate measurement, but still detectable qualitatively. It has been the practice in the latter circumstance to list the impurity as a "Trace." Obviously, there are always some impurities which are present in amounts too small to detect by any analytical method available, and which escape notice altogether. Consequently, it was these obscure "trace amounts" of manganese, boron, zinc, and copper—overlooked by the chemists, but easily found by the plants—which had been supplying plant needs for these elements during the average experimental investigation.

Mazé's great service in his work of 1915 was to call attention to the fact that the plant physiologist would have to turn his attention to special problems in chemical purification of plant growth media and other sources of contamination if he was to discover further knowledge of the mineral requirements of plants. Seeds of plants, water for irrigation, and the containers used to hold culture solutions were all to be regarded as possible sources for contamination. We now realize that one also has to pay attention to the atmosphere, which may provide, for example, sulfur, in the form of sulfur dioxide. Leaves can absorb sulfur dioxide readily, thus making usable sulfur available to the plant.

Chemical Purity

Although aside from the main theme, a few observations about chemical purification may be of interest. It has been found in our laboratories that freedom of major chemicals from micronutrient impurities is not necessary to quality standards set up for other purposes. For example, we have upon occasion found crude fertilizer grades of calcium nitrate to have a hundredth of the heavy metal impurity of a bottle of reagent grade calcium nitrate analyzed at the same time. Unfortunately, with other sacks of crude fertilizer and other bottles of reagent grade chemicals, the situation might be reversed entirely. It is conceivable that in the process of refining the specimens of reagent grade calcium nitrate, many more opportunities were provided for the salt to come into contact with heavy metals than was the case for the specimen of fertilizer grade calcium nitrate. Of course, the purity of the fertilizer grade also meant a fortuitous selection of raw materials very low in heavy metals.

The only certainty we have been able to establish in studying micronutrient contaminations in salts is that our own standards are special and that we cannot expect chemicals manufactured for other purposes to meet them. We have found at least two instances of commercially available chemicals which are consistently good and quite suitable for studies with the heavy metal micronutrient element nutrition of plants. These are special grades of nitric acid and sulfuric acid prepared commercially for use in lead and arsenic analyses. Whatever steps the suppliers of these acids have taken to prevent their contamination by lead and arsenic, they have succeeded at the same time in reducing other heavy metals to equally low values.

Microchemistry began to find its wider uses around 1930 with the result

that considerable attention was given to improving methods for analyzing very small samples for chemical constituents. Organic chemists provided many new dyes which reacted with heavy metals to form colored compounds. One of the more versatile of these was diphenylthiocarbazone, commonly referred to as dithizone. Dithizone is a chelating agent which reacts with any of a large number of heavy metals to form highly stable complexes.

This remarkable reagent is green in acid and yellow in alkaline solutions when not combined with heavy metals, but when complexed with heavy metals it is colored purple or red. Moreover, the metal complexes are much more soluble in chloroform or carbon tetrachloride than in water.

One microgram of soluble zinc, copper, or lead, for example, is very easily found in a liter of solution. One has only to add a milligram or so of the dithizone and 5 milliliters of chloroform to such a solution made slightly alkaline. Chloroform is immiscible with the water and settles to the bottom in a separate phase. However, if the chloroform is emulsified with the water by vigorous shaking the metal dithizone complex leaves the water and becomes dissolved in the chloroform phase. When set aside and allowed to stand quietly the emulsion breaks rapidly with small droplets coalescing into larger ones so that the chloroform again settles to the bottom of the container. If heavy metals such as zinc, lead, copper, nickel, or cadmium are present in the

Radioautograph of zinc in bean leaves, young pods, and mature seeds. Path of zinc up the petiole along veins is followed in detail. Seeds in pod are much brighter than surrounding flesh, showing that zinc is preferentially accumulated in them



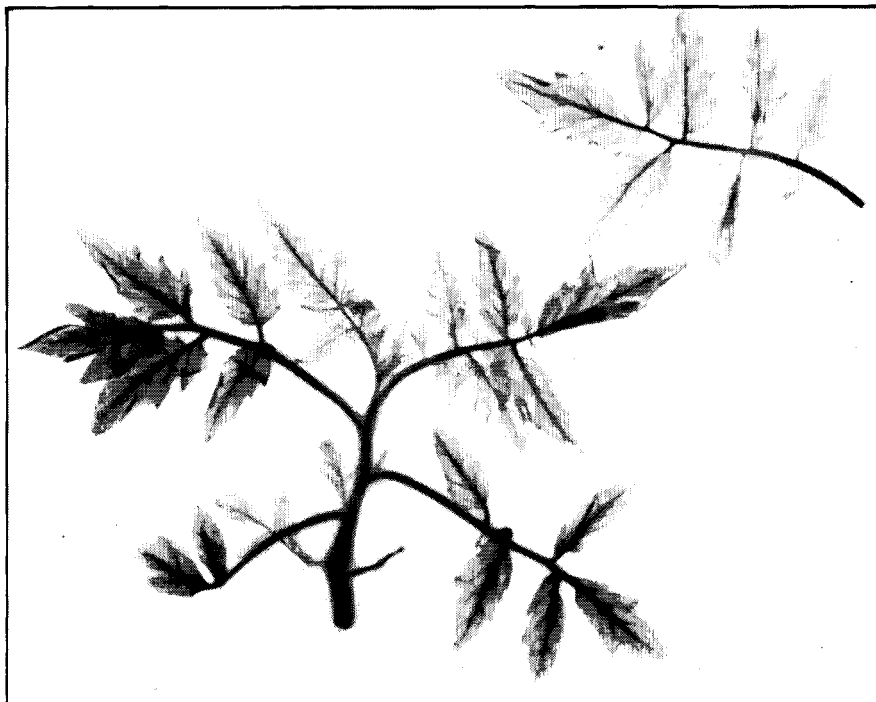
solution they are found to be neatly concentrated in the reddish colored chloroform solution accumulating in the bottom of the container.

Our first experiences with dithizone were most enlightening. Upon reading the account by Fischer of Germany of the synthesis of this organic compound and its colored reaction products with heavy metals, the exciting thought occurred that this might be the way to "see" zinc that was otherwise escaping detection. Zinc was a matter of great interest at the time because of the work of Chandler and Hoagland on the problem of the little leaf disease of fruit trees. Since there seemed no way to procure diphenylthiocarbazon, it was decided to synthesize the compound. There followed several discouraging months since it seemed that Fischer's reagent—which was supposed to be green—always turned out to be red when placed in contact with any ordinary reagents. However, it did not take too long to find out that reasons for securing red colors were not the fault of the synthesis but rather of our distilled water, salts, and other laboratory reagents, which had heavy metals in them.

Revelations Follow

This was a fascinating revelation. Having the means of greater perception at hand in the form of dithizone, it became possible first of all to produce water of 100-fold greater purity than the distilled water which came from our regular supply. Many other revelations quickly followed. It became apparent that zinc was very commonly used in all sorts of manufacturing processes. We are all familiar with galvanized wire, galvanized sheet, and similar materials which use zinc to protect iron from corrosion. Because of liberal use of zinc for this purpose, it seemed that everything which had passed very far along the route of ordinary human activities had had some zinc rubbed into it. Nevertheless, given a highly sensitive test for zinc—as well as for other heavy metals—the problem of providing a "low zinc" environment for the experimental growth of higher plants was helped very greatly.

This period represented the dawn of a new era in the control of plant growth media. It became possible to tell in advance through chemical testing whether or not solutions, water, or containers were adequate to demonstrate plant needs for zinc. Consequently, growth experiments with the micronutrient zinc became as controllable as others in which nitrogen or potassium was the subject for study. A further bonus came almost auto-



Radioautograph of chlorine in tomato leaves. Delicate appearance of leaves as compared with stems is caused in part by greater mass of tissue in stems and petioles. However, there is further indication that chlorine concentrates in the mid-ribs of older leaves near the base. Chlorine-36 was used

matically; it was found that in following procedures designed to remove zinc from the growth media, copper, manganese, and iron always disappeared along with the zinc. Copper also reacts with dithizone if present in amounts important in plant nutrition, and is detectable without great difficulty. Neither iron nor manganese reacts with dithizone, but chemical methods appropriate for the separation of zinc (usually co-precipitation of alkaline phosphates) seem always to remove iron and manganese quite effectively.

With the help of more effective chemistry applied toward problems in plant nutrition, attempts were made to look further into the basic mineral requirements for the growth of higher plants. There had been accumulated from many sources all sorts of suggestions of plant responses to additional chemical treatments. Unfortunately, one could not be certain of the status of the elements in the micro realm other than iron, manganese, boron, copper, and zinc. None of the work with higher plants had been conclusive for other elements.

At the University of California, D. R. Hoagland had composed supplementary solutions which contained large numbers of elements that at some time or other had been found in plants through chemical analysis, and which brought forth possibilities for nutrient relationships in plant growth. One of his often used "A to Z" supplements contained 22 different elements

which were provided to culture solutions in the order of 10 to 50 parts per billion. The elements included were molybdenum, tin, nickel, cobalt, cadmium, bismuth, chromium, lead, mercury, tungsten, vanadium, aluminum, titanium, barium, strontium, rubidium, lithium, arsenic, selenium, fluorine, bromine, and iodine. Chlorine was always present in these solutions in larger amount than any of the elements listed, because chloride salts provided the source of several of the elements.

Prof. Hoagland was a keen observer of plant conditions and had full confidence that this supplementary group contained a growth factor as judged from the many occasions when plants receiving the supplement seemed to enter a state of greater vigor—even if for only a few days during their growth. He was quite disinclined to rest his case on commonly used loose descriptions such as "beneficial effects" or "stimulus to plant growth," but in 1933, in speaking of one of his micro element supplements, he, and W. C. Snyder, did say: "From general appearance both observers obtained the distinct impression that the plants receiving this solution were superior to all others." Consequently, there was reason to believe that the only thing standing in the way of tangible proof of one or more micronutrient elements within this group was the chemical skill required to give adequate control to the culture solutions. With the coming of more sensitive reagents for

the detection of heavy metals it became possible to look more closely at the chemical environment in which plants were growing.

The search for new mineral growth factors was further narrowed by D. I. Arnon, who conducted experiments with a subgroup of Hoagland's "A to Z" supplements containing molybdenum, titanium, vanadium, chromium, tungsten, cobalt, and nickel. Arnon found that asparagus and lettuce plants responded greatly to additions of the supplementary solution. His experiments with lettuce were especially significant since he obtained nearly tenfold increases in yield from additions of the subgroup.

Chemical Purification Attempts Led to Moly's Role

Direct identification of molybdenum as a specific growth factor came about as a result of chemical purification processes directed toward the halides. To remove halides from salts used to compound culture solutions, silver nitrate was added in excess in order to precipitate the small amounts of contaminating chloride, bromide, and iodide ions. In this way removal of the halides was assured within the limits of the low solubilities of their silver halide salts. The excess silver had to be removed; this was done by passing hydrogen sulfide through the macronutrient salt solutions to precipitate the excess silver as silver sulfide. As an extra precaution in getting rid of trace amounts of remaining silver, an iron salt was added and this, in turn, was precipitated with more hydrogen sulfide. This succession of treatments gave rise to solutions as low in halide concentration as the solubility products of their silver salts would permit. It also lowered heavy metal contents below the amounts ordinarily dictated by the solubilities of metal sulfides, because formation of iron sulfide brought further co-precipitation of heavy metals.

A plant growth experiment was conducted with culture solutions made from these salts, using 20 different modifications of Hoagland's "A to Z" supplement—each with a different component element omitted. Unfortunately, no results were obtained from omission of the halides. As we now recognize chlorine as an essential element for plant growth, it is clear that even these experiments had not guarded sufficiently against all the sources of chlorine contamination. However, with these salts, acutely diseased plants were obtained from cultures in which either manganese, zinc, copper, or molybdenum had been omitted. More extensive experiments with purified culture solutions and

Arnon's groups of seven elements which contained molybdenum proved beyond doubt that molybdenum was the effective growth factor within the group, and that it was essential for the growth of the tomato plant, a fact which was announced in 1939.

It was found that culture solutions with 10 p.p.b. of added molybdenum could completely prevent the molybdenum deficiency disease in tomato plants. As a consequence, extreme care had to be exercised in all phases of experimentation if consistently reproducible results were to be obtained. Since such small amounts of molybdenum were involved, a great deal of work was done before it was felt that a valid announcement of molybdenum as a micronutrient could be made.

Molybdenum is the most recent arrival among the micronutrient elements to have significance for agriculture. For a while it appeared that information on the molybdenum nutrition of plants might remain in the realm of merely interesting knowledge,

but of no practical significance in the business of growing field crops. However, it was not too long before the discovery of molybdenum deficiencies in the field. In 1942, A. J. Anderson of Australia in a series of beautifully conducted field experiments demonstrated that some low-producing, hill-land clover pastures near Adelaide, South Australia, were molybdenum deficient; soil applications of $\frac{1}{16}$ ounce per acre were all that was needed to correct the deficiency. He showed also that the principal effect of molybdenum was to enable the symbiotic bacteria living in nodules on clover roots to perform their normal function of fixing atmospheric nitrogen. Anderson's work introduced a profitable new idea in associating these symbiotic organisms with molybdenum supplies. It is this interdependence between legumes and nitrogen-fixing microorganisms that has provided the greatest outlet for molybdenum as a practical agricultural chemical. The more common fertilization rates for

Virginia grower compares melon treated with molybdenum (left) with untreated melon. Sodium molybdate solution (1 ounce per gallon) was sprayed around hills



molybdenum deficient pastures are in the order of an ounce of molybdenum to the acre.

Straightforward molybdenum deficiencies in other crops were revealed in rapid succession. A most surprising feature of these revelations was that diseases correctable by molybdenum supplements had been recognized long ago by plant pathologists and in some instances had been corrected in the field without realizing the true nature of the disease. Whiptail disease of cauliflower is one of the more prominent ones. In Long Island this disease of cauliflower was described by Clayton in 1924 and suspected as a nutritional deficiency disease which Clayton found could be corrected through liming. It is now known that some soils fix molybdenum in forms difficult for plants to absorb and that liming may release adequate amounts of native soil molybdenum to provide plant needs for this element. The uses of molybdenum in agriculture are expanding so rapidly these days that it is difficult to keep fully informed of the areas and crops being benefited. The more spectacular successes are being reported in Australia and New Zealand where tenfold increases in yields are not uncommon when molybdenum is included with other required fertilizers, usually phosphate.

Chlorine is the latest element to become firmly established as an essential micronutrient element for higher plants. The eventual attainment of an adequate experimental arrangement to demonstrate its essentiality was accomplished by T. C. Broyer and a team of three associates in 1954. In retrospect, we must note that Julius Sachs, in 1860, had added a half gram of sodium chloride to each liter of his culture solutions and we are led to wonder as to his reasons for doing so. In the light of the considerable experimental difficulties which have had to be overcome to produce severe and repeatable chlorine deficiency symptoms in higher plants it would seem that the purity of chemicals in the 1860's must have been very good—if Sachs had really found it necessary to include a chloride salt in his culture solutions.

One member of the team demonstrating the need for chlorine in plant nutrition, C. M. Johnson, has recently extended the work to cover more than 10 other species. From these experiments we now know that the minimal concentration of chlorine required by plant tissues is in the order of 100 p.p.m. of their dry weights. Since chlorine is not fixed by soils and is leached freely into drainage waters, it must be concluded that the natural

source of chlorine for the land masses is the ocean. In years past, many analyses of rain waters have been made throughout the world. All such analyses show that chlorine is invariably a constituent of rain water and that the greater amounts are found near the seacoast. Annual acquisitions of chlorine from rain water range from about 5 pounds per acre per annum to several hundred pounds per acre; even several thousand pounds per acre have been reported for coast lands. Obviously, the stormier the seas and more rugged the coast, the greater will be the burden of sea salts picked up by the winds and carried over the land.

The smaller figures of 3 to 5 pounds per acre come into the order of amounts of chlorine needed by heavy crops. At present we are in the position of having to wonder whether there is a delicate balance in the more humid inland areas whereby the annual rainfall provides barely enough chlorine to meet the needs of the annual plant cover. We also wonder whether instances will appear where additions of more chlorine might result in better crop yields.

Since we must admit the importance of rains in supplying this micronutrient, we can raise similar questions concerning the role of rain water in supplying sulfur for land plants. In sea water the ratio of sulfur to chlorine is about one to 22. Even though the relative amount of sulfur supplied is small, it would seem important. We know of many cases of sulfur deficiency in the field and suspect that if we are to discover instances of chlorine deficiency, the chances of coming across them may be better if we look for possibilities of chlorine responses in known sulfur deficient areas.

While plant micronutrients make a very absorbing field of study in themselves, the micronutrient-element nutrition of animals and of microorganisms is an equally fascinating area for research and study. In trying to understand as much as we can about plant nutrition, we must realize that plants are only one fraction of the total cycle of life in which we ourselves are involved. From the standpoint of mineral nutrition of animals, we know that cobalt, sodium, and iodine are necessary but we do not know at this time whether higher plants have to have them. We are thus faced with the additional question, in considering plants as primary foods, as to whether we must expect them to secure these extra mineral nutrients for the convenience of animals, regardless of whether or not the plants need them for their own life processes.

Experiments with zinc, an established micronutrient, on apricots at University of California's Berkeley campus. Radiozinc is the material being used

