REQUIREMENTS AND FUNCTIONS OF MICRONUTRIENTS BY GREEN PLANTS WITH RESPECT TO PHOTOSYNTHESIS

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INTRODUCTION

The growth and normal biochemistry of green plants require the availability of from 15 to 19 elements. Eventually still others may be added to the list. Table 1 gives the list of these nutrient elements and indicates whether they are macronutrients or micronutrients. Some of the elements vary depending upon whether they are utilized by higher plants or by green algae.

Table 1

Elements Required by Green Plants

Macronutrients 10^{-2}-10^{-4} M	Micronutrients 10^{-5} M and less
C, H, O, N	Fe, Mn, Cu, Zn
P, S, K, Mg	Mo, V, B
Ca*	Cl, Co
Na**	

* except for algae where it is a micronutrient
** for blue-green algae

Plants require mineral elements for various functions. The macronutrients are used generally as building materials, whereas the micronutrients are commonly metal constituents of enzymes which enter into biological reactions.

A comparison between the mineral requirements for autotrophic and heterotrophic growth of Chlorella has shown some interesting differences (Table 2).

Except for Mg and NO_3 the critical quantities of macronutrients are quite similar for both autotrophic and heterotrophic conditions. The micronutrients such as manganese, iron, zinc, chlorine and vanadium are required in much greater amounts for autotrophic growth than for heterotrophic growth. This suggests a comparatively high concentration requirement for photosynthesis and a much lower concentration requirement for heterotrophic growth.

Table 2

Mineral Requirements for Chlorella pyrenoidosa Based on Critical Concentrations Required in the Culture Medium

Mineral	Heterotrophic Growth	Autotrophic Growth
NO_3	2.5×10^{-3} M	2.5×10^{-2} M
Mg	2×10^{-2}	2×10^{-3}
K	4.3×10^{-3}	4.3×10^{-4}
P	1.8×10^{-4}	1.8×10^{-4}
S	2×10^{-4}	2×10^{-4}
Fe	1×10^{-9}	1.8×10^{-5}
Zn	0.77×10^{-10}	0.77×10^{-6}
Mn	1×10^{-9}	1×10^{-7}
Cl	3×10^{-6}	3.4×10^{-2}

Macronutrients, based on critical concentrations in the culture medium, are required in the range of 10^{-2} M to 10^{-4} M. The quantitative requirements for micronutrients are less than 10^{-4} M. Of course the amounts added to a culture medium may be in excess of the critical concentration.

MANGANESE

McHarque (ref. 14) was the first to give definite evidence for the essentiality of manganese, and reported that plants deficient in manganese were stunted in their growth and produced no seeds. A mottled type of chlorosis accompanies manganese deficiency in higher plants. The essentiality of manganese for the growth of Chlorella was shown originally by Hopkins (ref. 13). Manganese deficient cells of Chlorella grown autotrophically are characterized by being abnormally clumped, by being about twice the volume of normal cells and by having a reduction in chlorophyll.

Manganese is one of the key elements in photosynthesis. The greater requirement for autotrophic growth than for heterotrophic growth shown for iron, zinc, chloride and nitrate also applies to manganese. The critical concentration for autotrophic growth of Chlorella pyrenoidosa has been determined to be about 1×10^{-7} M compared to 1×10^{-9} M for heterotrophic growth. This greater requirement for autotrophic growth in the presence of carbon dioxide, bicarbonate or carbonate is due to its role in photosynthesis.

Manganese deficient Chlorella were shown (ref. 16) to have a reduced level of photosynthesis which was restored to the normal level within a period of one or two hours upon the addition of manganese to the culture. Later these same results were obtained with manganese-deficient cultures of Ankistrodesmus (ref. 17). Furthermore, Arnon et al. (ref. 1,5,6) have reported that manganese increased the capacity of isolated chloroplasts to fix carbon dioxide.

One contribution of the Charles F. Kettering Research Laboratory has been to present evidence that manganese affects growth and photosynthesis by being required for Hill reaction activity which in the presence of an oxidant causes the formation of gaseous oxygen from water. The original experiments were based on the use of quinone as the Hill oxidant. More recently established has been the fact that manganese is required for the reduction of TPN by chloroplast particles of Chlorella in the presence of photosynthetic pyridine nucleotide reductase also derived from Chlorella cells. The manganese required for TPN reduction was shown to be associated with the chloroplast particles and did not seem to be a constituent of the reductase enzyme or necessary for the formation of the reductase enzyme. Unpublished results show that manganese is a general requirement for oxygen evolution regardless of the Hill oxidant used.

Growth, photosynthesis and Hill reaction show a close parallel relationship at each concentration level of manganese added to the culture medium. Most of the study involved the use of Chlorella pyrenoidosa, but was also extended to Nostoc muscorum, Scenedesmus quadricauda, Porphyridium cruentum, Lemna minor (duckweed) and sugar beets.

An interesting speculation has been that the size of the photosynthetic unit, which was reported to be about 2500 chlorophyll molecules, is regulated by the role of manganese in photosynthesis.

IRON

The essentiality of iron for plants has been known for more than a century. Iron deficiency results in a severe chlorosis of the leaves, especially in the younger leaves because iron is not readily transported within the plant. Iron is necessary for the culturing of Chlorella. More iron is required for autotrophic growth of Chlorella pyrenoidosa than for its heterotrophic growth. Chlorella must have a minimum of 1.8×10^{-5} M. At high light intensities in very active dense cultures the optimum iron concentration may be 10-fold higher.

The biggest problem of iron nutrition is associated with its availability to plants. On standing the dissolved iron changes to a colloidal form which reduces its availability. Fresh iron solutions need to be used for improved culturing conditions. Many prefer to chelate the iron with citrate or with EDTA (ethylene diamine tetra acetic acid). Although EDTA accentuates the need for more zinc, manganese and calcium in the nutrient solution for Chlorella it does not affect its iron requirement (ref. 21). The chelating effect of EDTA with iron must be very mild compared with the way it binds zinc, manganese and calcium. The application of iron as as potassium ferricyanide has proven very successful (ref. 21) under certain conditions. Potassium ferricyanide solutions, if stored in a cold dark place, do not deteriorate and aged solutions are as effective in nutrient media as fresh solutions.

The function of iron is associated with numerous iron-containing enzymes, e. g., peroxidase, catalase, cytochrome c, cytochrome oxidase, cytochrome f, and cytochrome b_6. Cytochrome f and cytochrome b_6 occur in chloroplasts of green leaves and are believed to be important in the transfer of electrons during processes involved in photosynthesis. Photosynthetic pyridine nucleotide reductase, which is a water soluble enzyme required for TPN reduction in green cells, has recently been reported to contain iron.

CHLORINE

Recently evidence has been reported to show that chlorine is a micronutrient element for higher plants. Tomato plants show the following nutritional deficiency symptoms: wilting of leaflet blade tips, chlorosis, bronzing and necrosis of the leaves proximal to the wilted areas. Compared with other micronutrients the chlorine requirement is not small. Bromine has been shown to be able to replace chlorine. However, neither fluorine nor iodine can substitute for chlorine.

Previously a chloride or anion effect had been reported for washed chloroplasts and for lyophilized Chlorella cells. Chloride has been designated as a "co-enzyme" of photosynthesis specifically concerned with oxygen evolution. Chloride is necessary for non-cyclic photophosphorylation and for the riboflavin phosphate pathway of cyclic photophosphorylation. For the vitamin K pathway of cyclic photophosphorylation chloride is not required. Chloroplasts have all of these pathways for photophosphorylation whereas the chromatophores of photosynthetic bacteria can carry out only the vitamin K pathway of cyclic photophosphorylation.

Non-cyclic photophosphorylation as it occurs in chloroplasts requires chloride and its biochemistry can be represented as follows:

$$2TPN + 2H_2O + 2ADP + 2H_3PO_4 \xrightarrow{PPNR} 2TPNH_2 + O_2 + 2ATP$$

If it is coupled with a ferricynaide Hill reaction the equation is as follows:

$$4Fe^{3+} + 2H_2O + 2ADP + 2H_3PO_4 \longrightarrow 4Fe^{2+} + O_2 + 2ATP + 4H^+$$

That two catalytic elements are known to be specifically concerned with oxygen evolution during photosynthesis should be emphasized. These two elements are manganese and chlorine.

When a special medium for Chlorella was formulated on the basis of critical concentrations of nutrients for autotrophic growth, the special medium was seriously inadequate and could be corrected best by the addition of chloride. The amount of growth of Chlorella could be doubled with the addition of merely 0.02 mg NaCl/100 ml (3.4×10^{-6} M). However, the critical concentration for sodium chloride was found to be about 3.4×10^{-2} M.

VANADIUM

Vanadium is one of the most recent additions to our list of micronutrients. Arnon and Bertrand (ref. 3,4,7,8) found that Scenedesmus obliquus produced symptoms of chlorosis when grown without vanadium in the culture medium. Furthermore, photosynthesis based on a unit-chlorophyll basis at high light intensity of 20,000 lux was about twice as high in the cells with vanadium as in cells deprived of vanadium. In weak light of 2,000 lux there was no significant difference.

The role of vanadium in photosynthesis was extended to Chlorella (ref. 22). There has been no extension of this study to other algae to indicate whether this essentiality for vanadium is general. Watching the literature on this subject during the next several years should prove very interesting.

It should be mentioned that vanadium can replace molybdenum as a catalyst in nitrogen fixation by Azotobacter.

ZINC

Zinc has definitely been established to be required for the growth of both higher green plants and algae. Zinc deficiency symptoms include leaf chlorosis, leaf necrosis, scant foliage and reduced leaf size, shortened internodes or rosette formation, and reduced fruit. "Mottled Leaf" of citrus, pecan "rosette" and "little leaf" or "rosette" of fruit trees are diseases known to be caused by a deficiency of zinc.

The following zinc metalloproteins have been characterized: carbonic anhydrase, carboxypeptidase, alcohol dehydrogenase, glutamic dehydrogenase and lactic dehydrogenase. The last three are pyridine nucleotide dependent metallodehydrogenases. Partial evidence has been obtained for the existence of four more pyridine nucleotide dependent zinc metalloproteins. These are glyceraldehyde-3-phosphate dehydrogenase, α-glycerophosphate dehydrogenase, malic dehydrogenase, and glucose-6-phosphate dehydrogenase. Hexokinase from Neurospora crassa may be a zinc metalloenzyme. Zinc may also play a possible role in the hexokinase of tomato plant tissues. The synthesis of tryptophan seems to require zinc. There is an important connection with auxin biosynthesis, in as much as tryptophan is a precursor. Zinc deficiency markedly reduces the ability of Neurospora, tomato plants and oat plant leaves to synthesize proteins. Deficient tomato plants contain about twice the total amount of amino acids and 10 times the amide content of normal zinc-supplemented controls.

Zinc-deficient Rhizopus nigricans has no pyruvic carboxylase activity. The conclusiong reached is that zinc is not a constituent of the enzyme but that zinc is necessary for its synthesis. The aldolase activity of higher plants is decreased by a zinc deficiency.

Warburg and Lüttgens (ref. 23,24) presented some evidence which caused them to speculate that zinc was a heavy metal intimately concerned with Hill reaction activity. They reported that the quantity of o-phenanthroline required to cause 100% inhibition was almost exactly equal to the amount required to completely chelate the zinc in the chloroplast fragments. The inhibitory action was corrected by the addition of zinc ions. Schwartz, (ref. 18) using lyophilized Chlorella cells, confirmed the reactivation of Hill reaction with zinc ions after removal of an unknown metal ion with phenanthroline. However, he could reactivate the phenanthroline inhibition by the addition of many divalent metal ions. Co^{++}, Cu^{++}, Mn^{++} and Fe^{++} as well as Zn^{++} were effective but Mg^{++} and Ca^{++} were completely without activity.

CALCIUM

Calcium is a macronutrient for higher plants. As calcium pectate, it is definitely known to be one of the components of the middle lamella of the cell wall. Furthermore calcium is necessary for the continued growth of apical meristems and may enter into the composition of protoplasm and certain types of protein in the cell. Anabaena cylindrica has been shown to require macro quantities of calcium for growth regardless of whether the algae were given molecular nitrogen or nitrate nitrogen. The optimum amount appeared to be about 20 p.p.m. calcium, with some indication that 20 p.p.m. calcium was quite necessary for optimum fixation of nitrogen whereas it was somewhat more than adequate for growth with nitrate in the culture medium.

Green algae require only microquantities of calcium or do not require any calcium. The calcium requirement for Chlorella has not been too certain. Chlorella does become calcium deficient when EDTA is present in the medium. Strontium is able to correct the deficiency better than calcium. A real calcium essentially would not permit the substitution of any other element. Also one cannot be sure what preferential bindings occur when EDTA is in contact with so many different cations. The problem of calcium essentiality among the various algae is one which should be pursued vigorously. Ultimately then one would be certain about its requirement and also be able to determine its biochemistry.

The amount of calcium required by Nostoc muscorum (a blue-green alga) to fix atmospheric nitrogen has been measured (ref. 11). The minimum amount in the modified Chu medium for maximum growth was reported to be 0.75×10^{-5} M or 0.3 p.p.m. (macro quantity). Neither nitrate nor ammonium salt were included in the medium. The cultures were bubbled with 5% CO_2 in air and illuminated with about 1000 ft-c of light.

BORON

Higher plants definitely require boron as a micronutrient. There is still some doubt about the essentiality of boron for algae except for its need by some algae for nitrogen fixation. Boron deficiency produces death of the apical meristems, failure of young roots to develop hypertrophy of the cambium, disintegration and discoloration of the phloem and breakdown of xylem tissue blocking the conducting system.

of 1 x 10^{-10} M molybdenum whereas no molybdenum is necessary for growth with an ammonium salt. To determine the critical concentration of molybdenum for nitrate utilization it is necessary to highly purify the reagents for the culture medium. This has been done by an electrolytic method. Ten-fold concentrations of nutrient medium were placed in an electrolytic cell which by means of a silver chloride reference electrode was provided with a voltage of -1.7 for 24 hours. At this voltage all of the cations were removed from the medium except K, Na, Mg and Ca. Special effort was observed to exclude oxygen from the system during electrolysis. The system was continuously bubbled with nitrogen gas which had been passed through oxysorbent to remove any oxygen. During the electrolysis nitrate was reduced to nitrite. Nitrite is poisonous to algae. To surmount this problem, the macronutrient minus potassium nitrate was purified. Fisher certified reagent grade potassium nitrite was found to be pure enough so that it could be used without further purification to demonstrate molybdenum deficiency in Chlorella. Fisher certified reagent grade compounds were used where possible for all the macronutrients. Also shown was that the only salt usually somewhat contaminated with cations such as molybdenum, manganese, iron, etc. was the magnesium sulfate. Micro elements added to the culture medium were the "Spec Pure" brand obtained from Johnson, Matthey and Co., Ltd.

COBALT

A cobalt requirement has been demonstrated for bacteria-free cultures of Calothrix parientina, Nostoc muscorum, Cocco-chloris peniocystis and Diplocystis aeruginosa. The nutritional function of cobalt seems to be associated with the role of vitamin B_{12}, and the cobalt requirement of Nostoc muscorum can be satisfied by the addition of relatively minute amounts of vitamin B_{12}. Forty parts per trillion of cobalt is sufficient to satisfy the needs of Nostoc muscorum for growth. Recently it has been established that cobalt is essential for soybean plants grown under symbiotic conditions for nitrogen fixation.

COPPER

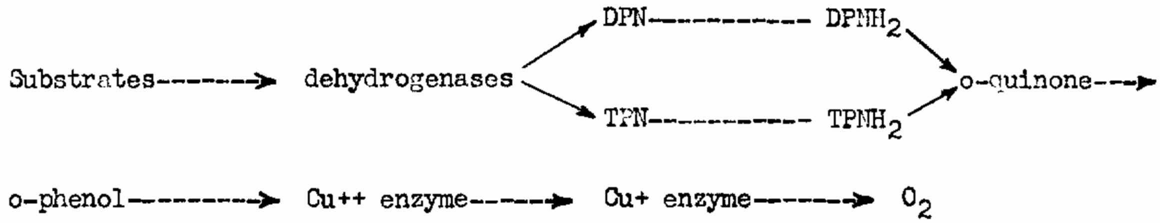
Sommer (ref. 19) was the first to clearly demonstrate that copper was an essential micronutrient for tomato, sunflower and flax plants. Tomato plants grown in nutrient solutions under controlled conditions showed stunted growth of shoots and roots, curling of leaves, failure to produce flowers and a bluish-green color of the leaves.

The metabolic role of copper in plants is not completely understood. Evidence to date links copper with oxidations in the plant. Laccase, ascorbic acid oxidase, and polyphenol oxidase (tyrosinase) are plant enzymes which have been shown to contain copper as a metal constituent. The function of laccase in the metabolism of the plant has not yet been explained. The latex of several species of lacquer trees contains laccase.

The role of ascorbic acid oxidase in plant metabolism has been explored. Some evidence exists that the enzyme acts as a terminal oxidase bringing about electron transfer between reduced ascorbic acid and atmospheric oxygen.

The ascorbic acid system in plants is quite similar to the cytochrome system in animal tissues and in yeast in that ascorbic acid is reversibly oxidized and reduced in the former and cytochrome c in the latter.

The present status of polyphenol oxidase in plant metabolism is that it catalyzes the oxidation of a variety of phenols aerobically and is considered to be a terminal oxidase in respiration. There is evidence that the enzyme catalyzes the oxidation of $DPNH_2$ and $TPNH_2$ according to the following scheme:



The afore mentioned copper oxidases make it obvious that copper is essential for the respiration of plants. There is indirect evidence that copper has a function in photosynthesis. Clover leaves were found to concentrate copper in the chloroplasts. Potassium ethyl xanthate, a strong inhibitor of the light reaction of photosynthesis and believed to be fairly specific for copper enzymes, was shown by Arnon (ref. 2) to have its inhibition counteracted either by dialysis or by the addition of copper which was the only metal effective in reversing the inhibition. Arnon postulated that "copper may prove to be at least one of the metals concerned in the light reaction of photosynthesis in green plants." Twelve years have elapsed and still no direct evidence has been reported that copper plays a direct role in photosynthesis. During this period of time manganese has been shown to be essential to photosynthesis, being concerned with oxygen evolution.

Walker (ref. 20) is the only one who has reported a copper requirement for the growth of green algae. He has shown, that Chlorella requires copper when grown in a glucose-urea (or nitrate)-EDTA-salt media deficient in copper. The copper requirement could not be satisfied by beryllium, magnesium, barium, cobalt, iron, zinc, manganese, molybdenum, nickel, thallium, gallium, aluminum, cadmium, vanadium, germanium, titanium, zirconium, arsenic, bismuth, tin, chromium, mercury, sodium, potassium, boron, silver, lead or gold when ions of these elements were added singly to growth cultures lacking copper. He could demonstrate no requirement for copper with the glucose-urea-salt medium; however, he found that copper was required for maximum growth in the glucose-nitrate-salt medium. This difference could have been due to a copper impurity in the urea or an increased copper requirement for nitrate assimilation. The problem of copper essentiality for algae is by no means solved. Information on the copper requirement of several more commonly cultured algae as well as confirmations about the copper requirement for Chlorella would be quite desirable.

SUMMARY

Presently only five trace elements seem to be directly concerned with photosynthesis of green plants. Manganese and chlorine each have a role in oxygen evolution. Vanadium accelerates photosynthesis under high light intensity but as yet this effect has been reported only for Scenedesmus obliquus and Chlorella. Iron is the metal part of cytochromes which enter into electron transfer, and is the metal constituent of photosynthetic pyridine nucleotide reductase, a water soluble enzyme required for TPN reduction. Although more zinc is required in light-grown than in dark-grown cultures of Chlorella its role in photosynthesis is uncertain. One might suspect zinc to aid in hydrogen transfer since zinc is definitely known to be the metal constituent of various dehydrogenases.

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