GROWTH AND CHEMICAL COMPOSITION OF *ANANAS COMOSUS* (L.) MERR., IN SOLUTION CULTURES WITH DIFFERENT IRON-MANGANESE RATIOS[1]

(WITH EIGHT FIGURES)

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Introduction

Pineapple plants grown in highly manganiferous soils become chlorotic and produce fruits which are small and of an inferior quality (**29**). Pineapple chlorosis is prevented in commercial plantations by the application of sprays of an iron sulphate solution.

Chlorosis of pineapple plants may develop, also in non-manganiferous soils with pH values higher than 5.5, especially when treated with fertilizers with residual alkaline reactions; high pH causing oxidation of Fe^{++} to Fe^{+++} with subsequent precipitation of the latter. Hence, ammonium sulfate with residual acidic reaction is preferred to sodium nitrate with residual alkaline reaction as a pineapple plant fertilizer.

Manganese chlorosis may be caused, according to JOHNSON (**29**) and KELLEY (**30**), by conversion of the water soluble ferrous to the water insoluble ferric iron by exchanges of electrons with manganese; a reaction comparable with that at high pH.

The results below, concerned with the growth of pineapple plants at different iron-manganese ratios and the chemical composition of the tissues under the various conditions, suggest that manganese chlorosis may result from a replacement of iron by manganese in the pyrrole ring of protoporphyrin 9, a precursor of chlorophyll.

Review of literature

JOHNSON (**29**) explains manganese chlorosis in pineapples as the result of the oxidation of ferrous to ferric iron by manganese at pH values above 5.5 whereby pineapple plants deprived of available iron fail to produce chlorophyll. KELLEY (**30**), discussing pineapple chlorosis, states that "yellow pineapples from soils containing a high percentage of manganese yielded poorly, had lower amounts of starch and chlorophyll but the xanthophyll content was the same in both chlorotic and green leaves." GILE (**16**) was of the opinion that manganese chlorosis of pineapples in Hawaii was due, in part, to iron deficiency in the plant, induced by manganese, while the lime-induced chlorosis in Puerto Rico was the lack of iron in the plant due to calcium carbonate diminishing the availability of iron in the soil.

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McGeorge (**37**) concluded from Johnson's and Kelley's data that pineapple chlorosis on manganiferous soils was due to a great assimilation of lime and that the principal physiological disturbance was the greater immobility of iron in the plant caused by the excessive lime content of the leaves and stalk, since iron was present in equally available form in both manganiferous and non-manganiferous soils of equal pH.

Other plants than pineapples have been found susceptible to manganese chlorosis. Rippel (**45**) observed that oats made chlorotic by high Mn in solution cultures contained almost equal amounts of iron as green plants, and concluded that Mn had not interfered with Fe absorption. Haas' (**20**) results indicate that excessive amounts of Mn caused chlorosis in citrus although Fe was added in comparable amounts, and, also, that when Mn was deficient in the leaves less iron had accumulated. Somers, Gilbert and Shive (**60**) and Somers and Shive (**61**) attributed manganese chlorosis in soy beans to certain iron-manganese ratios which, depending on the prevelance in the concentration of either element, may cause iron or manganese toxicity. Lindner and Harley (**32**) are of the opinion that in pear, apple and spiraea leaves, chlorosis may be induced by high potassium levels by replacing the iron on the enzyme responsible for chlorophyll formation, thereby inactivating the enzyme. Twyman (**64**), after reviewing the literature on the iron-manganese balance and its relation to chlorosis, concluded that "further investigations are necessary on the effects of this balance on the growth of plants and the occurrence of pathological symptoms, so that the deficiency disease concerned may be better understood."

Bennett (**5**) showed in prune trees that iron-manganese ratios may vary considerably (1.6–0.1) more than those indicated by Somers and Shive (**61**) without development of severe chlorosis symptoms but with Mn concentrations higher than 100 γ which presumably was toxic, chlorosis could be induced promptly. Sideris (**59**) observed more retardation of Fe translocation from roots to leaves in cultures with, than without Mn.

Experimental methods

Cultural methods.—Crowns (vegetative organs produced at the apical end of fruits) from a clone of uniform weight were grown in two sets of nutrient solutions, one containing ammonium salts and the other nitrate salts as sources of nitrogen but all other elements supplied in equal amounts to both sets. The ammonium-N set contained 0.132 g. of $(NH_4)_2SO_4$ and 0.172 g. $CaSO_4 \cdot 2H_2O$, and the nitrate-N set 0.236 g. of $Ca(NO_3)2 \cdot 4H_2O$ per liter of solution. Other salts equally applied to both sets per liter of solution were 0.246 g. $MgSO_4 \cdot 7H_2O$, 0.174 g. K_2SO_4, 0.068 g. KH_2PO_4, 0.0028 g. $ZnSO_4 \cdot 7H_2O$, and 0.0038 $Na_2B_4O_7 \cdot 10H_2O$. Iron and manganese were added to the various cultures as indicated in figure 1.

The various nutrient solutions placed in 4-gallon porcelain crocks, one

plant per crock, were aerated continuously and changed at three-week intervals. At the end and beginning of such intervals the plants were weighed and samples of the solution cultures were collected for determinations of the amounts of iron and manganese removed by the plants.

ANALYTICAL METHODS.—Iron and manganese were determined colorimetrically, the former by the nitroso-R-salt (**52**) and the latter by the periodate method (**3**).

Iron and manganese in the tissues were fractionated into sap soluble and insoluble fractions; the former extractable by acetone but not the latter.

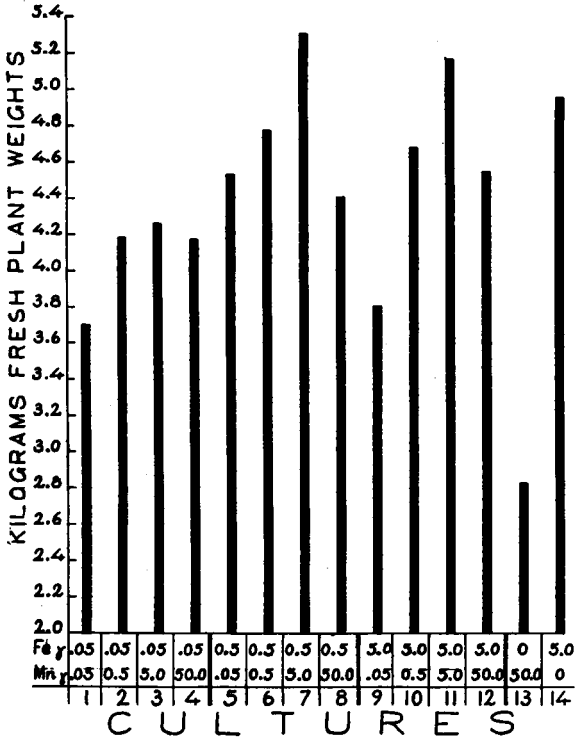


FIG. 1. Plant weights of cultures.

Also, protein bound iron or manganese fractions, the former reported previously (**58**), was obtained by extraction of the proteinaceous matter of the tissues (**57**) and determination of its iron and manganese content.

Sap soluble iron was obtained by mixing 10 grams of leaf tissues with 80 ml. of acetone in a Waring Blendor. The mixture after maceration for 15 minutes was filtered through No. 32 Whatman paper and the residual matter washed with more acetone until the removal of all green color was complete. The filtrate, after evaporation of the acetone in a platinum dish, was ashed and both iron and manganese determined by the methods above mentioned. Also, determination for total iron and manganese were made in different aliquots of the same leaf tissues after ashing in order to provide

means for the calculation of insoluble iron or manganese from the difference between total and acetone extractable (sap soluble) iron or manganese.

The acetone extraction method for soluble Fe and Mn was adopted after certain trials had showed that both elements were soluble in acetone in much greater amounts than those found in pineapple tissues.

Potassium, magnesium, and phosphorous were determined by methods reported previously (**50, 53, 55**), whereas calcium was determined by the official method (**3**).

Reducing sugars were determined by the method of QUISUMBING and THOMAS (**43**) and sucrose was calculated after treatment with invertase, from the difference between total and reducing sugars. For the estimation of starch the method of PUCHER and VICKERY (**42**) was employed. Ascorbic acid was determined with sodium 2,6-dichlorobenzenoneindophenol on a 10 ml. aliquot of the filtered extract obtained by mixing 10 grams of leaf tissues with 100 ml. of a 5 per cent. solution of oxalic acid and grinding in a Waring Blendor.

Chlorophyll was extracted and measured according to SCHERTZ'S (**47**) method.

Results

PLANT GROWTH.—Plant weights, in figure 1, varied considerably in the different cultures presumably on account of different iron-manganese ratios. The data indicate that in cultures 1, 5, and 9 supplied with very low manganese (0.05 γ) and in cultures 4, 8, 12 and 13 with very high (50.0 γ) manganese plant weights were depressed presumably in the former cultures by Mn-deficiency and in the latter by Mn-toxicity. The slight depressions in plant weights of cultures 9 and 10 with high (5.0 γ) Fe might indicate toxicity or improper balance between iron and manganese or between iron and other mineral nutrients. However, culture 14, with high Fe and minus Mn produced very satisfactory plant weights, indicating that neither the presence of high Fe (5.0 γ) caused toxicity or the lack of Mn, except the small contaminations in the C.P. nutrient salts, Mn-deficiency.

With respect to any definite amounts of manganese requirements for pineapple plants the data are inconclusive, because in certain cultures plant weights increased as the amounts were raised from 0.05 to 5.0 γ except culture 14 which, although lacking Mn, had almost as good plant weights as cultures 7 and 11. These results presumably suggest that extremely small amounts of Mn suffice in the presence of adequate Fe concentrations to produce satisfactory growth. Iron-manganese ratios ranging from 1 to 1 or from 1 to 10 as in cultures 11 and 7, respectively, may yield almost equally good results as long as the concentration of manganese is not abnormally high.

Under Hawaiian field conditions water soluble iron concentrations in most soils are ordinarily lower than 0.05 γ and manganese, in manganiferous soils, as high or higher than 50 γ. Total iron may range, according to

KELLEY (17), in manganiferous soils, from 18 to 26 and manganese from 2.5 to 9.0 per cent. and in the non-manganiferous soils iron from 20 to 35 and manganese from 0.06 to 1.17 per cent.

Because moisture conditions, water soluble iron-manganese ratios, the active plant nutrient fractions, and concentrations of other ions (PO_4^{---}, CO_3^{--}) reacting with Fe or Mn vary considerably in different soils at different pH values results obtainable by the solution culture procedure

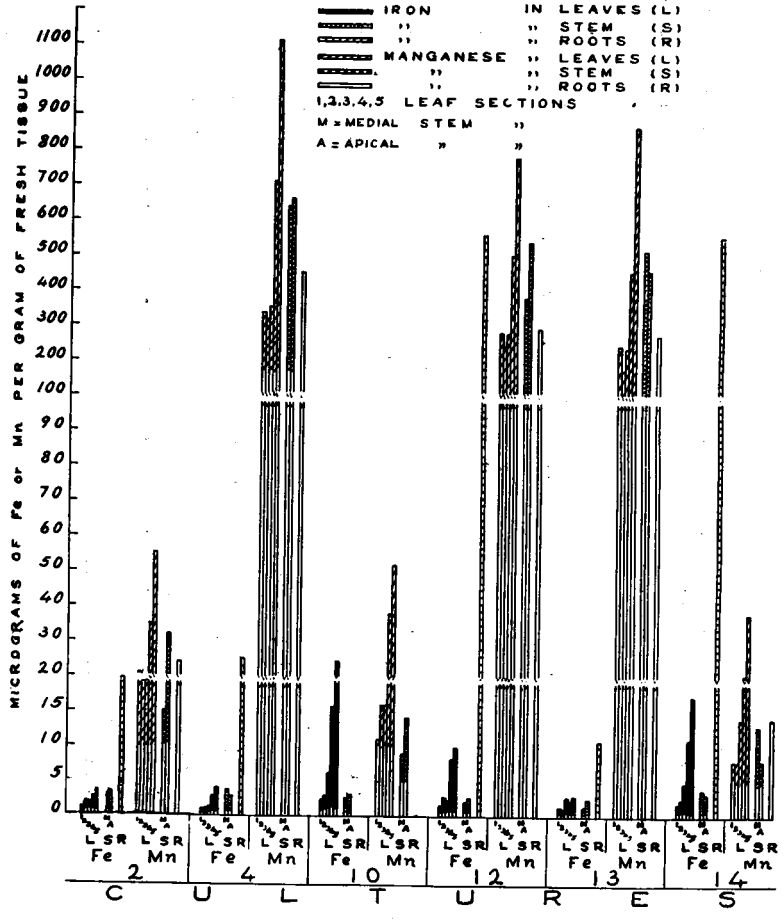


FIG. 2. Total Fe of Mn in leaf (L), stem (S), or root (R) tissues of cultures.

indicate approximations and not true conditions that plants encounter in soils.

IRON AND MANGANESE IN PLANTS.—The data in figure 2, depicting iron and manganese in different plant sections of the various cultures, reveal that Fe and Mn increased in the tissues with higher concentrations of these elements in the culture solutions. However, cultures 13 and 14, the former minus-Fe and the latter minus-Mn, contained small amounts of

these elements not supplied to the nutrient solutions, but which presumably occurred as contaminations in the C.P. nutrient salts.

The results indicate clearly that manganese did not accumulate in the roots but was translocated to the leaves, whereas iron was greatly immobilized in the roots as indicated in figure 2 and very small amounts moved to the leaves. The translocation of iron from roots to leaves, although very small in all cultures, was inhibited more in cultures 12 or 4 with high Mn than in cultures 10 or 2 with low Mn. In no case was iron translocation inhibited by manganese to a point of absence in the

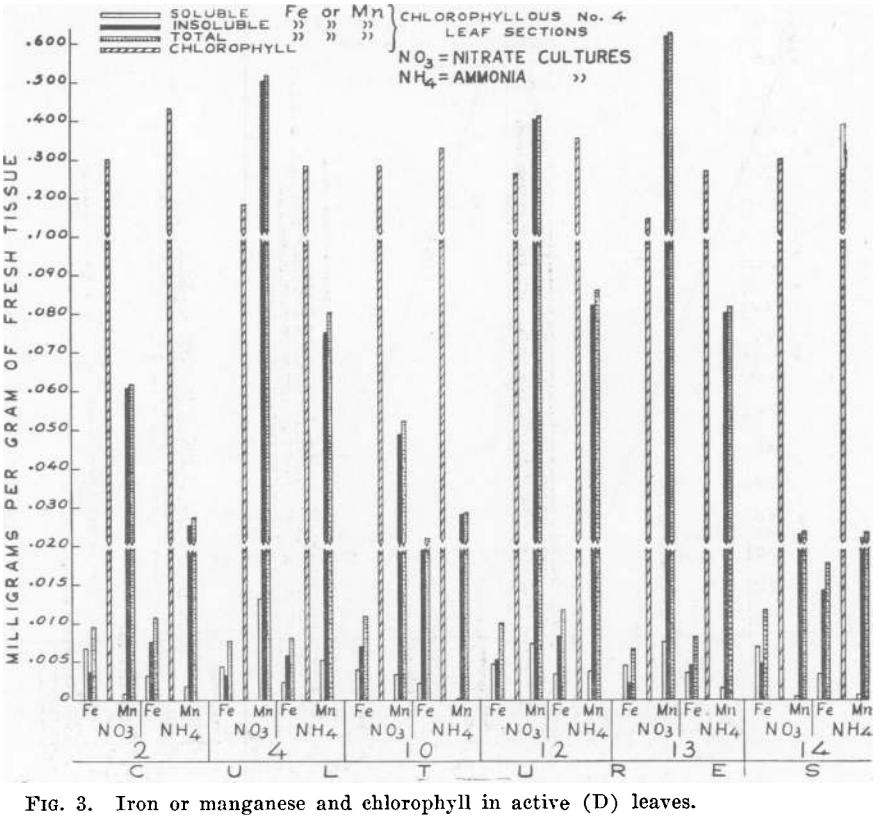


FIG. 3. Iron or manganese and chlorophyll in active (D) leaves.

leaves. However, the antagonistic effects of manganese, whatever the nature of their reactions in the plant cells, could be easily traced in the chlorophyllous regions of the leaves by the development of mosaic chlorotic patterns in the early stages which coalesced in later stages to produce general chlorosis.

Iron precipitation in the exodermal root tissues (**50, 53, 55**) is more pronounced in cultures with residual alkalinity than acidity. The slightly greater amounts of iron in the exodermal root tissues of cultures supplied with than without manganese may be of little or no significance concerning

the availability of iron to plants. However, such iron deposits may become subsequently available to plants when high H-ion concentrations or other elements with potentials capable of reducing Fe^{+++} to F^{++} are in the proximity of the rhizosphere (**50**).

The data, in figure 3, depicting amounts of chlorophyll, sap soluble iron or manganese and insoluble iron or manganese (protein bound and precipitated as inorganic salts) in the No. 4 sections of the active D leaves (**48**) of cultures with different iron-manganese ratios supplied with nitrate or ammonium salts, show that the tissues of the nitrate cultures contained,

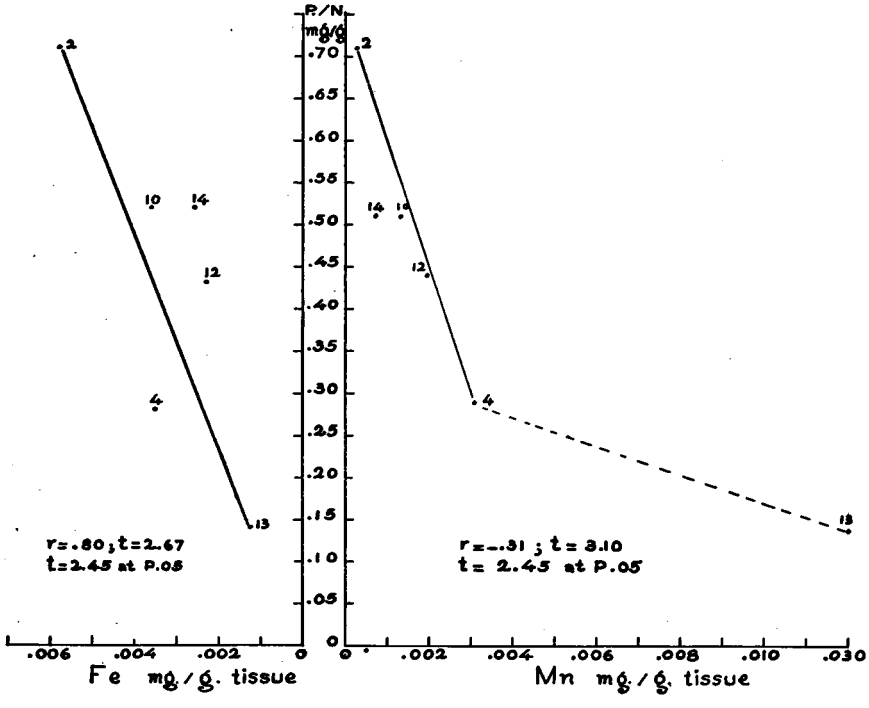


FIG. 4. Protein-nitrogen (P/N) and protein-bound Fe or Mn per gram of fresh tissue in cultures 2, 4, 10, 12, 13, 14.

in general, more manganese than those of the ammonium cultures, whereas iron and chlorophyll were reversed.

Approximately 95 per cent. of the total manganese and 50 to 80 per cent. of the iron in the tissues was insoluble (non-extractable by acetone). Certain tests indicated that precipitated manganese, presumably adsorbed or chemically bound to some colloidal organic matter, could be dissolved in dilute acetic acid solutions.

Manganese was considerably greater in the leaves of cultures 4 and 12, supplied with high-Mn, than in those of 2 and 10, with low-Mn. The differences in the total iron content of the leaves between cultures 2 and 10 or 4 and 12 in favor of 10 and 12, were very small and cannot be com-

pared with similar differences in Mn which were very great between cultures 2 and 4 or 10 and 12. The data clearly indicate that the rate of Mn intake by the roots and translocation to the leaves was considerably greater than that of iron, also, the interference of Mn in Fe absorption by roots and translocation to leaves was very small.

Cultures 13 and 14 with minus-Fe and minus-Mn, respectively, showed small amounts of both elements in the leaves, presumably occurring as contaminations in the C.P. nutrient salts. The Mn-content of the leaves in culture 13, without iron, being greater than in all other cultures with iron might have resulted from the lack of antagonistic effects by manganese or from concentration effects on account of the much smaller plant weights of this than other cultures. Chlorophyll and iron in the leaves correlated with the iron supplies in the nutrient solutions.

The data in figure 4, show that protein-N correlated positively with protein-bound iron but negatively with protein bound manganese. The correlation coefficient for the protein-bound manganese was not as great or statistically as significant as for the protein-bound iron. In culture 13, without iron, the amounts of protein-bound manganese were much higher than in cultures 4 and 12 supplied with equal amounts of manganese but, also, with some iron, suggesting that the presence of iron even in very small amounts prevented appreciable combinations of manganese with proteins.

Mineral nutrients in plant tissues

Potassium.—The amounts of potassium in the tissues of different plant organs, in figure 5, varied in the different cultures, being higher for culture 13 with small plant weights than in all others. The higher potassium concentrations in culture 13 presumably resulted from concentration effects rather than from greater rates of intake of this element by plant roots.

Calcium.—This element, also depicted in figure 5, was slightly higher in the culture with low than high manganese; although the differences in calcium between low and high manganese cultures were not statistically significant.

Kelley (**30**) observed greater calcium concentrations in pineapple plants grown in manganiferous than in non-manganiferous soils, which were thought of later by McGeorge (**37**) as the causes for the development of chlorosis in these plants.

The data in figure 3 and others previously reported (**50, 53, 55**) clearly indicate that manganese as well as calcium absorption is enhanced by NO_3^- but inhibited by NH_4^+. Also, Kelley (**30**) has shown that nitrification is greater in manganiferous than in non-manganiferous soils either because of higher pH in the former than the latter or of a greater rate of nitrate assimilation in the presence than absence of Mn, according to Burström (**12**). Therefore, the formation of NO_3 from nitrogenous organic matter more in manganiferous than non-manganiferous soils and the greater rates

of absorption of Mn and Ca in the presence of NO_3^- than NH_4^+ might explain the greater concentrations of Ca in the plants grown in manganiferous than non-manganiferous soils. McGEORGE'S (**37**) explanation of manganese chlorosis as resulting from high Ca may not interpret at all the actual conditions in the plant cells.

MAGNESIUM.—The concentrations of magnesium in the plant tissues, in figure 6, were greater in cultures 4, 12 and 13, with high-Mn, than in 2, 10 and 14 with low Mn. The per cent. difference between the high-Mn

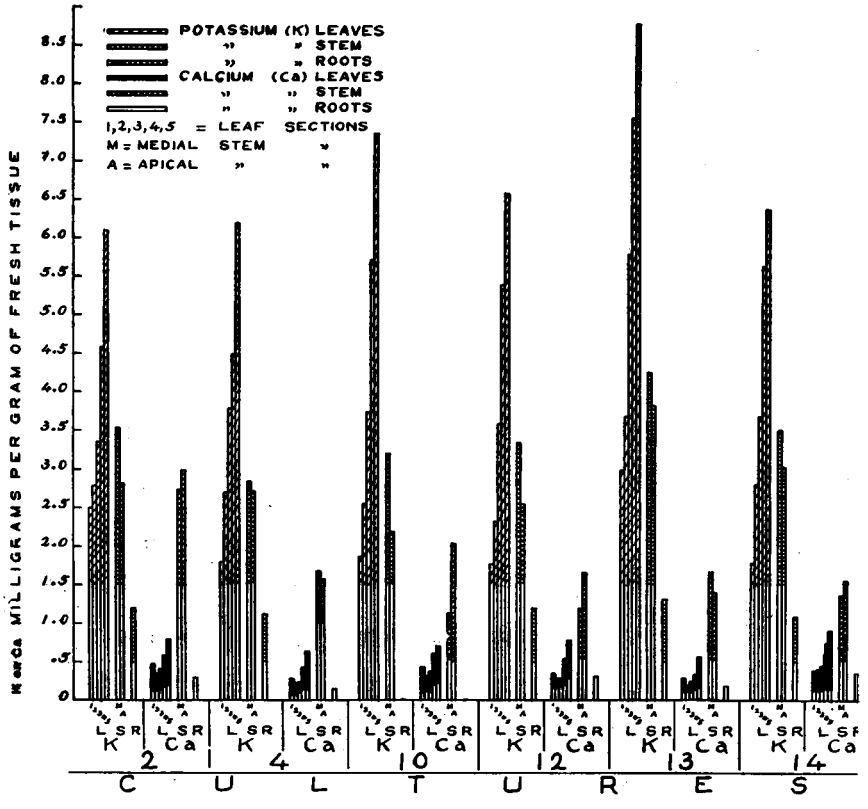


FIG. 5. Potassium and calcium in leaf (L), stem (S), and root (R) fresh tissues.

and low-Mn cultures, namely, 1.6 mg. per gram of fresh tissue equivalent to 24 per cent., was statistically significant at the P .01 level. The greater intake of magnesium by the plants of the high-Mn than low-Mn cultures cannot be explained. KELLEY (**30**) obtained similar results in 18 to 24 but not in 5 months old pineapple plants, suggesting that time is an essential factor for such accumulations.

PHOSPHORUS.—Concentrations of this element in plant tissues, in figure 6, were slightly greater for culture 13 than for the others. It is possible that the absence of iron in the solution culture might have rendered phosphorus more available to the roots than in all other cultures supplied with

iron. However, the interpretation offered for potassium, that the higher amounts of this element had resulted from concentration effects on account of smaller plant weights than from higher rates of absorption might apply equally well to phosphorus.

Products of metabolism

Chlorophyll.—Kelley (**30**) states that microscopic examination of plants grown in manganiferous soils shows fading of the green color in the

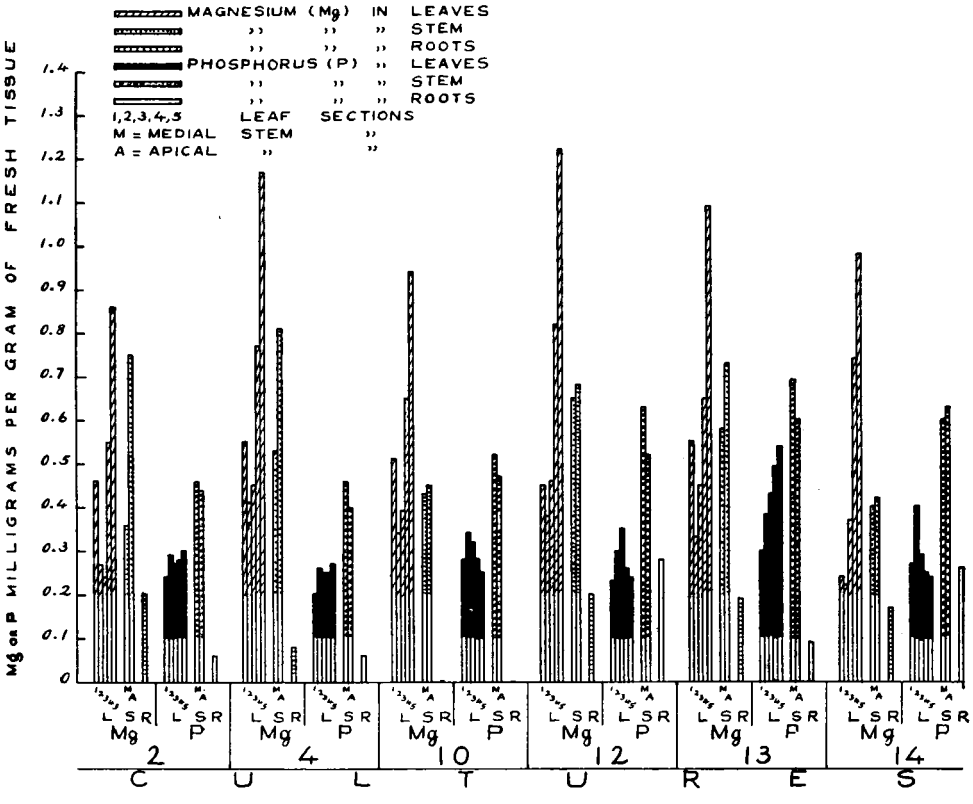


Fig. 6. Magnesium and phosphorus in leaf (L), stem (S) and root (R) fresh tissues.

chlorophyll granules, without their utter disintegration at first, but in successive stages the entire protoplasmic bodies in the cells and all organized arrangement of the protoplasm are broken up and in most advanced stages there is no trace of a granular structure, such as characterizes normal chloroplastids. He concludes that the etiolation of pineapples is caused by some fundamental change affecting the protoplasm and chlorophyll but not the yellow pigments.

The data in figures 3 and 4, the former depicting amounts of chlorophyll and the latter protein and protein-bound iron and manganese, show

that chlorophyll and protein increased with greater amounts of iron in the solution culture but decreased with greater amounts of manganese.

SIDERIS (**57, 59**) found positive correlation between chlorophyll and protein and chlorophyll and iron. The data in figure 4, revealing a similar correlation between iron and protein, suggest that iron is component

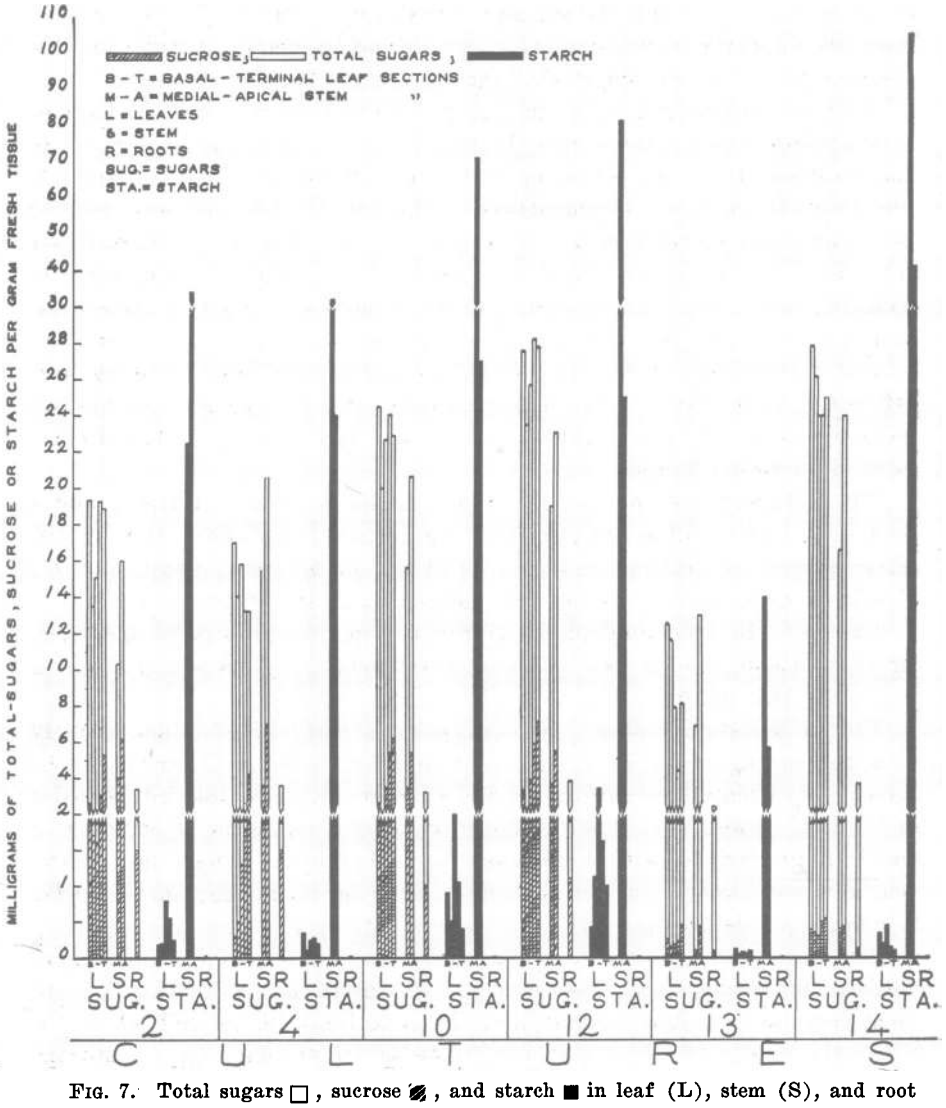


FIG. 7. Total sugars □, sucrose ▨, and starch ■ in leaf (L), stem (S), and root (R) fresh tissues.

of a system directly related to chlorophyll and chloroplastic proteins and that high concentrations of manganese in the cells, especially when iron is very low, affect adversely this system.

SUGARS.—The concentrations of total sugars and sucrose in the tissues,

in figure 7, varied for the different cultures. Total sugar concentrations were greatest in culture 12 and successively in a descending order in 14, 10, 2, 4 and 13. Culture 13, with high-Mn but minus-Fe, had considerably lower sugar than culture 4, also with high Mn but with low Fe (0.05 γ). The difference in sugars between cultures 4 and 13, namely, 41 per cent. in favor of the former and statistically significant at the P .01 level, emphasizes the importance of iron, in concentrations as small as 0.05 γ, in the acceleration of the carbohydrate synthesizing mechanism of plants.

Sucrose concentrations in the leaves depicted by the shaded portions of the columns for total sugars, in figure 7, were greatest in culture 14 and successively in a descending order in cultures 10, 2, 4, 14 and 13. The levels of sucrose concentrations in the various leaf sections were of the same order in cultures 12, 10 and 2, but not in 4 where sucrose was abnormally low in the basal leaf sections No. 1 and increased progressively from the basal No. 1 to the terminal No. 5 sections. Sucrose concentrations in the leaves of cultures 13 and 14 were extremely low, and were related presumably to iron deficiency, in the former, and to manganese deficiency, in the latter. No tests were conducted to associate the low sucrose values in cultures 13 and 14, with hydrolysis or with inhibitive processes in sucrose synthesis.

The differences of the means in total sugars between cultures 2 and 4 were very small and lacked statistical significance. However, similar differences between cultures 10 and 12 or 14 and 13 were very great, in favor of 12 and 14, and were statistically significant at the P .01 level.

STARCH.—In pineapple plants starch is deposited mostly in the stem, figure 7, although some may be found, presumably in a transitory state, in the leaves (**51, 54, 56**).

Starch deposits in the stem were greatest in culture 14 and successively in a descending order in 12, 10, 2, 4 and 13. Similar deposits in the leaves were greatest in culture 12 and successively in a descending order in 10, 2, 4, 14 and 13. The low starch values in the leaves of cultures 13 and 14 might indicate either extremely high amylolytic or very low amylosynthetic activity due, in the former, to iron deficiency and in the latter to manganese deficiency.

The data show positive correlation between sucrose and starch synthesis in the leaves with a coefficient r = .485 significant at the P .01 level. Such correlation might suggest that the conditions in the cell which favored the synthesis of sucrose did, likewise, of starch. The conditions favoring accumulations of both sucrose and starch in the leaves were adequate but not excessive amounts of both Fe and Mn.

Relationships between chlorophyll, on the one hand, and sugars and starch, on the other, were not strictly proportional. In cultures 2 and 4 with as much or more chlorophyll as in cultures 10 and 12 sugars and starch were not as high in the former as in the latter, presumably caused by variations in the physiological status of such plants as a result of dif-

ferences in the iron levels of leaves. GABRIELSEN (**15**) also observed that plants with chlorophyll levels above certain limits did not increase in photosynthetic efficiency if other synergistic factors were lacking.

ASCORBIC ACID.—The amounts of ascorbic acid, in figure 8, with ascending gradients from the basal-nonchlorophyllous to the terminal chlorophyllous sections (**50, 53, 55**) varied for the different cultures. With respect to different groups of leaves, ascorbic acid was highest in the terminal sections of the active D leaves, having lower values in the mature and less active C leaves. Similar values in the young E leaves, were lower than in the D

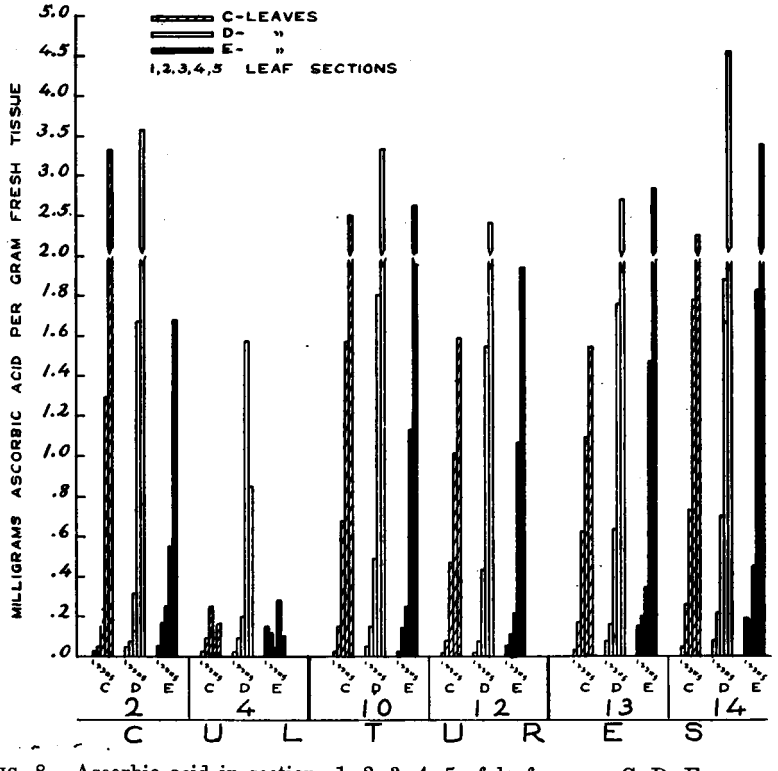


FIG. 8. Ascorbic acid in sections 1, 2, 3, 4, 5 of leaf groups C, D, E.

leaves, because ascorbic acid production, although rising, had not yet attained maximal efficiency.

Cultures 4 and 12 with high-Mn contained less ascorbic acid than cultures 2 and 10, respectively, with low manganese suggesting inhibition of ascorbic acid synthesis or oxidation by high-Mn. High ascorbic acid correlated positively with low manganese in cultures 2 and 10 vs. 4 and 12, respectively, with a coefficient of correlation $r = .61$ and $t = 3.280$, statistically significant at P .01

However, ascorbic acid in culture 13, with high Mn and minus Fe, was considerably higher than in culture 4 also with high Mn but with 0.05 γ

Fe. Moreover, in culture 13, the young E leaves contained more ascorbic acid than the active D or mature C, an anomalous order of distribution of this substance in the various leaf groups. These results suggest that ascorbic acid, like other growth promoting substances, may be utilized in plant growth and its accumulations in the young E leaves of culture 13 had presumably resulted from a lower rate of utilization than of production as attested, also, by the small plant weights of culture 13. The assumption explaining the accumulations of ascorbic acid in the leaves of culture 13 is supported by the findings of SAUBERT-V. HAUSEN (**46**) in peas with removed cotyledons, which showed that Vitamin C alone, in comparison with factors of the Vitamin B complex, biotin and yeast, caused good growth and flowering. Previous studies by SIDERIS, *et al.* (**56**) showed greater accumulations of ascorbic acid in the old B, mature C and active D but not in the young E leaves of low than high nitrogen plants, presumably resulting from a lower rate of utilization of ascorbic acid by the former due to reduced growth activity. Also, the young E leaves of minus-Fe cultures (**51**) contained more ascorbic acid than of plus-Fe cultures, due to reduced growth activity and lower rates of utilization of this substance by the former.

Negative correlation between ascorbic acid and amounts of nitrogen or combinations of nitrogen with potassium, both elements promoting plant growth, are claimed by BRACEWELL, *et al.* (**10, 11**) and WALLACE, *et al.* (**65**) in apples, HAHN and GÖRBING (**21**) in spinach, OTT (**40**), MACLINE and FELLERS (**36**), HAMNER, *et al.* (**23**) and LYON, *et al.* (**35**) in tomatoes, WYND (**67**) in sudan grass, REDER, *et al.* (**44**) in turnip greens, JONES, *et al.* (**28**) in grapefruit and SPEIRS, *et al.* (**62**) in sweet potato. In contrast with the above evidence HESTER (**24**), IJDO (**26**), ISGUR and FELLERS (**27**) and POTTER and OVERHOLSER (**41**) claim positive correlation between amounts of nitrogen and ascorbic acid.

HAMDALLAH (**22**) observed that plants grown without iron or magnesium contained as much or more ascorbic acid as with these elements. HESTER (**25**) found more ascorbic acid in plants supplied with than without manganese. ERKAMA (**14**) reported (his table 17) in pea seedlings somewhat similar results with our data in figure 8; pea plants grown in solution cultures with 100 γ Mn per liter producing smaller weights but containing greater amounts of ascorbic acid than the controls. According to ABERG (**1**), ascorbic acid production is conditioned by a light-independent synthesis, prevailing in sprouting seeds, and a light-dependent synthesis, which is probably connected with the assimilation of carbon dioxide. The same investigator, also, claims that ascorbic acid being translocateable from leaves to stem and roots, its amounts may depend on rates of translocation.

High light intensities, moisture deficiencies and high topographic elevations, presumably related adversely to plant growth, were observed to increase the ascorbic acid content of tissues (**8, 31**).

BONNER and BONNER (**9**) and DENNISON (**13**) consider ascorbic acid an essential factor in plant growth which is in agreement with our views and others cited above. Therefore, higher accumulations of ascorbic acid in the tissues of plants growing at lower than higher rates indicate non-utilized quantities in plant growth processes rather than greater rates of ascorbic acid synthesis by plants with lower rates of growth. JONES, *et al.* (**28**) have the same point of view of ascorbic acid accumulations in their diagram on possible factors related to the formation and utilization of ascorbic acid.

Discussion

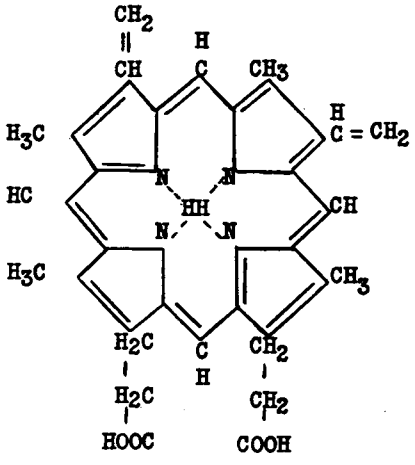
The above results, disclosing that the chloroplastic protein-chlorophyll system may be affected either favorably or adversely by different iron-manganese ratios, suggest that iron may be a component part of this system and the presence of manganese in the chlorophyllous cells in greater amounts than required presumably antagonizes the assimilation of iron by this system or interferes with the physiological functioning.

GRANICK (**18**) investigating the brown color of the cells of an x-ray mutant of *Chlorella vulgaris* found protoporphyrin 9 to be a metabolic precursor of chlorophyll. Protoporphyrin 9 has the basic plan of the ring structure and similarities of the side chain patterns of chlorophyll with Fe instead of Mg atom attached to the four N atoms of the pyrrol ring.

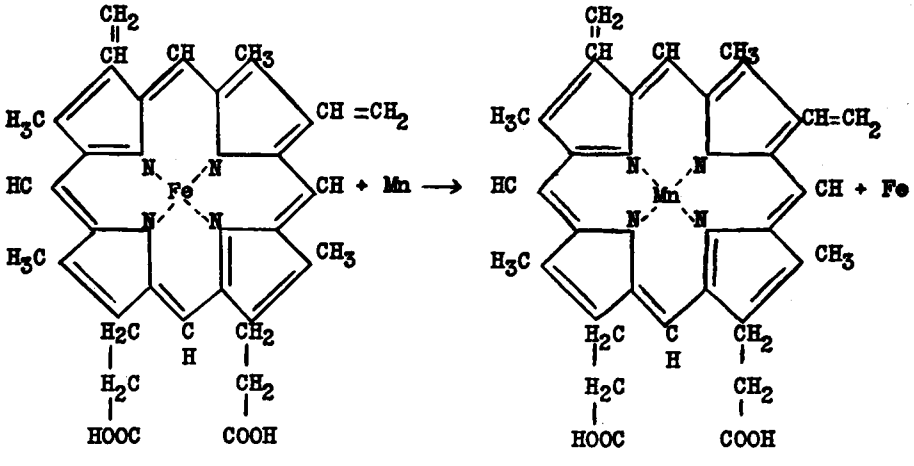
The relation of protoporphyrin 9 to chlorophyll, although shedding light on the role of iron in the synthesis of chlorophyll, does not explain satisfactorily the physiological mechanism of Mn in the development of chlorosis.

Porphyrins are basic structural units of the cytochromes, peroxidases, oxidases, etc., in addition to chlorophyll and, consequently, intimately associated with many essential physiological processes in the cell. They may undergo, *in vitro,* replacement of the Fe atom by other chemically related elements, according to TAYLOR (**63**), and exhibit similar or different properties. The preparations, in the laboratory by GJESSING and SUMNER (**17**), of the metalloprophyrins of Co, Ni, Mn and Cu, by substitution for Fe in the hemin from defibrinated cows' blood, were inactive except the manganese protoporphyrin which had 20 to 30 per cent. as much peroxidase activity as the iron protoporphyrin.

BAUDISCH (**4**) explains the physiological activity of the metal atom in the pyrrol ring as due to paramagnetic properties generated by the arrangement of a positive ion (Fe, Mn or Cu, etc.), in the center, surrounded by neutral or negative atoms (N) which tend to gain electrons by coordinating atoms, forming functional units in definite energy stages of electric or magnetic fields. Hence, any changes in the structure of porphyrins, which may result from replacement of Fe by Mn or other metals, might either increase or decrease the activity of the prosthetic substance, be it an enzyme or chlorophyll, and alter the rhythmic operation of processes on which depend most cell functions.

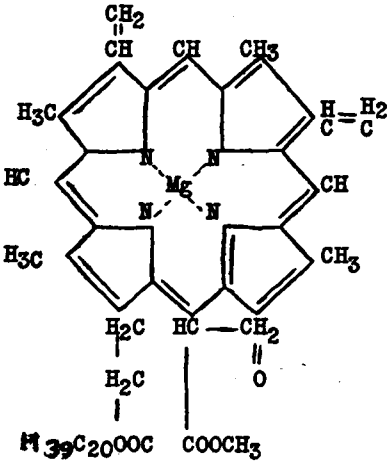


Protoporphyrin



Iron Protoporphyrin

Manganese Protoporphyrin



Chlorophyll∝

The findings of GRANICK (**18**) that protophyrin 9 is the precursor of chlorophyll, and of GJESSING and SUMNER (**12**) that substitutions of Mn for Fe, *in vitro,* are possible, suggest that similar substitutions may be possible in cells with extremely low-Fe but relatively high-Mn concentrations. GRANICK (**19**) reported lately that vinyl groups are necessary in protoporphyrins for the insertion of iron and that the latter is inserted only in completed porphyrin rings, by Haemophilus organisms, making implausible any consideration that porphyrin might be synthesized in piecemeal fashion around the iron atom.

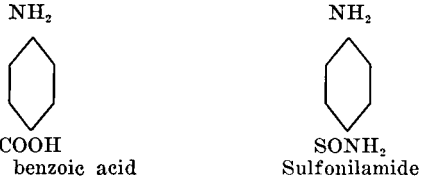
Our data in figure 4 showing positive correlation between protein-N and iron and negative correlation between protein-N and manganese suggest possible association of a protein prosthetic group containing iron, the formation of which and that of the associated protein are limited in the presence of very small amounts of iron and high ones of manganese. There are many known proteins and enzymes in association with prosthetic groups containing iron or other metals, such as haemoglobin, chlorocruorin, helicorubin, catalase, peroxidase and cytochrome, with iron, turacin with copper and chlorophyll with magnesium. In all these proteins and enzymes the prosthetic group containing the metal is a porphyrin.

The findings of GRANICK (**18, 19**), TAYLOR (**63**) and GJESSING and SUMNER (**12**) indicating conversion of porphyrins to chlorophyll and replacement of the metal in the pyrrol ring by other metals suggested a hypothetical mechanism of plant chlorosis resulting from high Mn in the presence of low Fe concentrations in plant tissues. This mechanism presumably associated with plant chlorosis due to Mn or other metals but not to nitrogen, may result from substitutions of Mn or other metals for 2H or Fe in the pyrrol ring of the protoporphyrin molecule, shown below, in cells containing inadequate amounts of Fe necessary to combine with the continuously forming protoporphyrin molecules necessary for the subsequent synthesis of chlorophyll. It may be further assumed that chlorophyll synthesis, if effected by substitution of Mg for Fe in protoporphyrins, releases protoporphyrin bound iron which is successively replaced by Mg and allowed to combine with newly forming protoporphyrin molecules. Thus, iron performs, in accordance with this assumption, the role of a catalyst which is in agreement with all theories heretofore advanced.

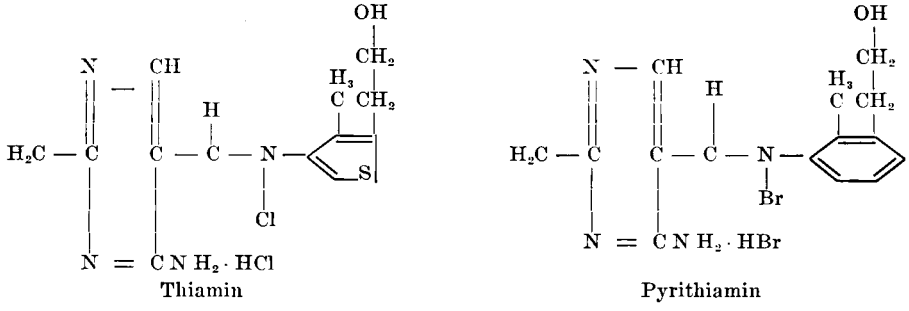
In line with the above thought, MILLIKAN (**39**), discussing the evidence obtained recently from experiments which indicated that the inhibition of symptoms of lower leaf scorch in flax by molybdenum was presumably caused by high manganese concentrations in the soil, states, also, that an excess of Mn, Zn, Co, Cu or Ni will induce iron deficiency chlorosis in plants. However, no explanation is offered on the nature of the antagonism between Mo and Mn whether resulting from the repelling forces of the ions Mn^{++} vs. Mo^{++} and MnO_4^{--} vs. MoO_4^{--} or from precipitation of Mn^{++} by MoO_4^{--}.

Replacement of Fe by Mn in protoporphyrins may produce physiolog-

ically inactive substances similar to those discussed by WOOLLEY (**66**) in his review of biochemical antagonisms where the substitution in a basic chemical structure, protein or vitamin, of a prosthetic unit for another changes completely the physiological activity of said structure. For example, the inactivation of p-aminobenzoic acid by sulfonilamide, an analogue of the former in which the carboxyl group is replaced by sulfonamide group, shown below, is effected by the sulfa drugs displacing p-aminobenzoic acid from its combination with specific proteins and thereby inactivate enzymes important in the growth of organisms.



Also, a similar case of biological antagonism is the inactivation of thiamin by pyrithiamin, an analogue of thiamin, in which the thiazole ring is replaced by a pyridin ring, or, more specifically the S by — CH = CH —, as follows:



The biochemical antagonism of Mn vs. Fe may be in many respects analogous to the antagonisms, mentioned above, of sulfa drugs vs. p-aminobenzoic acid or of pyrithiamin vs. thiamin.

The data, in figure 1, showing that plant weights increased progressively with the amounts of Mn from 0.05 to 5.0 γ, suggests certain beneficial or synergetic effects of Mn. Such effects, pertaining to agriculture, in general, were reviewed by MCHARGUE (**38**).

Investigations on the physiological functions of manganese indicate that it is an activator of enzymes. ADLER *et al.* (**2**) observed that Mn and Mg but not Ca, Zn or Cd activated coenzyme II for the oxidation, by the isocitric enzyme, of isocitrate to α Keto-β-carboxylglutarate; a reaction of paramount importance in plant metabolism, because it associates the function of the isocitric dehydrogenase system as a link between carbohydrate breakdown and protein synthesis as follows: carbohydrate ⟶ isocitrate⟶ α Ketoglutarate + NH_3⟶ α-iminoglutarate ⟶ glutamate. SHILS and MCCOLLUM (**24**) found that Mn-deficient rats con-

tained considerably reduced arginase activity in their liver, and were sterile. LIPMAN (**33**) states that Mn or Mg are essential factors for the catalysis of pyruvic acid dehydrogenation with oxygen as hydrogen acceptor. Both Mn or Mg, according to the same author (**33**), are essential components of the carboxylase system of Lohmann, whereby the function of thiamin is to catalyze the decarboxylation of pyruvic acid to acetaldehyde and CO_2.

Studies on the physiological functions of Mn in plants have not been as extensive as in animals. BURSTRÖM (**12**) and LUNDEGARDH (**34**) obtained information indicating that Mn accelerates the rate of respiration of and intake of nutrients by roots. BERTRAND (**6, 7**) has associated manganese with the composition of related oxidizing enzymes, such as laccase and oxidase.

The allegation that Mn favors the intake of mineral nutrients by roots from solution cultures or soil deserves greater substantiation as to quantities of Mn in various substrata and conditions of plant growth. KELLEY (**30**) claims that more lime (CaO) was taken up by pineapple and other plants from manganiferous than non-manganiferous soils without making allowance for the greater lime content of the former than latter soils. Manganiferous soils, because of their colluvial and alluvial origin, contain greater amounts of water soluble mineral elements and may be less acidic than adjacent non-manganiferous soils. The higher base status or percentage base saturation resulted in many but not in all cases from lower rainfall and less leaching of bases. KELLEY (**30**) shows that average per cent. composition of 10 manganiferous and 9 non-manganiferous soils, respectively, was: K_2O, 0.81 and 0.56; Na_2O, 0.37 and 0.28; CaO, 0.53 and 0.30; MgO, 0.45 and 0.40; Mn_3O_4, 4.95 and 0.35; Fe_2O_3, 22.2 and 26.0; Al_2O_3, 16.93 and 13.6; P_2O_5, 0.19 and 0.09; SO_3, 0.20 and 0.21; TiO_2, 0.87 and 2.22, etc. These figures clearly indicate that the greater uptake of Ca by plants from manganiferous than from non-manganiferous soils should be attributed to the greater Ca content of the former than the latter soils rather than to the influence of Mn. Nitrification, which is higher in manganiferous than non-manganiferous soils, might enhance the uptake by roots of more Ca^{++} from the former soils, because NO_3^- favors whereas NH_4^+ inhibits the absorption of both Ca^{++} and Mn^{++}.

Plant weights, sugars or starch and chlorophyll, all three or four presenting a chain of interdependent links, were best in cultures 7, with 5.0 γ Mn and 0.5 γ Fe, and in 11 with 5.0 γ Mn and 5.0 γ Fe. Such concentrations, although ranging in iron-manganese ratios from 1:10 to 1:1, respectively, were satisfactory because they were outside the deficiency or toxicity limits for Mn or Fe.

Manganese toxicity resulting in cultures with very low but not moderate amounts of iron may cause growth stunting or death to plants treated with high concentrations of this element. Eight of the plants in culture 4 and fourteen in culture 13 of a total number of 16 plants per culture died

at or before floral differentiation, and the fruits produced by the surviving plants were small and of poor quality. In manganiferous soils, where iron limitations are not as great as in solution cultures, plants may not die but produce fruits which often are shell chlorotic and split before or at ripening.

Ascorbic acid being higher in cultures 2 and 10 with 5.0 γ Mn and not in 4 and 12 with 50.0 γ Mn suggests possible partial inhibition of the formation of this substance under high Mn conditions. However, in culture 13, with equal amounts of Mn as in 4, ascorbic acid was high, which was contrary to our expectations, but this condition was explained as resulting from a decreased rate of utilization in plant growth which is evidenced by the small plant weights of this culture.

In previous studies (**58**) comparable results, as with ascorbic acid, were obtained in the utilization of sugars for plant growth; small plants containing greater concentrations of sugars due to lower rates of utilization than large plants. Under optimal conditions of plant growth the amounts of ascorbic acid in the leaves presumably should correlate with the sugars because the synthesis of the former depends on the latter. But under adverse conditions where growth and carbohydrate synthesis are interrupted, as in culture 13, the production of ascorbic acid from sugars, may continue to operate, whereas growth which depends on the utilization of both sugars and ascorbic acid, may stop and certain substances, such as ascorbic acid, accumulate.

Ascorbic acid accumulations under adverse growth conditions are associated more with cultures supplied with nitrate than ammonium salts as sources of nitrogen. For example, ascorbic acid accumulated more in the minus-Fe than in the plus-Fe cultures of the nitrate series, whereas, the levels of ascorbic acid, in the ammonium series, were approximately equal for the minus-Fe and plus-Fe cultures. Also plant weights in the latter series were approximately equal for both cultures. Although such results suggest that ascorbic acid might take part in nitrate reduction in the chlorophyllous tissues of pineapple leaves where the former is very plentiful and the latter disappears rapidly presumably after reduction and subsequent assimilation under optimal light and temperature conditions, the experimental evidence is more in favor of the utilization of ascorbic acid in growth than in nitrate reduction. However, certain preliminary tests, *in vitro,* employing leaf sap, ascorbic acid KNO_3 and toluene, showed in the course of 12 hours little or no nitrate reduction, but very pronounced reduction in the course of a week. These tests, although undecided, might have yielded different results had the conditions for the ascorbic acid-nitrate oxido-reduction system been better understood.

Summary

Pineapple plants grown in solution cultures with different concentrations of iron (0.05, 0.5 and 5.0 γ) and manganese (0.05, 0.5, 5.0 and 50.0 γ)

in various combinations and supplied with equal amounts of all essential elements and nitrogen as nitrate- or ammonium-salts developed under the various conditions, as follows:

1. Most satisfactory growth, as measured by plant weights, was obtained in culture 7 with 0.5 γ of Fe and 5.0 γ of Mn. The cultures with 0.05 γ Fe and 0.05 γ Mn or with 0.05 γ Fe and 50 γ Mn produced smaller plant weights presumably of Fe and Mn deficiency for the former or Mn toxicity for the latter. The plants of cultures minus-Fe failed to produce fruits and most died.

2. The concentrations of both iron and manganese increased in the tissues in proportion with the amounts in the nutrient solution of the cultures supplied with nitrates. In the cultures supplied with ammonium similar concentrations of Mn in the tissues were almost one half as great as those of the nitrate cultures, due presumably to antagonism between Mn^{++} and NH_4^+. Iron concentrations were greater in the tissues of the ammonium than of the nitrate cultures.

3. Certain amounts of iron and manganese in the leaf tissues were in combination with definite protein fractions; the amounts of protein increasing with iron but decreasing with manganese.

4. The tissues of the high-Mn cultures contained more magnesium than of the low-Mn cultures; but other elements, such as potassium, calcium and phosphorus were taken up from the nutrient solution at approximately equal rates by high-Mn or low-Mn cultures.

5. Sugars and starch were high in cultures with good growth and ample chlorophyll, but the latter was not proportionally related to the former in all cultures.

6. In cultures supplied with iron, ascorbic acid was higher in those with low-Mn than with high-Mn. But in the minus-Fe and with high-Mn cultures ascorbic acid accumulated in the young leaves. This condition was explained as resulting from lower rates of utilization due to decreased rates of plant growth.

7. The development of plant chlorosis from high manganese is explained by the hypothesis of biochemical antagonism of homologous substances, whereby manganese is presumably substituted for iron in protoporphyrin 9, the chlorophyll precursor, thereby inactivating the latter for subsequent conversion to chlorophyll.

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