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Determination of iron in nutrient solutions and its role in plant metabolism

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DETERMINATION OF IRON IN NUTRIENT SOLUTIONS

AND ITS ROLE IN PLANT METABOLISM

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Outline of Thesis

I. Purpose . . . . . page 3

II. Review of the Literature 4

1. Meaning of the Terms Used in Nutrient Solution Work 4

2. Significance of Iron 10

3. Factors Influencing the Hydrogen Ion Concentration 18

4. Discussion 33

Role of Iron in Plant Metabolism 45

1. Relationship of Potassium, Nitrogen and Iron in Assimilation 45

2. Nature of Light 48

3. The Action of Light on the Oxides of Nitrogen 50

4. The Nature of the Molecule of Potassium Nitrate and its Reduction 52

5. The Mechanism of the Reduction of Nitrates and the Properties of Iron Complexes . . . 53

III. Determination of Iron in Nutrient Solutions . 53

1. Volumetric Determination 58

a. Problem 58

b. Apparatus 60

c. Preparation of Standards and Materials Used 62

d. Volumetric Method in Nutrient Solutions 65

e. Standardizations of Permanganate . . . page 69

f. Washing of Precipitates 76

g. Determinations of Iron 78

2. Colorimetric Estimations 90

a. Problem 90

b. Apparatus and Procedure 90

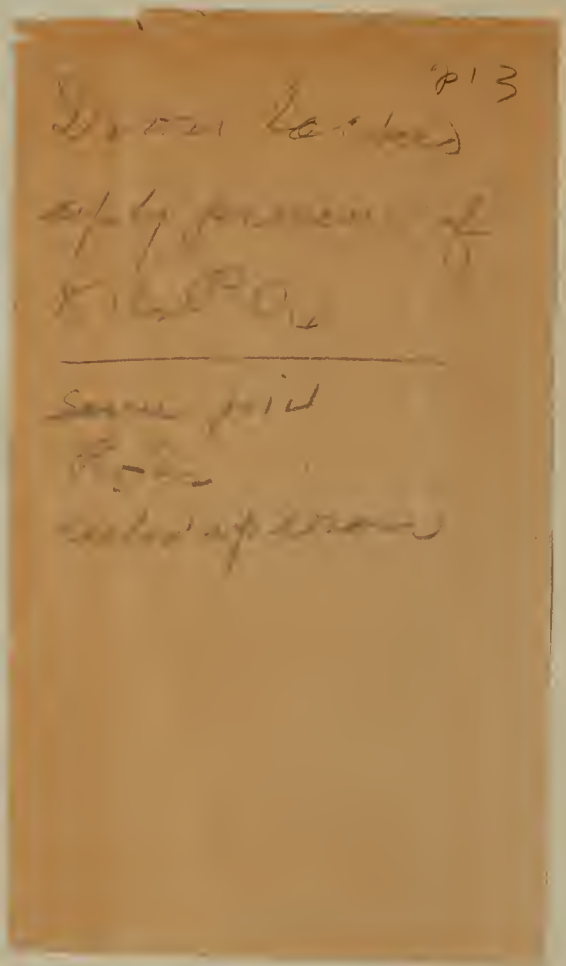
c. Studies on Interference 91

d. Data and Discussion 95

IV. Summary 102

V. Bibliography 104

Acknowledgements 113



PURPOSE OF THE INVESTIGATION

Iron is a very important element in plant nutrition. This was recognized by the first successful investigators in this field. In solution culture methods of research it is well known that growth in plants may be inhibited by an insufficient supply of available iron. To control this available supply of iron is apparently the most difficult of any of the nutrient elements. The quantity lies within relatively narrow limits; both too much, and an undersupply produce physiological disturbances in the plant which is indicated by chlorosis. Among the component salts of the culture solutions are those which produce very insoluble iron compounds. The solutions now considered "best" soon lose most of the iron so that frequent renewal of solution is necessary to keep up the supply of iron.

Since no satisfactory method for the determination of the iron present in such a system in unstable equilibrium has been produced it was undertaken to develop one and use it. This method, as far as it has beendeveloped, will be presented after a consideration of the solubility of iron in nutrient solutions, and the role it plays in the reduction of nitrates.

REVIEW OF LITERATURE

That iron is physiologically important has been proved by many demonstrations. The quantity in culture solutions necessary for normal plant growth varies with different forms of its salts, particularly between the ferric and ferrous forms. Hoagland[36]+ states that the presence of dissolved iron in culture solutions depends on the concentration and reaction of the solution and the time of standing. These three solution factors together with its physiological significance will be brought out by a review of the literature. Before this review is taken up some of the terms used in the description of culture solutions will be explained.

Meaning of the Terms Used in Nutrient Solution Work. We shall try, as far as possible, to bring together the references bearing on each of the previously mentioned solution factors. Often, however, the results of one investigation have a relationship to the others. Hydrogen ion concentration, solubility and rate of reaction are mutually influential and are treated separately for convenience only.

Hydrogen Ion Concentration. The reaction or acidity of a solution is determined by its hydrogen ion concentration. It is only the ionized hydrogen which is responsible for this. An equivalent weight of any acid contains one gram of hydrogen. Potentially all normal acids contain the same amount of hydrogen ions but, as a matter of fact, all acids do not ionize to the same extent so that the amount of hydrogen ions present in solutions of different acids vary.

(+) A bibliography of the literature referred to is given at the end of the thesis.

For instance hydrochloric acid, which is a strong acid, ionizes 97 per cent at .001 N (33), and acetic acid, which is a weak acid ionizes 13.6 per cent at the same normality. Hence the amount of ionized hydrogen in hydrochloric acid is seven times as great as in acetic acid, although their neutralizing power is the same. Both have the same capacity, but the former has the greater intensity.

To illustrate this intensity factor, we may consider a tenth normal solution of hydrochloric acid. Assuming that it would completely ionize, the concentration of the hydrogen ions would be 0.1 gm. in 1000 cc. This is conveniently expressed as a logarithm: $\log_{10} 10 = 1$. It has been suggested by Sörensen to express the hydrogen ion concentration as the exponent to the base 10 with the negative sign omitted. Hence the hydrogen ion ($\overset{+}{H}$) concentration of completely ionized N/10 acids would be expressed; pH = 1, or the normalities of acids as follows:

N	= pH0	$N/1 \times 10^{5}$	= pH5
N/10	= pH 1	$N/1 \times 10^{6}$	= pH6
N/100	= pH2	$N/1 \times 10^{7}$	= pH7
N/1000	= pH3	$N/1 \times 10^{10}$	= pH10 etc.
N/10000	= pH4		

This shows that the greater the hydrogen ion concentration the lower is the pH value. Often when the ($\overset{+}{H}$) concentration is referred to in later references it will be represented by the expression pH, meaning the logarithm of its reciprocal.

There are two methods of determining this ($\overset{+}{H}$) concentration. One, the electrode method, is a measure of the difference in electrical

potential between the hydrogen atom and the hydrogen ion. The hydrogen atom has the positive charge of electricity in its nucleus balanced by a negative charge in a surrounding orbit, while the hydrogen ion is the single positive charge. The atom has become positive by the loss of one electron. The negative charge of the atom has been carried away from the immediate proximity of the positive charge to a negative radicle of some other ion. This difference in voltage between the hydrogen ion and atom can be measured by a potentiometer and from it the pH value computed.

A second method of determining the ($\dot{H}$) concentration is by color. A series of chemical substances, indicators, have been synthesized whose colors depend upon the prevailing hydrogen ion concentration. Within certain limits each indicator changes its color with a slight change in acidity. When it is desired to take the acidity of any solution a few drops of indicator are added to the solution, and the color matched with a solution of known acidity containing the same amount of indicator.

Buffers. We shall also have occasion to refer to buffers and buffer action. Hydrogen ion concentration is of great importance in the growth and metabolism of plant and animal cells. To maintain the optimum or desirable acidity the plant fluids contain salts which resist its change. At least one salt of every good nutrient solution has this property. In our consideration of ($\dot{H}$) concentration we said that 0.001 N acetic acid was only 13 per cent ionized, which means that 87 per cent of the hydrogen ions are inactive or carry no charge independently. As soon as some of the 13 per cent of ($\dot{H}$)

ions are neutralized some of the undissociated 87 per cent dissociatesto maintain the equilibrium;

$$\underset{87\%}{CH_3COOH} \rightleftharpoons \underset{13\%}{CH_3COO} + \underset{13\%}{H}$$

when equilibrium has again been reached the total amount of $(\overset{+}{H})$ ions has slightly decreased, but the per cents dissociated, and hence the $(\overset{+}{H})$ concentration, remain the same. Generally the salt of a weakly ionized acid is used with that acid. Upon the addition of a hydrogen ion the acid radical from the highly ionized salt unites with the hydrogen ion to form the little ionized acid, and the original pH remains.

Concentration. A growing plant responds to both the chemical and physical properties of the nutrient solution in which it grows. Toxicity and hydrogen ion concentration are chemical properties while concentration in terms of osmotic tension is a physical property. This concentration may be expressed in four ways (48): (1) volume molecular salt proportions; (2) osmotic salt proportions (3) total volume-molecular concentration; and (4) total osmotic concentration.

From the study of gases, we learn that a molecular weight of a gas, at standard conditions, in a volume of 22.4 liters exerts a gas pressure of one atmosphere. This pressure is always caused by the same number of molecules or particles regardless of what gas is used. This general principal has also been applied to dilute solutions. A molecular weight of any chemical compound in a gram molecular volume (22.4 L.) gives an osmotic pressure of one atmosphere.

Osmotic pressure is of great interest in connection with the physiology of plants and animals, and has been used as a means of expressing the concentration of nutrient solutions by plant physiologists. The osmotic concentration is proportional to the total number of particles; ions, molecules, hydrated molecules, associated molecules, etc. Tottingham (87) derived the osmotic concentration by dividing the percentage of a salt by the percentage value of the molar solution of the salt considered and multiplying the quotient by the osmotic factor. The osmotic factors were taken from the table of conductivity measurements as determined by Jones in 1912 (44). When a compound of two ions dissociates completely the osmotic factor is two. When it ionizes 50 per cent, the factor is 1.5. Then the osmotic concentration is

$$\frac{\text{per cent of salt used}}{\text{per cent salt in a molecular solution}} \cdot \text{osmotic factor}$$

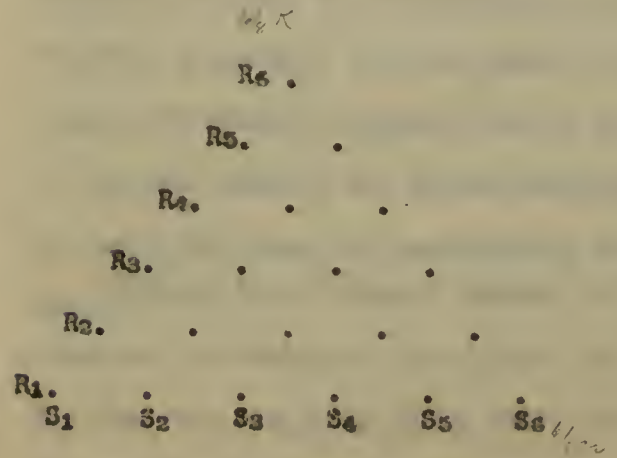
A convenient method of stating concentrations in nutrient solutions is the so-called total volume molecular concentration. It is found by dividing the number of cubic centimeters of a molecular solution of each salt of a nutrient solution used by 1000; or, it expresses the concentration of any single salt in terms of the number of cubic centimeters of a molar solution. This can be reduced to atmospheres by the above formula. The total osmotic concentration is proportional to the total number of particles per liter and is expressed in atmospheres.

Composition. The National Research Council in an attempt to arrive at the best solutions for culture work asked the cooperation of all workers in this field. In order to obtain comparable

results uniform cultures and methods of culture had to be followed. From the six radicals containing the inorganic salts essential to plant life, K, Ca, Mg, NO_3, H_2PO_4, and SO_4, six types of solutions, each containing three salts, have been developed and are shown by the arrangement below:

Type I	II	III	IV	V	VI
$Ca(NO_3)_2$	$Ca(NO_3)_2$	$Ca(H_2PO_4)_2$	$Ca(H_2PO_4)_2$	$CaSO_4$	$CaSO_4$
KH_2PO_4	K_2SO_4	KNO_3	K_2SO_4	KNO_3	KH_2PO_4
$MgSO_4$	$Mg(H_2PO_4)_2$	$MgSO_4$	$Mg(NO_3)_2$	$Mg(H_2PO_4)_2$	$Mg(NO_3)_2$

There is possible an infinite number of sets of proportions for each type, but to gain simplicity and uniformity 21 were chosen. By using solutions with a total osmotic concentration of one atmosphere and letting the volume-molecular partial concentration of each salt differ from solution to solution by increments of one-eighth of the total volume molecular concentration, the composition can be conveniently represented by triangular diagrams as first used by Schreiner and Skinner (81).



In the triangle let the base line represent an increment of the potassium salt, and let the left side represent one for the calcium salt. The right side then represents the magnesium salt. Each of the three

base lines represents a row of solutions in which the salt after which the base line is named gives one-eighth of the total volume molecular concentration. For instance, R_1 means that one-eight of the total volume molecular concentration is given by the potassium salt; S_2 that two eighths of the concentration is given by the calcium salt. In referring to a solution the Roman numeral designating the type is first wirtten, then the row R (K salt) followed by the Arabic numeral (number of increments stated in eighths). The calcium salt is then designated by S, and its concentration by a numeral. This places the solution on the diagram. III, R_2S_5 is a solution of type III ($Ca(H_2PO_4)_2$, KNO_3 and $MgSO_4$), two-eighths of the total volume molecular concentration being KNO_3, five eighths $Ca(H_2PO_4)_2$ and one-eighth $MgSO_4$. These designations will often be referred to throughout this work. In some solutions increments of tenths are used instead of eighths.

Significance of Iron in Nutrient Solutions. Form of Iron.

The significance of iron in plant nutrients was recognized as early as 1843, when Gris (32) concluded that the absence of iron inhibits the formation of chlorophyll. He also found that even in the presence of iron, the formation of chlorophyll may be partially or entirely suppressed when the plant is in an unhealthy condition. Sachs (80) brought chlorotic leaves back to a healthy color by means of ferrous sulphate and ferric chloride. In his culture solutions (79) he found that iron was precipitated and that ferrous sulphate was the best form of iron to use. Stohman (84) used ferric chloride in a solution containing no acid salts, and found that it turned alkaline. The iron was precipitated as ferric sulphide. Knop (45) in his first standard formula used ferric phosphate. Later many investigators

confirmed this. Lucanus (49) used ferric phosphate for leguminous plants. Crone (20) noted that excess of soluble phosphates induced chlorosis even with a liberal supply of ferrous sulphate. Also that ferrous sulphate was toxic while ferrous phosphate was favorable to plant growth. He, however, used an excess of the latter so that some undissolved salts were present. From this Crone concluded that no nutrient solution without undissolved salts was possible. Hartwell and Pember (34) observed that ferrous sulphate was toxic to barley and rye and was readily oxidized to ferric sulphate by the roots of these seedlings. Tottingham (87) in using ferric phosphate secured better yields with potassium dihydrogen phosphate than with the mono-hydrogen phosphate. Arndt (2) did not find ferric phosphate a suitable source of iron for corn grown in solutions of Type I. Mazé (54) concluded that chlorosis and growth of the plant depended on the relative proportions of all elements in a nutrient solution. Iwatsu (6) believes that ferrous salts produce greater physiological activity than ferric compounds. Arndt (2) further found that the optimum concentration of ferrous sulphate for corn was 0.0005 N or about 14 mgm. iron per liter. Twice as much ferric nitrate was required. The ferric nitrate became toxic before it furnished sufficient available iron for normal growth. In other cultures the availability of iron, as shown by growth, varied with the composition of the solution. Increase of ferric phosphate in the solution, which he called solution "A", gave increased growth, while increase of iron in solution "H" did not increase the yield. Thirty-five mgms. of ferric phosphate per liter were insufficient for growth of corn which

is greater than that required for wheat.

Solution "H" had the following composition:

$Ca(H_2PO_4)_2$	0.00005 M	$MgSO_4$	0.0008 M
$Ca(NO_3)_2$	0.0015	$Al_2(SO_4)_3$	0.000003
NH_4NO_3	0.001	$MnSO_4$	0.00001
KCl	0.0008	$ZnSO_4$	0.000005

The pH was 4.7 both with and without addition of ferrous sulphate over a range of normality of 0.00005N - 0.008N. The pH of the solution when ferric nitrate was added varied as follows:

0.00005N	-	pH 4.7
0.0005	-	3.9
0.001	-	3.5
0.002	-	3.2

The composition of Solution "A"

KH_2PO_4	0.0024M
$Ca(NO_3)_2$	0.0036M
$MgSO_4$	0.0035M

An increased amount of ferric phosphate in Solution "H" did increase the yield. Arndt further states that toxicity must be due to some other cause than acidity since ferric salts are more hydrolyzable than ferrous salts although they are less toxic.

Lack of iron does not normally depress root development as it does tops. In the article of Arndt the following suggestions are made: "Something in the solution "A" prevents the plant from absorbing iron. This is not due to any inherent property of the ferric phosphate. When in solution "H" the calcium acid phosphate is replaced by an equivalent molecular weight of ferric phosphate, the growth relative to the control was; tops, 95 per cent; roots, 68 per cent, and transpiration 96 per cent. The plants were well developed.... The behavior of ferric phosphate when formed as a

precipitate from adding ferric nitrate to solution "A", was interesting. A 0.001N concentration did not produce plants of normal green color. A 0.002N concentration was decidedly toxic and the ferric salt gives the better yield. This greater physiclogical activity of the ferrous iron may be correlated with its greater influence on catalytic activities. Other factors may be concerned. The ferrous salt is less easily precipitated than the ferric. There may be a difference in the solubilities of the two phosphates which may be influenced by the concentration of the phosphorus and calcium in the solution. Any attempt at an explanation of this difference can be only speculative until more data are obtained."

Jones and Shive (43) after an intensive comparison of the effects of iron salts reported greater differences are to be found with different forms of iron supplied to the plant in a Tottingham solution than any other factor. Ferric phosphate in quantities less than one mgm. of iron per liter of nutrient solution was not sufficiently available in the Tottingham solutions to supply the needs of the plants for iron during the early stages of growth. On the other hand, this form of iron in quantities of less than one-half mgm. of iron per liter of solution was ample to supply the needs of the plants for this element in the same solutions containing ammonium sulfate. Ferrous sulphate in quantities from .25 to .50 mgm. per liter was sufficiently available in the Tottingham solution to satisfy the needs of the plant for iron. However, ferrous sulfate in the solution containing ammonium sulfate produces a condition very toxic to the plants, the degree of toxicity

increasing with increase in the amounts of iron from .25 to 5 mgm. per liter of nutrient solution. The availability of an iron compound to the plant appears to be determined in large measure by the composition of the nutrient solution and by the nature of the reaction change induced by contact with the plant roots.

These workers (40) had previously found ferric phosphate unsuitable for use in Shive's solution, R_2C_2. Ferrous sulphate gave excellent results when used. When ammonium sulfate is used as one-half of the source of nitrogen instead of potassium nitrate the reverse was true. They believe that the greater availability of iron may be due to the greater acidity caused by the addition and differential absorption of ammonium sulfate or by the effect of ammonium sulfate upon the permeability of the absorbing membrane. Ferrous sulfate was also found by them (43) to be a better source of iron than ferric phosphate for soy beans under similar conditions. Corson and Bakke (18), using both ferric and ferrous phosphates as sources of iron in nutrient solutions, found differences in their efficiency and also differences in response by different plants. Gile and Carrero (28) in a study of the influence of iron upon rice found that reaction, concentration, form and amount of iron have a marked effect upon its availability. In making determinations for the solubility of iron they found less than one part per million after a period of 120 hours. Plants growing in a solution reduced the quantity of iron present. Ferric citrate was more soluble in neutral solutions, while ferrous sulfate was more soluble in acid solutions. They considered iron to be present in one or more of the following forms: (1) as precipitated ferric phosphate and ferric hydroxide;

(2) as colloidal ferric hydroxide; (3) as soluble undissociated iron compounds, and (4) as ionized iron." An equilibrium was perhaps existing between these forms of iron, as more was precipitated from the filtered nutrient solution on longer standing. From the determinations of iron in the filtered solutions it was evident that more or less than half the iron was precipitated as phosphate and hydroxide. The greater part of the remaining iron was probably present as colloidal ferric hydroxide. The available iron, which included the soluble undissociated and ionized iron, was undoubtedly extremely small and was governed chiefly by the completeness of the hydrolysis of the dissolved iron. They say further; "The amount of iron hydrolyzed would depend on the reaction of the solution, being less in acid solutions, and would also depend on the form in which the iron was added, being less with the less ionized organic salts. The effect of the form of iron and the reaction of the solution on the assimilation of iron by rice is thus easily comprehended.

"When judged by the growth of plants ferrous sulfate, ferric nitrate and ferric tartrate afforded sufficient iron when used in proper quantities in the acid or neutral solutions. Ferric chloride was an inferior source of iron and dialyzed iron utterly inadequate. Only ferric tartrate furnished sufficient iron in alkaline solution."

These writers cite Crone (21) as observing chlorosis when soluble phosphates were added to a solution in which ferric phosphate was the source of iron.

Toxicity. Turning our attention to toxicity we find that Wolff (93) considered iron to be a catalyst, acting very advantageously in the growth of barley. Only a small part is absorbed by the plant.

In his experiments neither nickel nor chromium could replace iron, Miyake (61), by comparing the effects of the same normalities of aluminium chloride and hydrochloric acid upon plant growth, concluded that toxicity was not caused by acidity. Kuprecht (76) reported toxicity of ferrous sulfate to clover seedlings when more than four parts per million of iron was present. Hartwell and Pember (34) found ferrous sulfate toxic to barley in Knop's solution in quantities of five parts of iron per million or more. Rothert (74) found the toxicity of salts dependent upon the method of application. They were most toxic when used alone in distilled water, less toxic in Knop's solution and least in soil solutions. Maquenné and Demoussy (51) found that the addition of calcium salts and phosphates to toxic solutions of both ferric and ferrous salts reduced the toxicity by precipitating the iron salts as insoluble phosphates.

Chlorosis. In our last paragraph the significance of adding too much iron producing toxic effects was reviewed. The lack of iron also produces chlorosis as the following citations will bring out. Pfeffer (70) claimed that the chlorosis due to the absence of iron is not caused by its being directly concerned in the formation of chlorophyll, but is the result of malnutrition. MacCallum (50) identified iron as a constituent of the chromatin of the nucleus. Benoley (12), working with MacCallum found it in the pollen cells of several plants. Gile and Carrero (29) after an exhaustive study of the literature, both from soil surveys and from positive experimental results, reported by others and also from their own results, were convinced that chlorosis is due to a depression of available iron in the soil. Russel (77) cites evidence that plants tend to become chlorotic when the content of calcium

carbonate becomes too high. Mazé (56), by adding calcium carbonate to a solution containing neutral salts, some of which were hydrolyzable, produced marked chlorosis. Treatment of the affected cultures by organic acids, restored the green color in most cases. Lipman (47) believes that lack of useable iron produces chlorosis and that the alkalinity induced by carbonates may depress growth without affecting the color. Arndt (3) found chlorotic corn plants were commonly found in cultures containing toxic concentrations of aluminium salts. The nitrate usually produced the most severe chlorosis. Hoffer and Carr (38) found iron accumulated in the nodes of diseased corn stalks, and with the upper leaves chlorotic the plants contain considerable amounts of immobile iron compounds. These authors ascribe this to a low ($\overset{+}{H}$)concentration of the plant sap. Sufficiently high ($\overset{+}{H}$) concentration keeps the iron compounds moving and chlorosis does not occur. Marsh (52), in a critical study of the availability of iron in nutrient solution, its physiological effects, and its distribution in normal and chlorotic plants, has found that when the iron supply was adjusted from day to day to meet the requirements of the plants, large healthy vigorous plants were produced regardless of the type of culture solution or the iron compound employed. Chlorosis may result from both toxicity and from a lack of iron. The latter produces stunted chlorotic plants, but the percentage iron content in the ash of the stems is as high or higher than in normal plants while it is low in the leaves. When iron is available, slightly above the optimum amount necessary for growth, the percentage iron content of the plant may be high throughout the plant. An increase in the toxic amount results in the lodgement of iron in the roots and stems thus preventing its distribution to the leaves in

amounts sufficient to provide adequately for chlorophyll formation. Small additions of iron supplied as the plant appears to need it tend to produce equal distribution throughout. Thatcher (86) states that iron is absolutely essential for the formation of chlorophyll. Palladin (67) states; "Plants need iron, the lack of which prevents chlorophyll formation; they finally become pale and chlorotic, even in the light, when grown without this element."

Factors Influencing the H-ion Concentration. Barnette (8) observed that a decrease in the ($\overset{+}{H}$) concentration was soon followed by chlorosis in wheat plants. He states; "The higher availability of iron to the plants in solutions containing ammonium sulfate is undoubtedly due to the fact that in this solution the ($\overset{+}{H}$) concentration is maintained at a higher level as the result of contact with the plant roots thus giving a higher degree of solubility to the ferric phosphate. The ($\overset{+}{H}$) concentration of the solution containing ammonium sulfate is increased as a result of contact with plant roots." Many others hold the opinion that increased acidity increases the available iron. Gilo (28) has found that the reaction of a solution has marked influence upon the availability of iron to the rice plant. The data of Arndt (2) showed that for corn, culture solutions with a pH above pH3.6 had little effect on growth when the acidity was caused by the addition of acids. The toxic effects of ferric salts in solution were more nearly related to the ($\overset{+}{H}$) concentration than ferrous and aluminum salts were because the former were immediately precipitated and the precipitate formed was more soluble in a solution of lower pH. Van Alstine (92) when comparing wheat plants grown in solution with ferric phosphate to those grown in a solution with a soluble form of iron in

equivalent amounts per liter, found the plants unable to obtain a sufficient amount of iron. Judging from the chlorotic conditions the availability of iron was proportional to the pH. Acidity increases the availability of the iron. McCall and Haag (58) believe that a high ($\overset{+}{H}$) concentration may favor the availability of iron. They varied the pH of different solutions and noted that it had a marked effect upon the rate of growth of wheat plants and is an important factor in chlorosis. Reed and Haas (73) state that the iron of ferric tartrate soon changes to insoluble compounds when added to nutrient solutions. A number of organic compounds, when added to an alkaline medium increase the amount of soluble iron. They suggest that this fact may be of significance in maintaining an adequate supply of soluble iron in solution for the growth of plants. Their results agree with the earlier mentioned work of Gile and Carrero (28). Duggar (25) states that the composition of all salts employed determines the ($\overset{+}{H}$) concentration, the influence of which is very complex. Gordon and Starkey (30) found the ($\overset{+}{H}$)concentration an important factor affecting the solubility, and, therefore, the availability of some ions like calcium and potassium. Weir and Halsted (60) state; "Solutions of high ($\overset{+}{H}$) concentration may be efficient on account of their correspondingly high phosphate content, and resulting low buffer capacity, rather than because of the acidity per se." Atkins (4), in working out the curves for the solubility of normal phosphates, found that the solubility of tertiary calcium phosphate increases from 114-786 parts per billion over a pH range from pH7-5.1. The solubility of magnesium phosphate is 450 parts per million at pH 7.7 and 1233 parts per million at pH 5.8. The mono and di-acid phosphates are very much more

soluble. The readily hydrolyzed ferrous and ferric phosphates appeared to be more soluble in neutral solutions than in slightly acid solutions. It is of interest, also, to note that in an earlier article (5) this writer ascribes the color of Hydrangia hortensis to the available iron in the soil. In a soil with pH range from 5.7 - 6 the flower is blue, in less acid habitats some flowers may be blue and others pink, but above pH 7.5 all flowers are pink. Even in neutral solutions some ferrous iron remains in solution, but all the ferric iron has precipitated; hence the plant must use ferrous iron. The difference in color is not due to the $(\overset{+}{H})$ concentration, but to the available iron. Haematoxylin tests for iron show inorganic iron in the blue flowers and only traces in the pink. Colorimetric estimation gave the blue flowers 140 parts per million, and the pink 60 parts per million. The increased acidity liberated enough iron to produce blue flowers unless elements other than iron can produce this same coloration of flowers.

In a culture solution the pH value generally has a tendency to shift. Duggar (25) believes that this depends in part upon the composition of the nutrient solution used and in part upon the plant grown. That both plant and solution are mutually influential is evident from a summary of reports from various investigators. Rautenberg and Kuhn (72), in substituting an ammonium salt for a nitrate as a source of nitrogen found that the acidity increased rapidly when ammonium chloride was used, but not when the sulphate, phosphate, or nitrate of ammonium were used. Breazeale (14), in growing wheat seedlings found that a sodium nitrate solution became alkaline and a potassium chloride or sulphate solution became acid, while a potassium nitrate solution remained constant. Arrhenius (3) found

the direction of the change to vary with the plant used. Conner and Sears (17) found that acid nutrient solutions became less acid when in contact with the roots of rye and barley. Rudolfs (75) in germinating seeds obtained a pH value constant for each species of plants when all are grown in the same solution. When the nutrient solutions vary the final pH values vary for each species.

The change in pH also varies with the composition of the solution used. Olsen (65) found the direction of the change to depend primarily upon the source of nitrogen in the solution. Those containing ammonium nitrate became more acid after exposure to plant roots while those containing sodium nitrate became alkaline. Arndt (2), in comparing the effects of sulfuric acid and iron salts upon the wheat plant, found that an acidity due to the presence of sulfuric acid alone was more easily shifted than the same solution with aluminium and iron salts present. The roots of the plants developed better in a solution containing sulfuric acid than in solutions containing equivalent amounts of hydrochloric acid and nitric acid, especially in solution "A". The sulfate solutions, except ferrous sulfate, are all shifted toward neutrality by the plant roots. The reverse holds true when iron is supplied as ferrous sulfate. With small plants the change is small, but increases with the size of the plant grown. Arndt comments; "It seems fairly evident that the plant in some manner accelerates the hydrolysis and precipitation of the ferrous sulfate, and is the main agency which produces the change, giving a high initial acidity to the solution. The ferrous salt is less readily precipitated, but when it is a similar acidity is produced."

Jones and Shive (42), in studying the change in ($\overset{+}{H}$) concentration from the effect of plants, obtained data indicating that: (1) with a constant number of plants in a constant volume the rate of change of pH decreased with an increase in total osmotic concentration; (2) with a constant number of plants and a constant concentration the rate of change of pH decreased with increase in volume of solution; (3) with a constant concentration and a constant volume, the rate of change increased with the number of plants per culture. The maximum rate of change occurred soon after the plant roots were placed in the solution, and decreased gradually after the maximum had been reached, the rate depending on the kind of plant, the composition, concentration, and volume of the solution. In a series of differential absorption tests, by Prince, Jones and Shive (71), it was found that the nitrate radical was more rapidly absorbed than the calcium radical thus tending to rise the pH; potassium was absorbed more rapidly than the H_2PO_4 radical, thus tending to lower the pH and the rates of magnesium and sulphate radicals were about the same. The first tendency was stronger and the curve on the graph given in the original representing the nitrate absorption, has the same general trend as the curve for the pH values. The same was also found when the source of nitrogen was ammonium sulphate.

Buffer Action. The preceding paragraph indicated that the acidity of a nutrient solution could be altered by preferential ion absorption of the plant grown. That it is also largely due to the composition of the solution is evidenced by the following citations.

Tottingham (87), in growing wheat cultures, found that potassium dihydrogen phosphate produced 17.8 per cent better growth of tops than did the potassium mono-hydrogen on the basis of yields relative to the control cultures. The controls grew in distilled water. Arndt (2), in studying availability of iron and ($\overset{+}{H}$) concentration says that the buffer action of a solution is more important than the initial acidity.

Some of the solutions found best for plant growth and their ($\overset{+}{H}$) concentrations are given below. Jones (41), in studying the rate of reaction change, notes that of the solutions with an initial pH below 5, Shive's solution R_5C_3 exhibited the greatest resistance to reaction change, while Tottingham's solution $T_3R_1C_4$ showed only slightly lower buffer properties. The solutions of Schreiner and Skinner and of Hartwell, et al, possess relatively low buffer properties. He further states; "In general it appears that the resistance offered to reaction change resulting from contact with roots of growing plants, is dependent largely upon the volume molecular proportions of the soluble phosphate salts contained in the solutions ..... One striking exception to this general rule as exhibited by the solution of Birner and Lucanus which has a volume molecular proportion of dihydrogen phosphate equal to that in Tottingham's solution, $T_3R_1C_4$, and about two and one-half times higher than Knop's solution or Pfeffer's solution yet there showed a higher resistance to reaction change as influenced by wheat plants than did the solution of Birner and Lucanus."

It was further noted that the final pH values of nutrient solutions approached each other and the neutral point when the solutions were exposed to growing plant roots, hence there was a greater change in solutions with a high initial acidity. The time required to effect this

change varies with the buffer properties of the culture solution when the same species of plant is grown. When ammonium sulfate is used as a source of nitrogen the reaction tendency is toward a lower pH value which helps to keep some iron soluble.

"Formulae of nutrient solutions commonly used for plant cultures, all with total osmotic concentration value of approximately 1.75 atmospheres as determined by Method of Freezing Point Lowering" (79).

Author of Solution	Initial+ pH	Final++ pH	Volume Molecular Partial Concentration of Salts Employed									
			KH_2PO_4	$Ca(H_2PO_4)_2$	$Fe_3(PO_4)_2$	$Ca(NO_3)_2$	KNO_3	$NaNO_3$	$MgSO_4$	K_2SO_4	KCl	NaCl
Birner and Lucanus	4.3	5.5	0.0108	--	0.0043	0.0133	--	--	0.0061	--	--	--
Hartwell, Wheeler and Pember,	4.0	5.7	--	0.0027	--	0.0136	--	--	0.0077	--	0.0075	
Knop,	4.6	5.7	0.0044	--	--	0.0145	0.0059	--	0.0050	--	--	--
Pfeffer,	4.7	5.8	0.0041	--	--	0.0136	0.0056	--	0.0046	--	0.0037	--
Schreiner and Skinner,	4.2	6.1	--	0.0066	--	--	--	0.0278	--	0.0030	--	--
Shive, R_5C_2	4.5	5.3	0.0180	--	--	0.0052	--	--	0.0150	--	--	--
Tottingham, $T_3R_1C_4$,	4.6	5.5	0.0108	--	--	0.0101	0.0034	--	0.0081	--	--	--

+ Initial pH is the pH of the solution before exposure to plant roots.
++ Final pH is the pH of the solution after exposure to wheat seedling roots for a period of 52 hours.

These pH values were not part of the original table, but were taken from other tables by the same author and inserted in the above for comparison.

"Formulae of some Livingston-Tottingham three salt nutrient solutions (Types 1 - VI) and of Tottingham's solutions $T_1R_1C_5$ and $T_1R_3C_5$, modified (Jones and Shive) by substituting $(NH_4)_2SO_4$ for KNO_3 in equivalent osmotic concentrations. All solutions had total osmotic concentrations value of approximately 1.00 atmosphere" (42).

Type	Number	Initial H^+	Final H^+	Volume Molecular Partial Concentrations									
				Livingston Tottingham Three Salt Solutions									
				KH_2PO_4	$Ca(H_2PO_4)_2$	$Mg(H_2PO_4)_2$	$Ca(NO_3)_2$	$Mg(NO_3)_2$	KNO_3	$MgSO_4$	$CaSO_4$	K_2SO_4	$(NH_4)_2SO_4$
I	R_3S_2	4.2	5.7	0.0072	--	--	0.0048	--	--	0.0072	--	--	--
II	R_3S_2	3.8	5.8	--	--	0.0019	0.0037	--	--	--	--	0.0094	--
III	R_4S_1	3.6	5.6	--	0.0025	--	--	--	0.0099	0.0074	--	--	--
IV	R_4S_1	3.7	5.6	--	0.0019	--	--	0.0057	--	--	--	0.0076	--
V	R_3S_4	3.6	5.4	--	--	0.0025	--	--	0.0074	--	0.0098	--	--
VI	R_4S_2	4.3	5.6	0.0093	--	--	--	0.0047	--	--	0.0047	--	--
				Modified Tottingham Solutions									
	$T_1R_1C_5$	4.8	4.2	0.0021	--	--	0.0073	--	--	0.0071	--	--	0.0014
	$T_1R_3C_5$	4.8	4.2	0.0021	--	--	0.0073	--	--	0.0024	--	--	0.0042

\+ The (H^+) concentration of the solution before exposure to plant roots.
++ The (H) concentration of the solution after exposure to the roots of wheat seedlings for a period of 52 hours.

Meier and Halsted's (60) Partial Volume Molecular Concentrations in Six Solutions of Type I, differing by increments of one-eighth in the salt proportions, and having a total osmotic concentration of approximately one atmosphere at 25°C. Six best solutions were selected from a list of twenty-one.

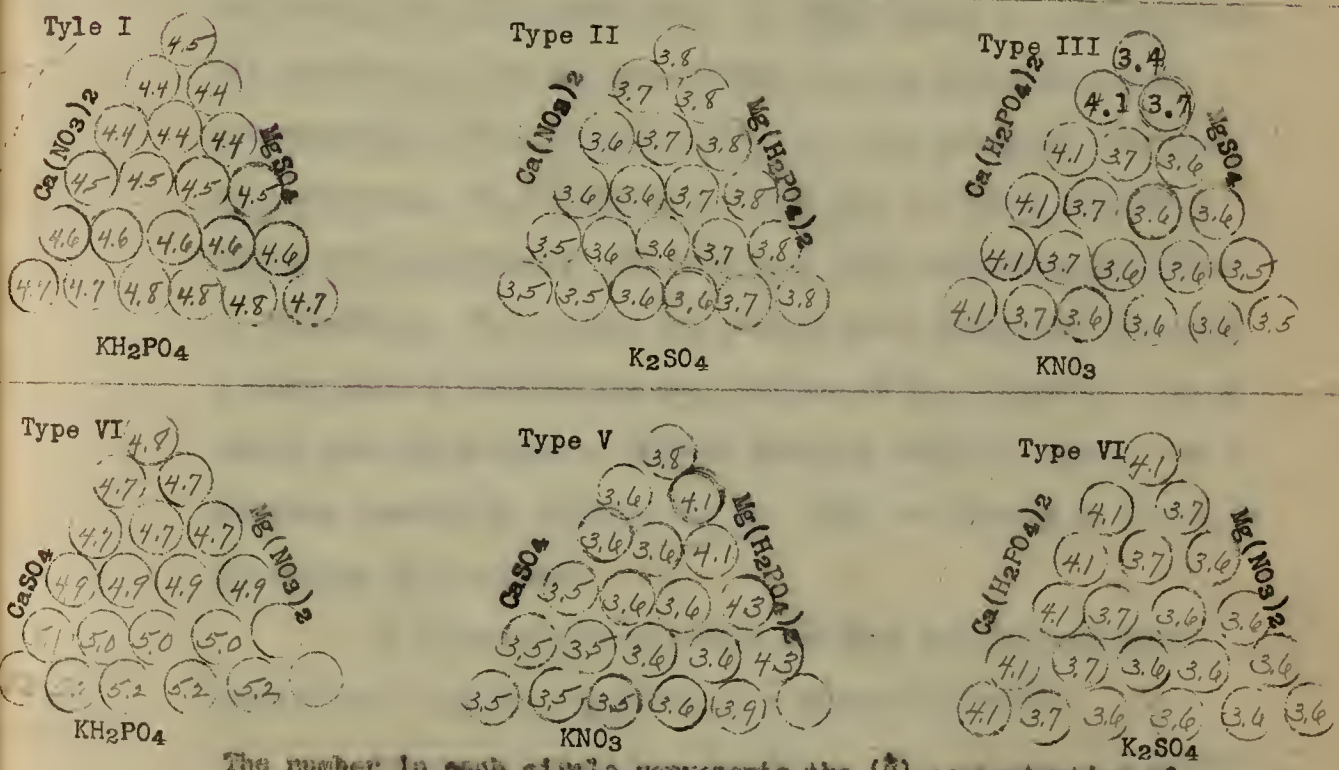
Solution Number	Initial+ pH	Final++ pH	Partial Volume-molecular Concentrations KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
R_2S_2	4.9	5.8	0.0049	0.0049	0.0099
R_2S_3	4.9	5.1	0.0047	0.0071	0.0071
R_2S_4	4.9	4.9	0.0045	0.0090	0.0045
R_3S_2	4.9	5.3	0.0072	0.0048	0.0072
R_3S_4	4.9	4.9	0.0065	0.0086	0.0021
R_4S_2	4.9	5.0	0.0094	0.0047	0.0047

+ Initial pH of solution.
++ pH after plants had grown in solution 3 1/2 days. The wheat plant at this stage had grown from July 1 to August 5, a period of 36 days.

These workers remark that the difference between the initial and final pH for a given 3 1/2 days is greater when a nutrient solution contains only small amounts of the phosphate radical or those insufficiently buffered by KH_2PO_4. The more highly buffered solutions, because of the increased quantity of the phosphate salt present, are also more highly acid because of the dissociation of the hydrogen-ion in this salt. They further state that the solutions that are poorly buffered support minimum weight yields because of the small quantity of phosphate present.

McCall and Haag (58) determined the ($\overset{+}{H}$) concentration of all of the three-salt solutions of the six types recommended by the National Research Council. Their figures, arranged so that the acid salts of the same cation can be compared, are given below.

Diagrams Representing the pH Concentration of Six Types of Three-salt Nutrient Solutions.



The number in each circle represents the ($\overset{+}{H}$) concentration of that particular solution. The bottom line of each triangle is the base line for potassium, the left side for calcium and the right side for magnesium. Each of these three base lines represents a row of solutions, each of which has one-eighth of its

total volume molecular concentration derived from the salt for which it is named, the salt proportions increasing by increments of one-eighth from row to row until the apex of the triangle is reached. The solution at each apex has six-eighths of its total concentration derived from the salt indicated at the opposite base line.

McCall and Haag remark; " . . . the $(\overset{+}{H})$ concentration is in general a function of the volume molecular proportion of the di-hydrogen phosphate salt used. In other words, as a general rule all solutions of any one type having the same volume molecular concentration of the phosphate salt also have about the same $(\overset{+}{H})$ concentration. It is also to be noted that the types containing KH_2PO_4 are considerably less acid than those containing $Mg(H_2PO_4)_2$ or $Ca(H_2PO_4)_2$. The sulfate and nitrate salts apparently play only a minor part in determining the reaction of the solution." The pH values were determined on freshly prepared solutions since a day's standing materially effected the pH. This was thought to be due to the action of the glass.

In a further study McCall and Haag (58) emphasize the importance of buffer properties in a nutrient solution.

Effect of Acidity on the Plant. The acidity of a solution, whether produced by the plant or by the hydrolysis and ionization of the component salts of the solution, affects the plant. Tarr and Noble (65) grew wheat seedlings for a period of five weeks. Maximum growth was obtained in a solution with a pH value of 5.0, greater acidity being harmful and lesser acidity unharmful until a pH 6 was reached when the cultures became chlorotic. Tottingham and Rankin (67)

using solution RgC_1, renewed frequently, produced wheat seedlings with short stubby roots with a pH below 6.4. A pH which was endured in intermittently renewed solutions became intolerable when the solution was continuously renewed. Certain pH values which restrict the elongation of roots and stem appear to favor the production of dry matter in these organs. In studying $(\overset{+}{H})$ concentration as an independent factor Arndt (2) found that a pH below 3.6 was harmful to corn, but above this it seems to have little effect on its growth. Addoms (1) by microscopic examination, found that the protoplasm in the root hairs of wheat seedlings precipitated in a solution of pH 3.6 to 3.8 or lower. This precipitation rendered the root hairs unfit for feeding purposes. Van Alstine (92) found that soy beans in a culture solution of pH 3.8 or lower died within ten days. Buckwheat plants did not die in solutions of pH values from 3.3 to 4.0, but all showed acid injury. Hoagland (37), by quantitative analysis of the culture solution after exposure to plant roots, found that the absorption of phosphate and nitrate ions was greater in an acid solution, pH 5 to 5.5, than in a neutral solution.

Acid-base Equilibrium. In the study of plant sap, Hurd (39) found the $(\overset{+}{H})$ concentration of the juice expressed from different varieties of wheat to be uniformly at a pH from 6.0 to 6.2 for all varieties at all stages of growth except when the plants begin to dry out. Hoagland found the pH to be 6.1 for sand, water and soil cultures even though grown in widely different concentrations of nutrients and under different atmospheric conditions. The experiments of Brezeale (15) indicate that the plant absorbs carbonate and exudes carbon dioxide to maintain the acid-base equilibrium. Since the carbonate radical plays

an important part in the chemistry of the iron nucleus and also because the plant sap affects the acidity of a nutrient solution this work will be considered at some length. It was noticed that the ash of plants grown in a nutrient solution containing carbonates, bicarbonates or an alkali nitrate contained carbon dioxide. When these nutrients were not used there was no effervescence of carbon dioxide upon the addition of hydrochloric acid. This is explained by the following hypothesis. The plant seems to feed upon ions only. The carbonate radicals are directly absorbed by the roots. Under normal conditions the plant feeds more heavily upon nitrates than upon the alkali with which it is in combination. When the nitrate ion of sodium nitrate is absorbed the alkali reacts with the water to form sodium hydroxide and the solution turns basic. The solution reacts with the carbon dioxide dissolved in it to form sodium carbonate or bicarbonate which in turn is ionized. The system thus tends to restore the equilibrium disturbed by the requirements of the plants. There are other strong experimental indications that the plant absorbs the carbonate ion from solution by means of its roots, but this is not taken up unless some soluble base is present. Carbonic acid seems to be too little ionized to furnish the required carbonate radical when growing plants are placed in it.

In a series of wheat cultures in which one set could not obtain carbon dioxide from the air it was evident that the carbonate ion could not furnish the carbon necessary for building up cellulose or organic compounds. The carbon for this seems to be derived from the carbon dioxide of the air. Yet it seems evident that the plant demands the carbonate radical. Its function is to maintain acid-base equilibrium.

The plant in the absorption of potassium, a necessary element, must also absorb an equivalent amount of an acid radical or the plant sap must become basic. To maintain a constant pH a radical of opposite charge must also be absorbed. If no more desirable ion is present in the solution then the carbonate is selected. If, on the other hand, an acid radical as the nitrate is absorbed the plant sap tends to become acid, provided an equivalent amount of base is not absorbed. To keep the pH of the sap constant apparently carbon dioxide is exuded as it is in animal physiology.

The alkalinity of plant sap may be overcome in two ways, first by exudation of some base not needed in the plant, and second by the absorption of some acid. The plant has selected the latter method and uses carbonate as the negative radical that is absorbed. Similarly, the acidity of the sap may be overcome by the absorption of a base or by the exudation of an acid. The plant has the power to do both. The acid carbon dioxide being exuded if necessary.

Hoagland (37), in a study of the absorption of ions by chemical analysis of the residue after exposure to plant roots verifies the work of Brezeale. In all cases where an anion is absorbed more rapidly than the cation a bicarbonate ion replaces the nitrate ion lost by absorption.

We have emphasized the fact that the equilibrium of the plant sap is continually disturbed. Other theories to maintain it are presented by Truog (90) and Dickinson (24) who believe that calcium can be absorbed to neutralize the organic acids produced in

protein synthesis. Mazé (58) believes that malic acid is secreted by maize roots, and Czapek (22) that acid salts, principally potassium acid phosphate, are excreted by the plant roots.

Discussion of the Availability of Iron. Solubility.

In our historical survey we have endeavored to develop the significance of iron in plant metabolism, the factors affecting its solution, and have brought together evidence for each factor. The amount of iron in a solution will depend upon the solubility of the iron salt added to the initial solution, the speed of reaction of the iron salts with the salts present in the solution, and the solubility of the iron compounds formed in the solution when it has come to equilibrium. In dilute solutions the presence of one salt does not usually affect the solution tendency of other salts. The solubilities of the final products of the reactions in nutrient solutions may be taken as these products in water of the same acidity. Solubilities taken from Olsen (66) are given below:

$FePO_4$, insoluble.
$Fe_3(PO_4)_2.8H_2O$, insoluble
$Mg_3(PO_4)_2.4H_2O$, .0205 gm. in 100 cc. cold water.
$MgHPO_4.7H_2O$, .3 gm. in 100 cc. cold water.
$Ca_3(PO_4)_2$, .003 - .008 gm. in 100 cc. cold water.
$CaHPO_4$, .028 gm. in 100 cc. cold water.
$Ca(H_2PO_4)_2$, 4.0 gm. in 100 cc. cold water.
$FeC_4H_4O_6$ (tartrate) 0.877 gm. per 100 cc. water, 15.6°
$FeC_2O_4.2H_2O$ (oxalate) 0.022 gm. per 100 cc. cold water.
$Fe_2(SO_4)_3$, s.soluble.
$FeSO_4.7H_2O$, 32.8 gm. per 100 cc. 0°C.
$MgCO_3$, .0106 gm. per 100 cc. cold water.
$CaNH_4PO_4.7H_2O$, insoluble.
$CaCO_3$, .0013 gm. per 100 cc. cold water.
$Ca_3(C_6H_5O_7)_2.4H_2O$, 0.085 gms. per 100 cc water, 18°C.
$MgC_4H_4O_6.4H_2O$, 0.8 gm. per 100 cc. water, 16°C.
$CaC_2O_4.H_2O$, 0.000554 gm. per 100 cc. water, 18°C.
$CaC_4H_4O_6.4H_2O$, 0.016 gm. per 100 cc. water, 15°C.
$CaSO_4$, 0.179 gm. per 100 cc. water,)0°C.
$CaSO_4.2H_2O$, .241 gm. per 100 cc. water, 0°C.

From a study of these solubilities we should expect the phosphates of iron, calcium and magnesium to precipitate first depending upon their relative solubilities and concentrations. If the nature of the solution is altered by the addition of ammonium or organic compounds or increase in $(\overset{+}{H})$ concentration the system is very complex. Considering concentration and solubility only we should not expect ferric phosphate to be soluble in a nutrient solution. Until accurate solubilities have been determined nothing can be definitely stated. Ferric phosphate is insoluble, and when added to a solution already containing a phosphate ion its solubility should be still less. However, since the addition of phosphate is always in the form of an acid salt the $(\overset{+}{H})$ ion produced upon ionization makes the resulting solution acid which produces a higher solubility of the phosphate. In other words, furnishes the $(\overset{+}{H})$ ion to form phosphoric acid. The plant roots reduce the acidity when in contact with the solution, and a precipitation of ferric phosphate occurs. This reduction of acidity and precipitation of iron depends upon two reactions tending to lessen the amount of iron present. Even though they may be rapid, the iron available to the plant during this time may be sufficient for the needs of the plant for several days. This will be indicated in a later part of the article. In a study of Knop's solution, Tottingham (87) found that calcium sulfate was precipitated from a standard Knop's solution of 2 per cent strength or more. This precipitation of calcium sulfate was confirmed in our laboratory. The solubility of calcium sulfate as listed above is 1.79 gm. per liter at 0°, while in a 2 per cent Knop's solution where 1.05 gm. of calcium sulfate as calcium radical plus sulfate radical, is present some precipitation (4.52 per cent of the total calcium present) was found. As the concentration of Knop's

solution increased the percentage of calcium precipitated also increased. This discrepancy in the solubility as given by Olsen and found in nutrient solutions cannot be explained until further data are produced. That calcium sulfate precipitates instead of calcium phosphate is due most likely to the fact that the phosphate is added as a di-hydrogen salt. The dissociation factor of this salt is 1.86, by calculating from Tottingham's formula, showing that a negligible amount of PO_4 ion is produced, and therefore no calcium phosphate can be formed. Iron was not added in Tottingham's (87) determinations. Since ferric phosphate is more insoluble than calcium phosphate we can expect the more insoluble compound of the ferric salt to combine with the negligible concentration of PO_4 ion. As this low concentration of PO_4 ion still exceeds the solubility product of ferric phosphate some PO_4 ion is removed by precipitation with iron. As fast as this is removed the acid salt dissociates to keep up the equilibrium and the reaction with the formation of ferric phosphate as an end product moves forward. This seems to be a slow reaction, the time required depending upon the factors affecting the speed of such reactions.

$$KH_2PO_4 \rightleftharpoons K + H_2PO_4$$

$$H_2PO_4 \rightleftharpoons H + HPO_4 \rightleftharpoons H + PO_4$$

$$Fe + PO_4 \longrightarrow FePO_4$$

Among the individual factors effecting the speed at which iron is precipitated is the acidity. If acidity is the limiting factor and a common ion, the hydrogen ion, is present in a system there is less tendency for the acid phosphates to dissociate. The H_2PO_4 ion and HPO_4 ion do not have to ionize to bring about the equilibrium that is attained in a solution when the phosphates are dissolved in the

solution. In other words, when acid phosphates are dissolved they dissociate until a certain acidity is reached. If a solution has this acidity the hydrogen ions are present, the phosphates do not have to decompose and the PO_4 ion is not liberated.

The success with which ferric phosphate is used in solutions in which calcium acid phosphate and magnesium acid phosphate are used can perhaps be explained by this hypothesis. When few or no PO_4 ions are present, due to the acidity of the di-hydrogen solutions, the ferric phosphate that is added can dissolve and dissociate until the solubility and dissociation constants have been reached. When other compounds of iron than the phosphate are added the iron of these compounds will also precipitate more slowly because the PO_4 ion concentration is lower.

Iron Ammonium Compounds. Among other factors that affect the solubility of iron is the presence of an ammonium salt. Arndt (2) remarked that something in his solution "A" kept the plant from absorbing iron. His solution "H" contained ammonium nitrate while solution "A" did not. This effect has already been referred to (71) and the reason ascribed to one or both of two possibilities, increased permeability in the membranes of the plant cells, or increased availability of iron from a higher acidity. In our laboratory work it was noticed that in washing a precipitate containing ferric phosphate with ammonium hydroxide more iron passed through the filter than when washed with water. This was believed to be due to the colloidal state after some of the ferric phosphate was changed into ferric hydroxide. This kind of a transformation resulting in colloidal condition may go on in a nutrient solution as well and thus render iron available to the plant. Whatever may be the cause more iron is available to the plant when ammonium salts are present in the nutrient solution. The presence

of ammonium hydroxide in a solution does not keep all forms of iron dissolved or iron dissolved in all solutions. It is the standard precipitant of iron in analytical chemistry. The lowest acidity at which iron and ammonium hydroxide or sodium hydroxide can exist in a hydrochloric acid solution is pH 3.3 (68). On the other hand the addition of neutral ammonium citrate to a calcium phosphate solution increases the solubility of the calcium salt (69). Calcium phosphate settles out at pH 2.3, under certain conditions, while upon the addition of neutral ammonium citrate no precipitation occurs until a pH of 5.5 is reached, when calcium citrate is precipitated. The solution tendency of the phosphate seems to be greater in an ammonium citrate solution, due either to the ammonium or to the citrate radical. To which of the three radicals $\overset{=}{PO_4}$, $\overset{+}{NH_4}$ or $\overset{=}{C_6H_4O_8}$ or combination of radicals this peculiarity is due does not seem to be known. A similar reaction may tend to keep ferric phosphate in solution in nutrient solutions. The elements phosphorus, nitrogen and carbon, are those which have special and peculiar properties. A compound, calcium ammonium phosphate, is given as an insoluble compound. We do not know its solubility product, but if it exceeds that of ferric phosphate, or after once being formed, acts as a non-dissociating complex ion the PO_4 ion that would otherwise react with the iron, will be removed and the iron left in solution and available to the plant. Any one of these factors, permeability, increased $(\overset{+}{H})$ concentration, displacement of the PO_4 ion by citrate or complex compounds, may be responsible for the increased iron supply of plants. Further knowledge must be gained from experimental investigation.

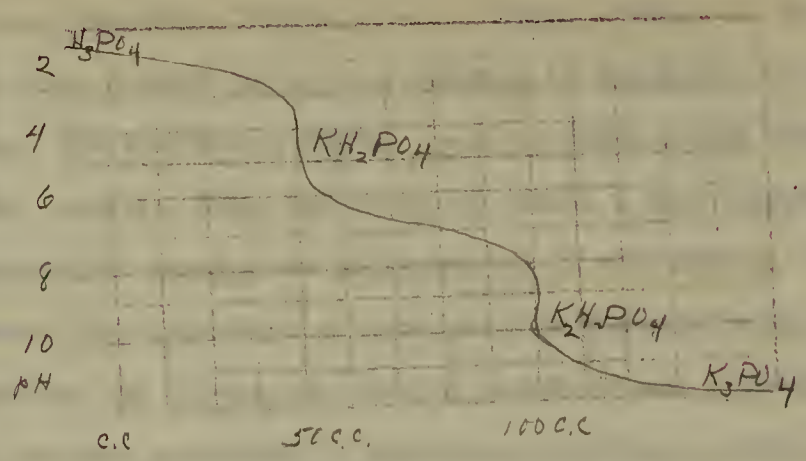
Preferential Ion Absorption. Beside a possible direct influence in solubility of several of the salts in a nutrient solution, the presence of ammonium salts also changes the nature of the solution by preferential ion absorption of the plant. When ammonium is added to certain solutions, the NH_4 ion seems to be preferred to the NO_3 ion as a source of nitrogen by certain plants. When a less essential ion is added as the anion of an ammonium salt such as ammonium sulfate or ammonium chloride the differential ion absorption is greater. The cation is removed which leaves the sulfate to form an acid with water, thus increasing the acidity. The result of the differential ion absorption and its consequent effect upon the nutrient solution has already been given in the literary survey of the subject.

Hydrogen-ion Concentration. The initial acidity of a nutrient solution depends upon the kind and amount of the component salts employed. This acidity is produced by ionization of the acid salts, hydrolysis particularly of those salts of ferric iron which have an ion in common with another salt of the solution, and buffer action. The acid salt largely determines the acidity of the solution and also its buffer capacity. This is evident from a consideration of three sources of data: (1) the pH values of nutrient solutions; (2) the ionization of acid salts, and (3) the buffer curves of acid salts. From the triangular diagrams of McCall and Haag (58) it can be seen that if two-eighths or more of the total concentration of a culture solution is due to the acid salt the pH is about the same for all other possible combinations.

Composition and pH Ranges of Three-salt Solutions (59)

Type	Acid Salt	pH-ranges
I	KH_2PO_4	4.4 - 4.8
VI	KH_2PO_4	4.8 - 5.3
III	$Ca(H_2PO_4)_2$	3.5 - 4.1
IV	$Ca(H_2PO_4)_2$	3.5 - 4.1
V	$Mg(H_2PO_4)_2$	3.5 - 4.3
II	$Mg(H_2PO_4)_2$	3.5 - 3.8

From this table we note that the same acid salts give about the same pH range except types I and VI, where potassium di-hydrogen phosphate gives a higher pH for solutions of type VI. Dickson (24) gives the osmotic factor of potassium di-hydrogen phosphate in a 0.1 N solution as 1.88. Complete dissociation of potassium di-hydrogen phosphate would give two if only ($\dot{K}$) and ($H_2\bar{P}O_4$) were formed. That (H_2PO_4) dissociates very slightly and depends on the acidity is evident from the buffer curves of Clark (16) which are reproduced here. To get the curve 50 cc. of N/10 phosphoric acid were titrated with 50 cc. N/10 potassium hydroxide. The pH is represented as the ordinate and the cubic centimeters of N/10 potassium hydroxide used as the abscissa. From the curve we see that pure N/10 potassium di-hydrogen phosphate should have a pH 4.5 It then has its maximum buffer value. Below pH 4.5 some free phosphoric acid must exist. Above pH 4.5 some di-potassium hydrogen phosphate must be present as a mixture in the salt. The variation in the pH of solutions which have the same acid salt can be due to one or both of two reasons. The amount of potassium di-hydrogen phosphate added is so small that sufficient hydrogen ions are not present to raise the acidity to pH 4.5. The buffer action of the cations of the other salts present may reduce the acidity as rapidly as the acid salt is ionized. The curve shows the H-ions present in a 0.1 N solution of potassium di-hydrogen phosphate. The buffer capacity of the salt will allow great dilution to be made before the pH will change. When the dilution in a nutrient solution is considered it is doubtful that



Titration Curve of Phosphoric Acid.

the same pH can be maintained when the smallest increments of acid salt are added. For example, in solution I Ri8c only 2 cc. of a molar solution of potassium di-hydrogen phosphate are used per liter of nutrient solution. The total quantity of hydrogen in a liter of molar solution is 2 grams or 0.002 gm. per cc. Since only 2 cc. of this solution are used per liter the nutrient solution has an amount of hydrogen of 0.004 gm. per liter, the amount present as ions depending on the extent of the ionization of the H_2PO_4 and HPO_4 ions. The ionization of the second hydrogen of H_3PO_4 is very small, its dissociation beginning at pH 4.5 or when 0.000005 gm. hydrogen are dissolved in a liter of solution, the third hydrogen at pH 9.4. This third hydrogen can not be expected to be present dissociated to any extent. Since the third plays no part in the acidity the quantity of active hydrogen per liter has been reduced one-half or to 0.002 gm.

per liter. But the actual ionization of 0.1 N potassium di-hydrogen phosphate ($KH_2PO_4 \longrightarrow K + H_2PO_4 + HPO_4 + H$) is only 1.88 where a total of four is possible if ionization is complete. The ionization $KH_2PO_4 \longrightarrow K + H_2PO_4$ occurs before $H_2PO_4 \longrightarrow H + HPO_4$, and we cannot expect a high percentage dissociation because the potassium ion is very active. This leaves a low ionization of hydrogen from the acid salts possible. Magnesium and calcium di-hydrogen phosphate each contain twice the hydrogen equivalent of potassium di-hydrogen phosphate, and can be expected to be more acid. By a study of the triangular diagrams one can see that one increment of potassium di-hydrogen phosphate does not reduce the acidity of the nutrient solution to that of a 0.1 molar solution of the salt, but two or more increments being the acidity almost uniformly to that of the acid salt except those of type VI. No curves for calcium and magnesium phosphates have been made, but it is quite evident that their maximum buffer value will be at a pH of about 3.5.

Not only is the acid phosphate salt a factor in the acidity of nutrient solutions, but the acid carbonate is also. Breazeale (15) and Hoagland (37) both found the bicarbonate radical in a nutrient solution when nitrate had been removed by the plant. If carbon dioxide is secreted by the roots it will form bicarbonates in the solution. The maximum buffer value of molar calcium bicarbonate as given by Kugelmass (46) is pH 6.3. He gives pH 7.00 for the maximum buffer value of calcion phosphates. This agrees with Clark's graph where the maximum is pH 7.00 when both primary and secondary phosphates are considered as one buffer. As there is only

one acid carbonate its maximum value is the pH which a pure calcimum bicarbonate solution would give in a molar solution. Carbon dioxide from the plant roots and from the air, if vessels are open, would deplete the buffer capacity of calcium acid phosphate solutions, the rate depending on the vapor pressure of the carbon dioxide, and the dissociation tendency of the phosphates.

A study of the tables of Meier and Halsted (60), Jones (42) and McCall and Haag (58), indicates that there is some relation between the ratio of calcium and magnesium to the potassium dihydrogen salt in determining the pH of the solution. To illustrate this some of the data will be reproduced here.

Solution	Final pH +	Initial pH	Partial Volume Molecular Concentration of Salts Used						
			KH_2PO_4	$Ca(NO_3)_2$	$CaSO_4$	$Mg(H_2PO_4)_2$	$MgSO_4$	$Mg(NO_3)_2$	$Ca(H_2PO_4)_2$
Detmer,	5.9	4.7	0.0035	0.0149	--	--	0.0051	--	--
Knop,	5.7	4.6	0.0044	0.0145	--	--	0.0050	--	--
Pfeffer,	5.8	4.7	0.0041	0.0136	--	--	0.0046	--	--
Schimper,	6.1	4.8	0.0028	0.0018	--	--	0.0040	--	--
Shive, R_3C_2,	5.3	4.5	0.0180	0.0052	--	--	0.0150	--	--
$T_1R_1C_5$,	4.2	4.8	0.0021	0.0073	--	--	0.0071	--	--
$T_1R_3C_5$,	4.2	4.8	0.0021	0.0073	--	--	0.0024	--	--
V, R_3S_4,	5.4	3.6	--	--	--	0.0025	--	--	--
II, R_3S_2,	5.8	3.8	--	--	--	0.0019	--	--	--

+ The final pH values of the solutions were taken after 52 hours of exposure to growing plant roots.

From the above data and from data previously given it is evident that the original acidity is due to the amount of acid phosphate present. When 2.1 cc. and 2.8 cc. of molar potassium di-hydrogen phosphate are used the nutrient solution has an acidity of pH 4.8. A solution containing 3.5 and 4.1 cc. gives a pH of 4.7; 4.4 cc. a solution of pH 4.6, while 18 cc. of molar potassium di-hydrogen phosphate gives a nutrient solution of pH 4.5. McCall and Haag report values of the same magnitude while those of Meier and Halsted are uniformly higher.

When there are two buffer salts in the same system and the maximum buffer capacities of the two salts are not at the same pH, the salt which ionizes more freely will tend to repress the ionization of the other. In nutrient solutions of type III, for example, the carbon dioxide which forms the carbonate is absorbed from the air and given off by the plant roots. The dissociation of each is represented by the equations and the pH given

$$Ca(H_2PO_4)_2 \rightleftharpoons Ca + 2H_2PO_4 \quad (-\uparrow\downarrow\ 2(H + HPO_4)) \qquad pH\ 3.5$$

$$Ca(HCO_3)_2 \rightleftharpoons Ca + 2HCO_3 \quad (\uparrow\downarrow\ 2(H + CO_3)) \qquad 6.3$$

When any one of the ions, H_2PO_4, HPO_4 or H, is removed from the system there is a tendency for the pH to rise.

These equations are given to show that more than the acid phosphates must be considered in preparing buffered nutrient solutions.

McCall and Haag took their pH values immediately after making up the solution because they found that the pH values changed

quite materially while standing one day. They thought it to be due to the action of the glass. When the buffer capacities of the solution are considered it is very improbable that the values are changed by the action of the glass. Nutrient solutions set in liter flasks in our laboratory did not noticeably change for a period of three and a half days when they had not been freshly prepared. In making titration curves of calcium acid phosphate electrometrically, however, it was readily observed that some time was required for the acid-base reaction to come to equilibrium. When those ions are present which form insoluble compounds with the PO_4 ions it is very likely that the reaction is very slow and does not effect the pH of a buffered solution. Jones does not report data for changes of pH in shock solutions. How far the bicarbonates of magnesium and calcium effect the pH of a nutrient solution is not known. In solutions which do not contain the ammonium radical the plant roots have a tendency to change the reaction to neutrality.

Role of Iron in Plant Metabolism

Relationship of Potassium, Nitrogen and Iron in Assimilation.

Thus far we have discussed largely the solubility of iron in the nutrient culture solution. We shall now turn our attention to the function of iron in the plant. The method in which iron is utilized by the plant is not definitely known, but that the metabolism of nitrogen, potassium and iron are closely related is indicated by a study of the literature. In the first part of the literary review it will seem that we diverge from the subject of iron, but it will

later be evident that the facts presented are significant.

Schreiner and Skinner (81) observed that in the early growing period of wheat the absorption of potassium was rapid while phosphates and nitrates were more rapidly absorbed later. Nägeli (63) found that potassium salts were better adapted to catalytic work than sodium salts in the synthesis of carbohydrates. Noble (64) believed that carbohydrates were formed only in the presence of potassium. Hartwell and Pember (35) observed that when the supply of potassium was deficient, but not sufficiently low to interfere with the apparent health of the plants, the addition of sodium to the nutrient solution gave an increase in yield from 19 to 30 per cent. Russel (77) states that potash-starved plants are the first to succumb or suffer in a bad season, while Turner (91) reports that a culture of high nitrate content produced plants very susceptible to fungous diseases, and solutions of low nitrate content are more resistant. In making up his solution Turner varied the amount of nitrate ion, while the others were maintained. This varies the ratio of nitrate to potassium. Where more nitrate was used and potassium was constant the ratio of nitrate to potassium was high or unbalanced and the plants were susceptible to disease. Where the ratio of nitrate to potassium was balanced more resistant plants developed. Gericke (27) studied culture solutions in which the plants were fed on successive days on only one of each of a three-salt solution for a period of one day, thus giving the plant all the essential salts in three days; he concludes that the utilization of the potassium ion is closely related to the nitrate ion and the nitrate closely to the potassium.

Russel (77) states that the lack of nitrogen causes a yellowing of the leaf, absence of growth and a poor starved appearance while an abundant supply of nitrogen leads to a bright green color, to a copious growth of tissue and retarded ripening. Duggar (26) states that the lack of iron is only one of the causes of pathological chlorosis. Coulter (19) believes that iron salts and nitrates are favorable for chlorophyl formation. Saccharoff (78) in seeking to explain oxidation in living processes, thought iron to be a compound which was easily oxidized, and yielded compounds which were easily reduced again or further decomposed with comparative ease. He put forward the hypothesis that various vital phenomena of protoplasm are set up by the oxidation of a minute trace of iron contained in the living substance, with subsequent or concurrent hydrolysis.

The material and data beginning here have been taken from Haas and Hill (33). They give the original references for their statements in the footnotes. Haas and Hill have demonstrated potassium as an important factor in the synthesis of plant products. Beet seedlings under similar culture conditions showed seven times more protein and eighteen times more carbohydrate when grown with potassium than without. "When grown under sterile conditions with a culture medium containing a sugar, and supplied with carbon dioxide, it was found that those grown in the light were independent of potassium as regards the synthesis of protein, and that the addition of sugar to the culture medium resulted in an increase of protein. In darkness, on the other hand, a less vigorous development obtained, and only those plants supplied with potassium salts showed protein

formation." Stoklasa suggests that the activity in the dark when potassium is present is due to its radio-activity.

It has long been known that light is an important factor in the photosynthesis both of carbohydrates and proteins. Schimper found that nitrates were destroyed in green leaves exposed to daylight, but were not destroyed if the leaves were kept in the dark. Also that leaves in the shade were richer in nitrates and that etiolated leaves, perhaps iron-free, exposed to sunlight reduced no nitrates.

On the reduction of nitrates Laurent noted that nitrates were reduced to nitrites by the plant. Thiele found that potassium nitrate exposed to the rays from a quartz mercury lamp was reduced to potassium nitrite and oxygen ($2KNO_3 \longrightarrow 2KNO_2 + O_2$). Baly and his coworkers obtained formaldehyde by exposing potassium nitrate solution saturated with carbon dioxide to ultra-violet light. In this case activated formaldehyde is formed for which the formula H.C.OH is suggested, showing two unsatisfied bonds. They state that further synthesis of proteins in the plant is not photosynthetic, as all the other intermediate products of carbohydrate and protein synthesis can be synthesized without the use of light.

Nature of Light. The further consideration of some of the products of photosynthesis is based on the work and articles of Bandisch (9) (10) and (11). After a statement of the observations which interested Bandisch in taking up the study of nitrate reduction, the following topics will be considered in the order given. (1) The nature of light. (2) The action of light on the oxides of nitrogen. (3) The nature of the molecule of potassium

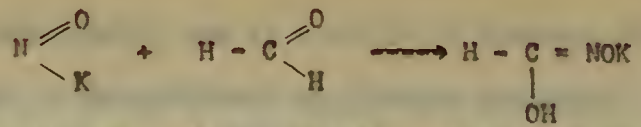
nitrate and its reduction. (4) The mechanism of the reduction of nitrates and the properties of iron complexes.

Baudisch set out to find the explanation of three observations, viz: (1) Schimper's observation that nitrates were reduced in the sunlight and not in the dark. (2) Laurent's observation that sterilized potassium nitrate decomposed into potassium nitrite and oxygen by the action of sunlight; and (3) his own observation that iron was very important in the reduction of nitrates by cholera bacilli. The bacilli taken from a victim immediately after death reduced nitrates in a peptone culture, in the dark as rapidly as in the light, the reduction being proportional to the iron content and oxygen respiration of the bacteria.

We have frequently referred to the importance of light in our discussion. Maxwell asserted that light is caused by electromagnetic phenomena. Planck and Einstein hold that light is emitted when the electrons moving about the central charge of one atom change their orbits or energy levels from a higher to a lower plane. One such change or shift causes an electromagnetic disturbance or release of energy. This unit of energy is hurled into space and is designated as a "light quanta". The reaction is reversible, electronic shifts producing light and a lower total energy value in the atom or molecule. Light, on the other hand, causes electrons to shift to a larger orbit and raises the energy level of the compound with the absorption of energy or electromagnetic units. The electrical energy absorbed in the form of light need not be radiated as light, but may be given out from the

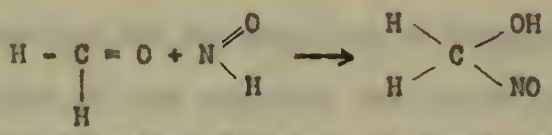
system as a phenomenon of heat, chemical energy or any form or combination of forms of energy. Part or all of it may be retained in the system. The orbits of each electron describe paths each with a definite relation to the nucleus of the atom and each electron having a different orbit. When electronic shifts are made and light is radiated, each shift gives light of a certain wave length and vice versa; light of a certain wave length is necessary to change an electron orbit from a lower to a higher energy level thus increasing the energy value of the atom. In passing through media light is absorbed. The wave length of the light which is absorbed depends upon the electronic structure of the media through which the light passes. Light is absorbed by the compounds which have electrons whose shifting involves quantities of energy corresponding to the wave length of light that it absorbs. In summer sunlight and at high altitudes ultra violet and violet rays of short wave length are plentiful. These bring about the reduction of nitrates. Bluish-yellow and red light of long wave lengths brings about the formation of aquo bases.

Action of Light on the Oxides of Nitrogen. At high altitudes, the gases, nitric oxide, nitrous oxide, and ammonium nitrite, found in the lower atmosphere are decomposed by the violet and ultra violet light rays, and an extremely unstable compound, nitrosyl, NOH, is formed. Because the nitrosyl is so extremely active, it cannot be isolated, but its properties are studied from its alkali salts. Angeli, an Italian chemist, observed that nitrosyl reacts with aldehydes to form hydroxamic acids.



Potassium nitrosyl | Formaldehyde | Potassium salt of hydroxamic acid

Under the conditions of the experiment the alkali salt is hydrolyzed and formhydroxamic acid, H - C = NOH, is formed. When formaldehyde and nitrosyl combine an unstable intermediate product, nitroso-methyl alcohol is formed.



Formaldehyde | Nitrosyl | Nitroso-methyl alcohol

This is very unstable and rearranges itself into Formhydroxamic acid

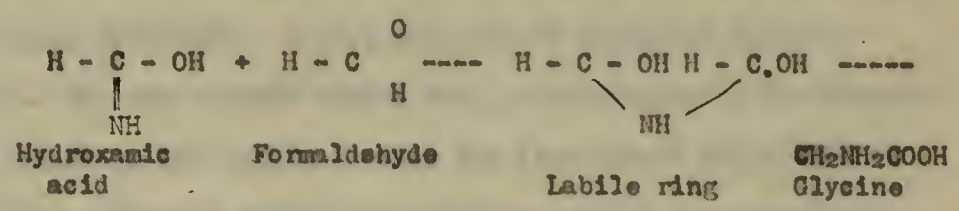
which on exposure to light partially rearranges itself into aci-nitro methane.

Formhydroxamic acid | aci-nitro methane

The formhydroxamic acid by the loss of a molecule of oxygen becomes

a cyanhydrin which is important in the formation of cyanogenetic glucosides. The photosynthetic activity of the production of nitrogen compounds ceases after activated formaldehyde is produced

by chlorophyll, but it would be of interest to complete the steps in the synthesis of nitrogen compounds. The cyanhydrin can condense another molecule of activated formaldehyde to produce a labile ring which rearranges itself to give glycine.



By condensation and rearrangement of these simple proteins and by the addition of ring compounds the higher proteins are built up. Very little of this is known.

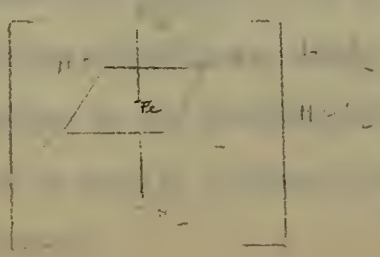
Baly and his collaborators are confident that proteins are synthesized in the manner described above. Baudisch suggests that it is possible because they can be thus synthesized experimentally, but does not feel certain that this is the only way.

Nature and Reduction of the Potassium Nitrate Molecule.

Instead of producing the nitrosyl from the decomposition of the gases in the air by light it can also be produced by the reduction of nitrates such as potassium nitrate. We have mentioned that sunlight broke down sterilized potassium nitrate into potassium nitrite and oxygen; also that bacteria reduced nitrates in the presence of oxygen and iron and leaves reduced nitrates only when they contained iron. Baudisch found an analogy to this in the reduction of nitrites by grape sugar in the presence of small traces of iron. Grape sugar does not reduce nitrites or nitrates even at high temperature and under great pressure without the presence of iron, but when iron is present nitrites are immediately reduced in a warm alkaline solution. Nitrates, however, remain unattacked. This is explained by the

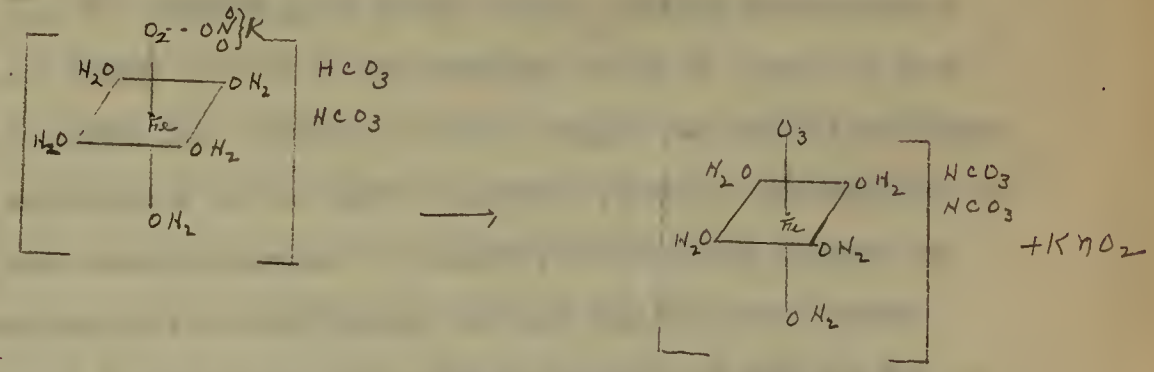
secondary or auxiliary valences of nitrogen. Iron has a strong affinity for nitrogen. In the molecule of potassium nitrite ($\overset{O}{\underset{O}{N}}$ --)K, a primary valence of the nitrogen atom links with an iron nucleus. This linkage will be explained in the next paragraph. In the molecule of potassium nitrate ($\overset{O}{\underset{O}{NO}}$ --)K, the nitrate oxygen atom, the affinity of the nitrogen is masked by the oxygen so that the iron cannot unite with it and bring about a subsequent splitting off of an oxygen atom.

The Mechanism of the Reduction of Nitrates and the Properties of Iron Complexes. It has been emphasized that oxygen must be present in the reduction of nitrates by bacteria. Further work by Bandisch showed that a ferrous salt with oxygen instantly reduced nitrates, even in the cold. He states (11); "It is known with considerable certainty that the catalytic action of the iron in respiration and many other important biological processes is especially related to the properties of the ferrous atom or ferrous ion and not so much to the ferric atom or ferric ion." In the absence of oxygen nitrates are not reduced by ferrous iron. When present the amount of reduction is proportional to the partial pressure of the oxygen. Ferrous bicarbonate in the presence of air or oxygen forms a compound with a co-ordination formula:



This compound is formed by absorption of an oxygen molecule into the complex ion. The oxygen thus absorbed and "attached to the iron nucleus forms a new center of forces".

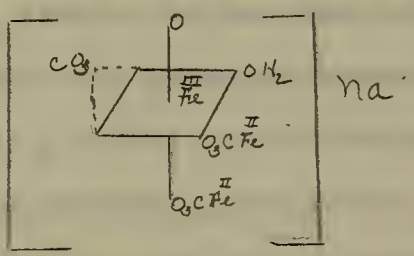
It can link to itself both organic and inorganic groups. With potassium nitrate the following reaction takes place:



The nitrite is further reduced to the active nitrosyl, KON, by the same reaction. We thus see that iron in the presence of oxygen has played the role of light. Baudisch believes that the energy required in reduction of nitrates is derived from light by the activation of the auxiliary valence powers of the nitrate oxygen atom, and the oxygen of the water. Where iron plays the rold of light the energy is transmitted to the nitrate molecule by the strongly magnetic peroxide compound.

That light has an influence on iron compounds is illustrated by potassium ferrocyanide. When the neutral salt is exposed to sunlight an alkaline compound is formed in a few seconds. Longer exposure in the presence of air gives ferrous iron and an extremely active compound, potassium pentacyano-aquo-ferroate, $(Fe\overset{OH_2}{(Cn)_5})K_3$. This compound can absorb oxygen from the air to form potassium penta-cyano-peroxo-ferroate, $(Fe\overset{O_2}{(Cn)_5})K_3$. It too acts as an iron catalyst to compounds which show affinity for iron. These iron catalysts can fertilize themselves by the absorption of an oxygen atom, and give this absorbed oxygen to readily oxidizable substances. The two processes constituting respiration.

The properties of this ferro-magnetic iron have been investigated by Baudisch and his coworkers. The change $Fe^{II} \rightleftharpoons Fe^{III}$ involves great energy changes, and the reverse action is not likely to occur in the catalytic action of iron. The iron of a complex ion, however, is easily brought into each of the stages of oxidation by violet light or hydrogen peroxide. The divalent iron is more reactive because its secondary valences are stronger and therefore able to draw various radicals into the inner sphere. Baudisch believes that the magnetic properties of iron and its power to reduce nitrates are closely related. "Practically non-magnetic, white ferrous hydroxide becomes black and strongly ferro-magnetic on the absorption of oxygen, but on further absorption of oxygen it turns into red and weakly magnetic ferric hydroxide." This shows that there must be several stages in the oxidation of the nucleus. If sodium bicarbonate is used in the precipitation of the iron complex instead of the hydroxide, the iron goes into solution to form polynuclear magnetic compounds of the constitution



The molecular oxygen stands in direct relation to the formation of polynuclear complexes and magnetic properties of iron the same as it does to the reduction of nitrates to nitrites. These different iron complexes are isolated and identified by their solubility in 50 per cent acetic acid, and by their power to absorb water.

We state again that all this work on the catalysis of iron is based on the articles of Baudisch. The generalizations that he makes are backed by experimental evidence. He notes further that it is the concentration of each of the components oxygen, ferrous salts and carbon dioxide that determine the rate of oxidation. The carbon dioxide protects the ferrous compound from oxidation until a nascent ferrous hydroxide has been formed from ferrous bicarbonate by hydrolysis and then oxygen can be absorbed to form the active compound. Many compounds that can be reduced by catalytic iron, because their auxiliary valences are stronger than those of oxygen, are mentioned in his articles.

In commenting on the relation between catalytic iron and nutrient solutions we can say that ferrous iron is most available to plants in culture solutions. Ferric citrate has been found a good source of iron for plants. Citric acid, however, is a reducing agent and when ferric citrate is formed some iron may be reduced to the ferrous condition. In a test for ferrous iron in a sample of ferric citrate, ferrous iron was shown to be present. It gave a distinct blue color when potassium ferricyanide was added. Carbonates added to a culture solution increase growth. It is possible that their function may be more than maintainance of equilibrium. They perhaps form ferrous carbonates which are more soluble in excess carbonate solution than ferric compounds, and also retard the rate of oxidation of the ferrous iron by oxygen. The solubility of iron phosphates have been reported as more soluble in a neutral solution than in an acid solution. Our laboratory work shows that this is not true for all nutrient solutions. It may be true for some solutions. If it is we note an interesting and most

likely a very important coincidence of six facts. (1) The acidity of an important buffer salt in nutrient solutions is pH 6.30. If this salt is not originally added it is soon formed. (2) The acidity of plant sap is pH 6.0 to 6.2. (3) The HCO_3-ion is important in the formation of the complex iron nucleus which reduces nitrates. (4) Carbonates added to a nutrient solution increase growth. (5) Ferrous iron is not oxidized to an inactive ferric form as rapidly when bicarbonates are dissolved in the solution. (6) Ferrous carbonates are more soluble in solutions containing ammonium carbonate than in solutions which contain no carbonate.

DETERMINATION OF IRON IN NUTRIENT SOLUTIONS.

Volumetric Determination. Problem. The study of the literature and some tentative culture work made it evident that a measure of the iron present in a nutrient solution was very important. This iron may be present in the form of a suspension and as a true solution. No adequate method had been worked out for the determination of iron in quantities such as those present in culture solutions.

Two types of analytical procedure, the colorimetric and volumetric are used to determine iron. The colorimetric methodis used for determining small quantities. It is a comparative method influenced by several factors. The volumetric is a stoichiometric determination, that is, the chemical reactions involved are each complete. The work of Gile and Carrero (28) indicates that the amount of iron present in nutrient solutions is very small within and beyond the limit of the colorimetric range, while Tottingham and Rankin (89) report solubilities well within the range of titration with $KMnO_4$. When such a volumetric method is used, enough iron must be present so that a volume of permanganate is required which is large enough to overcome the end point error of the solutions titrated. On the other hand the permanganate must be concentrated enough to make the end point evident in the volume of iron solution that is used. It is known that phosphates present in an iron solution interfere with its colorimetric determination. Nitrates also interfere, forming a yellow color which is

difficult to distinguish from the red color given by the iron complex.

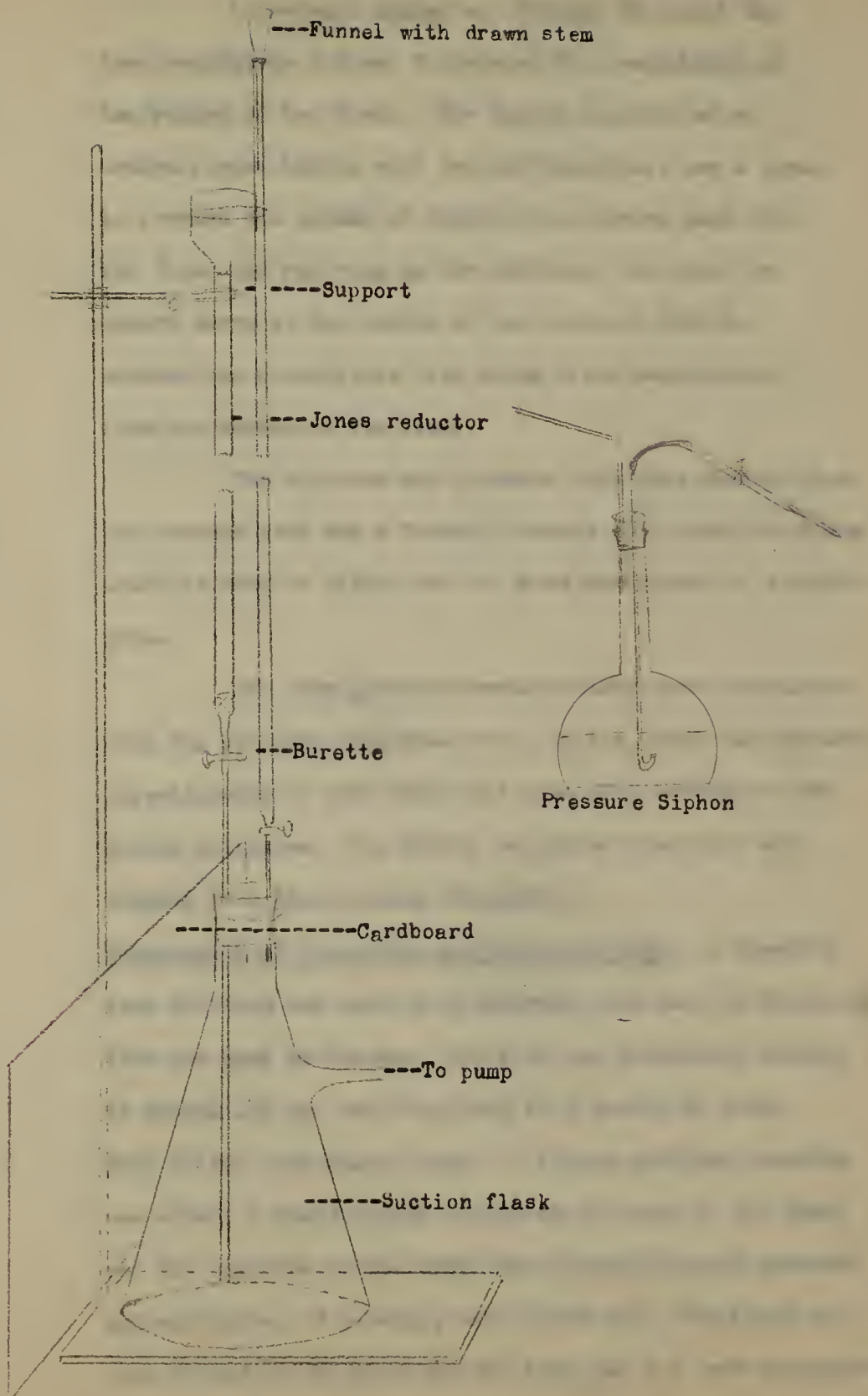
It was our purpose to gain information on both the iron in solution and the iron in colloidal suspension. This eliminated the colorimetric method since the limit of its range is beyond the quantity present in a ferric phosphate suspension. This was assumed as a fact. Murray (62) described a method which was selected as the basis for this work. In the determination of the iron content of blood she reports quantities of iron which were within the range of the quantities of iron given by Tottingham (89). Murray's method runs as follows: after incinerating the blood sample, she dissolved the ash in HCl. The acid is removed by heating the solution with strong H_2SO_4 and the iron determined by a reduction and oxidation process. She used a special reducer. Instead of the ordinary Jones reductor the column of amalgamated zinc was replaced by "cadmiumized zinc," made by submersing zinc in a 2% cadmium sulphate solution for five minutes. The quantity of reduced iron in the solution is measured by oxidation with eightieth normal $KMnO_4$ in an atmosphere of hydrogen. Her apparatus consists of a modified Jones reductor fitted into a suction flask by means of a two-hole rubber stopper. The nozzle of a small burette to contain the $KMnO_4$, is inserted through the other hole. The flask is connected to a suction pump. The iron solution, after proper dilution, is drawn through the reductor and while the pump is still running is oxidized by the permanganate. Eightieth normal $KMnO_4$ was found the most convenient for

this work altho stronger or more dilute concentrations are also satisfactory.

Apparatus. In our work the apparatus used by Murray, modified to suit our purpose, was used. The representation is given in the diagram below. An ordinary Jones reductor with a (32 cm.) column of amalgamated zinc is fitted into a half liter suction flask of clear white glass. The upper end of the glass tube is enlarged to form a funnel (5 cm. x 5 cm.). Glass beads covered with a mat of glass wool prevents the zinc almalgam from escaping thru the stopcock. The end of the reductor tube comes almost to the bottom of the suction flask.

A small burette is attached to the reductor. The nozzle of the burette projects several centimeters below the stopper in order to prevent the permanganate from creeping upward and adhering to the stopper. The burette is of 10 c.c. capacity. It is graduated to 1/50 of a c.c. and easily read accurately to 1/100 of a c.c.. A small glass funnel with a long stem of small diameter is inserted in the burette so that the permanganate will run down the sides of the burette. If this funnel is not used, air bubbles, difficult to remove, enter the burette.

The reductor is supported by a ringstand and clamp. When the clamp is attached just below the cup of the reductor the flask can be removed after each titration by raising the reductor and clamping it when the lower end of the reductor tube is above the mouth of the flask. The flask is attached to a suction pump with heavy rubber tubing. The suction flask is set on white paper and a white



Titration Apparatus

cardboard placed back of it.

A pressure siphon was devised to decant the iron suspension without disturbing the precipitate at the bottom of the flask. The siphon consists of an ordinary wash bottle with two modifications, one a clamp to prevent the column of liquid from flowing back into the flask and stirring up the sediment, the other an upward curve at the bottom of the delivery tube to prevent the precipitate from being drawn mechanically from the bottom of the flask.

The burettes and pipettes used were standardized. The balance used was a Troemner balance upon which weighings could be made to within two to three hundredths of a milligram.

The hydrogen-ion concentrations were determined with the hydrogen electrode and a type K, Leeds and Northrup potentiometer or with Clark and Lubs (16) indicators and buffer solutions. The buffer solutions when used were checked with the hydrogen electrode.

Preparation of Standards and Materials Used. A standard iron solution was made by dissolving iron wire in dilute H_2SO_4, five per cent by volume. The iron was accurately weighed to within .03 mg. and dissolved in a two-liter flask half filled with dilute acid. A little hydrogen peroxide was added, a small funnel placed in the neck of the flask and the solution boiled until the excess hydrogen peroxide and acetylene, if present, had boiled off. The flask was then filled to the mark and the iron per c.c. was calculated

from the per cent purity, 99.858%, given by the maker. The standard from which dilutions to make up samples are made must not contain more than 0.05 gm. per liter, otherwise an error noticeable in titration will result from making the dilution.

The sulphuric acid used to dissolve the iron wire and the iron precipitates in the determinations of the iron in nutrient solutions was the five per cent acid recommended for the Jones reductor by Blasdale (89). This was also used for washing the reductor before determinations were made and to wash the reductor immediately after a sample was run thru. The purity of the sulphuric acid seemed to vary with different bottles. The impurities are due to the oxides of nitrogen and iron. Reduction and titration by this method can be used perhaps to determine the quantity of such impurities present in sulphuric acid.

The nutrient solution used is that represented as III R2S1. It has the following partial volume molecular concentrations: potassium nitrate, 0.0054; calcium acid phosphate, 0.0027, and magnesium sulphate 0.0135. The calcium acid phosphate, as it was purchased, contained enough free phosphoric acid to give a twentieth molar solution, a pH 2.96. Before the tenth molar solution was added to the complete solution, the pH was brought to pH 4.0 by the addition of saturated lime water, $Ca(OH)_2$. This culture solution with three grams ferric phosphate has been found best for growing wheat plants by Erdman and Bakke.

The ferric phosphate used was passed thru bolting cloth (100 mesh), heated at 100°C. for twelve hours, cooled in a desiccator and set in the balance case with an open bottle of sulphuric acid. Before this treatment and method was used, it was difficult to weigh the ferric phosphate because of its hygroscopicity.

The ferric phosphate was weighed and transferred to the flasks. While the flasks were being filled they were rotated briskly to insure a uniform suspension. The suspension is necessary for rapid solution when the flasks stand for short periods.

The diphenylamine, $C_6H_4(NH_2)_2$, used to detect nitrates can be made so that it is clear by putting the crystalline salt in a Gooch crucible. The crucible which has a perforated bottom is dipped into pure sulphuric acid. When the first traces of color appear at the bottom of the crucible the salt remaining in the crucible is rejected and a new portion used. This is repeated until the desired strength of solution is obtained. Three four-gram portions were sufficient to produce 100 c.c. of the reagent for the nitrate tests in this work. The 100 c.c. acid are diluted with water in the ratio: one part water to five parts acid diphenylamine. The solution must be kept cold when the water and acid solution are mixed.

Single salt solutions that were used in making up the culture solution, III R2S1, were dissolved. Blanks containing 50 c.c. to 200 c.c. of a molar solution of each, except KnO_3, were passed thru the reductor and titrated.

The addition of the salts gave results no different from those of the blanks.

Dilute acid was passed through several filter papers but no reducible matter sufficient to affect the titration was dissolved from the filter.

The iron wire that was used as a standard contained both ferric and ferrous iron. The ferrous was oxidized to ferric before standardization in the nutrient solution because the ferrous is not precipitated by ammonium hydroxide.

The ammonium phosphate used to wash the precipitate was made by adding phosphoric acid and ammonium hydroxide in the ratio: 69 c.c. (85%) phosphoric acid to 411 c.c. (Sp. Gr. 90) ammonium hydroxide. Five c.c. of this solution were used in a liter of water.

A tenth normal permanganate solution was diluted to approximately N/80. The final dilution was standardized against the iron solution already described.

Volumetric Method in Nutrient Solutions. Before making a determination, the reductor is washed with dilute sulphuric acid (5% by volume). The volume of acid necessary depends upon the condition of the reductor. When it has stood idle for some time, more acid is required to wash the zinc amalgam free of iron. Usually about one liter of acid is required before blanks of dilute acid check. Three hundred c.c. of dilute acid can conveniently be used as a blank. After this is drawn through the reductor into the flask, it is titrated with the $KMnO_4$ (N/80) from the burette. The amount

of oxidizable substances in terms of the permanganate equivalent varies with different samples of sulphuric acid. For 300 c.c. of dilute acid, used as a blank in our work, it varied from .10 c.c. permanganate to .25 c.c.. The acid put out by the J. T. Baker Company has been found best and uniformly the equivalent of .10 c.c. of $KMnO_4$ (N/80).

When determining the iron in a sample which has been dissolved in a liter of dilute acid, 250 c.c. can be measured conveniently in a volumetric flask. At the dilution used in this work, two 25 c.c. portions of dilute acid wash out all the iron from the reductor after the sample has passed thru. The quantity of iron present was found by subtracting the volume of $KMnO_4$ necessary for a blank from the total volume required and from this difference, calculating its iron equivalent. The iron equivalent is obtained by standardization of the permanganate against standard iron solution. Before restandardization of the permanganate, the effects of different shades of light on the detection of the end point were studied. The titrations thus far had been made under favorable lighting arrangements. The work was done in a north room with the snow on the ground. Diffused light came in thru four windows, two at the rear and two on the right. A white cardboard was set behind the flask, reflecting the light diffusing thru the four windows. The best reading could be made when the cardboard was set an angle of about 45° to a ray of light from the window on the right.

During a period of foggy weather, it was difficult

to read the end point. The titrations did not vary with the intensity of the light but the blanks did vary as much as .02 c.c. of $KMnO_4$. Incidentally, the effect of shades of light were tried. No difference was found in the volume of $KMnO_4$ required to oxidize a blank when using daylight, an artificial blue light, a yellow light and both the yellow and blue lights together. No artificial light is as satisfactory as diffused sunlight, at least as long as the experimenter is accustomed to titration with daylight. All titrations recorded in the work were made with daylight.

A sample of a nutrient solution is taken by inserting the delivery tube of the pressure siphon into the flask containing the solution. Air pressure is applied and the clamp released. When 250 c.c. have been siphoned over, the clamp is again attached before the pressure is released. This afforded a very convenient means of decanting the supernatant solution without disturbing the sediment on the bottom of the flask. From one flask triplicate samples were usually taken and the solution remaining in the flask measured, in order to compare the quantity of iron recovered by determination with the quantity of iron originally dissolved.

The iron in a nutrient solution is determined by precipitation of the iron, washing the precipitate free from nitrates and redissolving the precipitate in dilute acid. The sample is poured into a 400 c.c. beaker, five c.c. of concentrated H_2SO_4 added and the iron precipitated with strong ammonia. Five c.c. of H_2SO_4 have been used but a

smaller volume may be sufficient. When NH_4OH is added, not only the iron precipitates, but a heavy white flocculent precipitate is dispersed thru the solution. This dispersion is settled by heating on the steam bath for a period of twenty minutes or more. The supernatant liquid is decanted thru a filter. The now crystalline precipitate is washed with water by decantation several times. When the washings are free from nitrates, the precipitate on the filter is dissolved in 100 c.c. of dilute acid. The acid solution passing thru the filter is run back into the beaker in which the sample was precipitated, in order to dissolve any iron which may adhere to the walls of the beaker and to the stirring rod. The iron in the sample is then determined by reduction and oxidation.

Since a liter of solution was made up for each sample, the value for the number of c.c. of permanganate is generally the average of four titrations.

It is interesting to note that the size of the precipitate on the filter is not increased correspondingly by an increase in the quantities of iron present in the solution because of the bulk of the other precipitate brought down with the iron. The color of this precipitate, however, varies and is a good index of the quantity of iron present. Noting the color, if white little iron is present, is helpful in approximating the number of c.c. permanganate necessary in the subsequent titration. Tottingham and Rankin (89) report a white flocculent precipitate upon the addition of ammonium hydroxide to a filtered nutrient solution. From

our work it seems that a white precipitate is not consistent with the quantities of iron which they report.

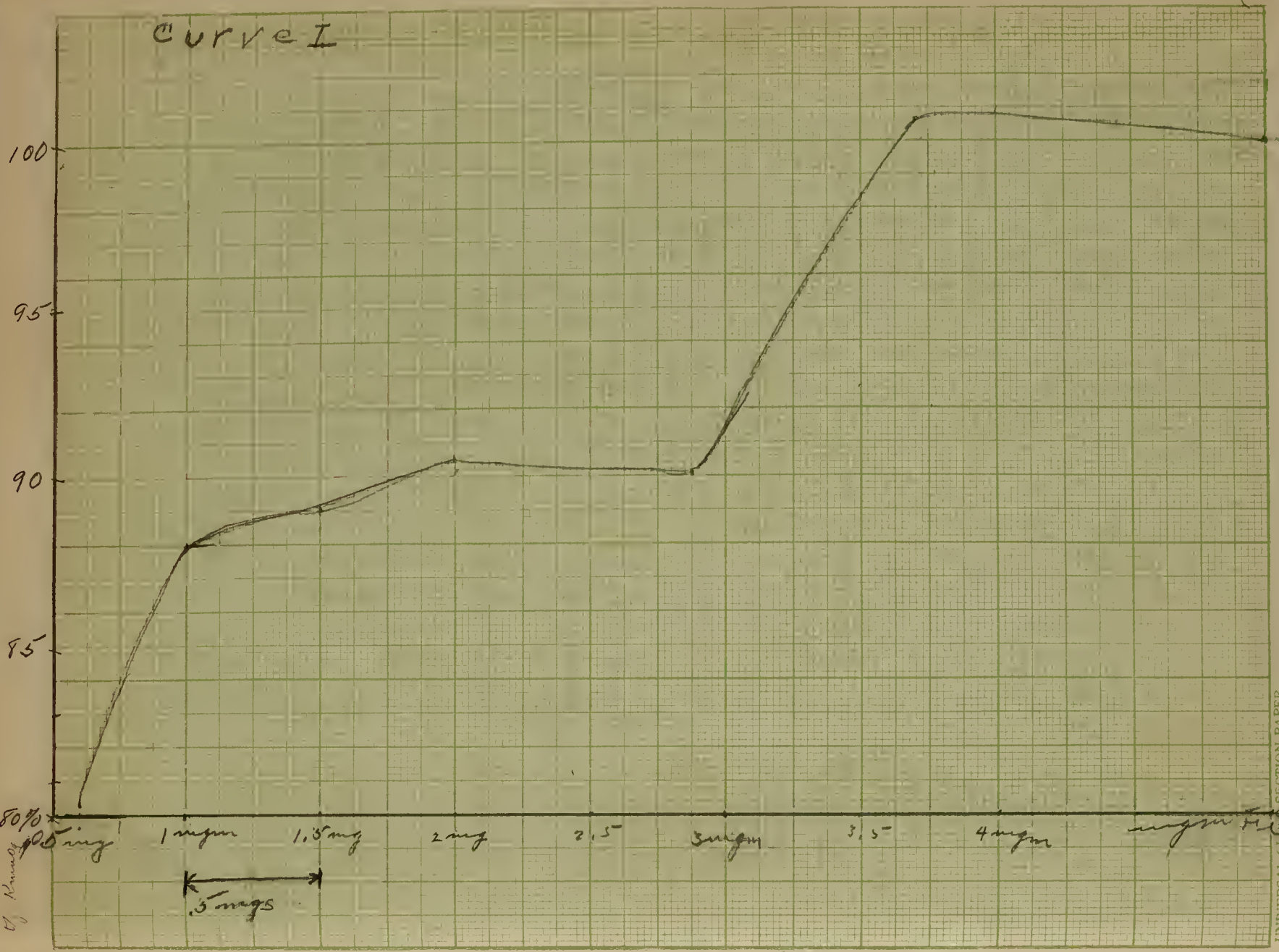
Standardization of the Permanganate. Table I and the curve from the data of Table I show the results of the first standardization. Quantities of iron, .62 mg. to 4.98 mg., were dissolved in two-liter flasks. These quantities cover the same range as the amounts of iron usually dissolved in nutrient solutions. Where more than five mg. were dissolved, liter flasks were used. The figures in the first column of Table I give the quantities of iron present per liter of solution. From the percentage purity of the iron, the value in terms of pure iron was calculated. The second column gives the number of c.c. of $KMnO_4$ (N/80) required to oxidize the ferrous iron to the ferric condition. From the c.c. of permanganate used its iron equivalent was determined. The equivalents are given in column three. Since 250 c.c. were used for each titration, the value of iron in each sample is the mgs. iron per liter, divided by four. These values are given in column four. The per cent normality on the basis of N/80 is given in column five. It is derived by the division of the actual amount of iron present by the permanganate equivalent. In the sixth column is given the average percentage normality of the titrations made for each set of iron samples. They vary for different amounts of iron present in the sample. In the last column, figures are given which show the variation which one drop of permanganate can give with the indicated amount of iron. They show that the end point error decreases as the quantity of iron present increases.

Table I.

Standardization of Permanganate.

Fe Mgs./L	$KMnO_4$ c.c.N/80	Fe equivalent of $KMnO_4$ Mgs.	Value of iron by weight	$KMnO_4$ % N/80	Average %	Approximate variation for one drop
0.62	.26	0.18148 Mgs.	0.15456 Mgs.	85.28		
	.24	.16752		92.28		
	.29	.20242		76.39		
	.30	.2094		73.84	81 36	
	.28	.19544		79.08	80.54-	11.44%
0.1035	.40	0.2792 Mgs.	0.25838 Mgs.	92.76		
	.41	.28618		90.29		
	.42	.29316		88.13		
	.43	.30014		86.06	88.13	8%
1.575	.66	0.46068 Mgs.	0.393191 Mgs.	85.35		
	.60	.4188		93.89		
	.64	.44672		88.01	89.06	5.3%
1.98	.79	0.55142 Mgs.	0.493277 Mgs.	89.46		
	.77	.53746		91.78		
	.78	.5444		90.61	90.61	4.5%
2.83	1.10	0.7678 Mgs.	0.706 Mgs.	91.95		
	1.15	.8027		87.95	89.95	3.+%
3.72			0.92868 Mgs.			
	1.32	0.93036 Mgs.		99.82		
	1.29	.89842		103.34		
	1.30	.9164		101.34	101.50	3.+%
4.98	1.78	1.24244 Mgs.	1.243 Mgs.	100.05		
	1.74	1.21425		102.40		
	1.80	1.2564		98.96		
	1.82	1.2703		97.97	100.00+	2.4%
3.7	1.29	0.89942 Mgs.	0.923686 Mgs.	102.70		
	1.305	.90989		101.53		
	1.32	.92136		98.89	101.53	
7.93	2.92				102.79	
9.38	3.36				100.00	
4.09					98.85	
4.8					93.60	
9.6					98.22	
3.375					98.48	

(Page 71)



UNIVERSAL CROSS SECTION PAPER

One drop of permanganate from the burette was equal to .04 c.c.. The volume added beyond the end point can be estimated to .01-.02 c.c. by the depth of the color of the tinge produced when the end point was overstepped. Since the iron equivalent of one drop of permanganate remains constant, while the amount of iron present increases, the relative error grows less. This can be illustrated by the formula, $\frac{K}{X} = Y$. If K represents the error which is constant and X the amount of iron which is variable, then Y will increase and decrease inversely to X.

Curve I is a graph of the data of Table I. The normality factor in terms of eightieth normal as the ordinate has been plotted against the weight of iron in the solution as the abscissa. The irregularities in the curve are due to two sources of error, the errors of weighing and the errors due to the inaccurate reading of the end point. These are the results of the first determinations that were made. The uniformity with which weighed quantities of iron could be estimated when dissolved in acid was encouraging. At first some difficulty was found in reading the end point. After some experience, one drop of permanganate gives a decided color and estimations can be made to one or two hundredths of a c.c.. If we allow the end point error to be two hundredths c.c., the actual weight of iron caused by this error is very small. A calculation will show this. The equivalent of one c.c. of N/80 permanganate is 0.000698 gms iron, then .02 c.c. are equivalent to 0.0000139% gm. iron. An average of a

number of titrations gives an error no larger than two hundredths c.c.

To approximate actual working conditions, the permanganate was also standardized against iron dissolved in acid nutrient solution. Iron wire was not weighed out for each sample but the sample was made by dilution from a stronger standard iron solution. Much time was spent in learning the best method of washing the precipitate. When it was learned how to treat it, the N/80 permanganate was restandardized. The results are given in Table II in the same order of arrangement as in Table I. The double line indicates where a change was made from a dilute to a more concentrated iron solution. With larger amounts of iron one can again see the variations of the normality factor.

Table II.

Second Standardization of Permanganate.

Weight of Fe/L	c.c. $KMnO_4$	Fe (by Wt.)	Fe (by Detm.)	% N/80	Average
0.1132 Mgs.	0.04 c.c.	0.0283 Mgs.	0.02792 Mgs.	101.40	
	0.05		0.03490	81.11	91.25
0.22647 Mgs.	0.08 c.c.	0.056619 Mgs.	0.05604 Mgs.	101.40	
	0.09		0.06282	90.13	
					95.71
0.4529 Mgs.	0.19 c.c.	0.113238 Mgs.	0.13202 Mgs.	85.36	
	0.20		0.1396	81.12	
					83.24
0.679 Mgs.	0.27 c.c.	0.1698 Mgs.	0.18846 Mgs.	90.13	
	0.28		0.19544	86.89	88.51
0.9059 Mgs.	0.37 c.c.	0.2239 Mgs.		86.72	
	0.38			84.44	85.58
1.0194 Mgs.*	0.42 c.c.	0.25485 Mgs.		86.93	
	0.43			84.93	85.93
1.043 Mgs.*	0.40 c.c.	0.26075 Mgs.	0.2793 Mgs.	93.40	93.40
2.0861 Mgs.*	0.78 c.c.	0.52152 Mgs.	0.5444 Mgs.	95.79	95.79
3.129 Mgs.*	1.15 c.c.	0.7822 Mgs.	0.8026 Mgs.	97.45	97.45
4.179	1.51	1.043	1.043	98.98	98.98
5.214	1.88	1.3035	1.3035	99.33	99.33

It is not practical to take the normality factor from a curve such as that plotted for the data of Table I. To illustrate this let us take the greatest variation in percentage from Table II. This is from 101%-80%, with a change of .04-.05 c.c. permanganate. The difference

*The average of two or more titrations.

in the normality factor is 20%. The iron equivalent of .05 c.c. permanganate is calculated as follows:

1 c.c. $KMnO_4$ = 0.000698 gm. iron
.05 c.c. $KMnO_4$ = 0.0000349 gm. iron
20% Error = 0.000000698 gm. iron.

With increase in iron in the sample determined, the percentage error grows less but the net error remains the same. Iron in such quantities is beyond the range of this method. The normality factor of the permanganate found, when 2-5 mgs. iron per liter are used in standardization, is accurate enough for all quantities of iron of a lower value.

The results of the standardization of the permanganate against iron in acid nutrient solutions are given in Table III. The range, 1 mg.-5 mgs. iron per liter, was used. Permanganate of the same strength as in Table II was used. The data is arranged as in the other tables.

Table III.

Standardization by Precipitate Method.

Fe per L	N/80 c.c. $KMnO_4$	Fe (by Wt.)	Fe (by Detm.)	% N/80
1.043 Mgs.	.30 c.c.	0.26075 Mgs.	0.26524 Mgs.	98.31
2.0861	.74	0.5215	0.1565	100.94
3.129	1.10	0.7825	0.7678	101.92
4.170	1.51	1.043	1.054	98.98
5.214	1.88	1.3035	1.3132	99.33

From the results of Table III and from the discussion of error it is apparent that the same normality factor can be used in determination of iron in acid

(?) Recommend

found to find Fe^{+++} in

tests p 76 (H_3PO_4

washing) because of the

large amount of PO_4 in

solution?

[illegible] 0.3 cc of pho. acid

[illegible] N [illegible]

[illegible] could [illegible] color

[illegible] .6 cc, [illegible]

[illegible]

H_3PO_4

1 cc per lit

1.7

$Ca(H_2PO_4)_2$

40
4
62
128

234

117

1.178 0.1

.01178 grms per cc N.1

N

1

nutrient solutions as in pure dilute acid.

Washing of Precipitates. Some preliminary work on the washing of the precipitate formed by the addition of NH_4OH to a nutrient solution indicated that more knowledge of this was desirable. In an effort to further study this problem, four cold and hot (80° C.) washing solutions were used. These were: (1) water, (2) ammonium hydroxide, one per cent, (3) ammonium phosphate and (4) phosphoric acid, a few drops per liter of water. The results are given in Table IV. Column one gives the number of c.c. of permanganate used in the titration. Column two gives the presence or absence of iron in the filtrate. The usual test for iron was made by adding potassium thiocyanate to a solution acid with hydrochloric acid (53). Column three gives the presence or absence of nitrates. Nitrates were detected by adding a clear solution of diphenylamine to the filtrate from the washings after the required number of washings had been made. Column four gives the solution used to wash the precipitate; column five, the number of times the sample was washed; column six, the weight of the sample of iron taken; column seven, the iron equivalent of column one and column eight, the error on the basis that the iron by weight was exact.

Table IV.

Effect of Washing.

c.c. $KMnO_4$ 99.33% N/80 av. of 4 titrations	Test for Fe^{+++}	Test for NO_3	Washings Solution	No. of times	Fe by Wt.	Fe by Detm.	Error
1.88 c.c.	-	+	Cold water	2	1.3035 Mgs.	1.3034 Mgs.	-0.0001 Mgs.
1.88	±	-	" "	5	1.3035	1.3034	-0.0001
1.88	±	-	Hot water (80°C.)	2	1.3035	1.3034	-0.0001
1.87	+	-	" "	5	1.3035	1.2965	-0.007
1.94	+++	-	Cold NH_4OH	2	1.3035	1.3452	+0.417
1.89	+++	+	" "	5	1.3035	1.3103	+0.0129
1194	-	-	Hot $(NH_4)_3PO_4$	2	1.3035	1.3452	+0.417
1.90	++	+	Cold "	5	1.3035	1.3173	+0.0118
.77	-	-	Hot H_3PO_4	2	0.6268	0.5406	-0.0862
.63	-	-	Cold "	5	0.6268	0.4423	-0.184

This data shows that cold water used two or three times is best for washing the precipitate of this nutrient solution free from nitrates. The samples washed with hot and cold water give less error than any of the other solutions. The iron and nitrate estimations show that the colorimetric tests are more sensitive than the titration method. Enough nitrates may be present to be detectable in the washings or enough iron lost to give a color with sulphocyanate without being of any consequence in titration. Ammonium hydroxide is ineffective in washing out the nitrates and dissolves some of the iron on the filter. This seems contrary to general knowledge. The only explanation found for this fact is that some iron passes through the filter as a dispersion when the ferric phosphate on the filter changes to ferric

hydroxide. Ammonium phosphate is found also to be ineffective in washing out the nitrates and like NH_4OH dissolves some iron from the filter. Phosphoric acid dissolves a relatively large amount of iron. The errors in the last column show that determinations by reduction and titration can be made very accurately and the ferric iron in nitrate solutions can be determined accurately by precipitation with NH_4OH if cold water is used as the solution to wash the precipitate free from nitrates.

Determination of Iron in Nutrient Solutions. A satisfactory method of handling the precipitate from acid solutions containing nutrient salts had now been developed. Less delay and difficulty had been anticipated and flasks containing culture solution III R261 with varying quantities of $FePO_4$ had been set out December 5, 1924. These solutions were analyzed for dissolved iron after standing for a period of two months. The results are given in Table V. The data is of little significance other than that it shows that there was little iron in solution at the time of analysis and that very nearly all the iron originally weighed out can be recovered from the precipitate.

Some important improvements in technic were made. The clamp had not been attached to the delivery tube of the siphon, described in the apparatus, when the decantations from the first samples were made, consequently the sediment in the bottom of the flask was stirred up when the column of liquid in the delivery tube flowed back into

the flask. This agitation was immediately noticed and the order of the decantations, as they were made, was taken. The order is given in column one of Table V. When the end point was overstepped in titration, the second sample was run into the first sample without disconnecting the reduction apparatus. This was possible because only 100 c.c. of dilute acid are used to dissolve the precipitate. Whenever one sample was run into the next in this way the fact has been indicated by parenthesis in column two, where the c.c. of permanganate required for the oxidation of the iron solution are given. The blank to be subtracted from the c.c. of permanganate of column one varies between .10 c.c.-.12 c.c.. It varies because the same quantities of acid were not used for all samples. Column three gives the grams $FePO_4$ recovered by determination, column four the grams $FePO_4$ weighed out and column five the error in terms of ferric phosphate, assuming that the weighed quantity was the correct value. Before the ferric phosphate equivalent of the c.c. of permanganate could be determined, the exact percentage of iron in the phosphate originally dissolved was necessary in the calculation. The iron by determination divided by the per cent iron in ferric phosphate gives the ferric salt of column three. The per cent iron in the sample of ferric phosphate was determined by dissolving the phosphate in dilute sulphuric acid, precipitating with NH_4OH, washing and dissolving of the precipitate, reduction and titration. This procedure was necessary because the ferric phosphate, as it was bought,

was not nitrate free. The analysis gave 24.3% iron in the sample. After three portions had been decanted, the remaining 250 c.c. was put into a beaker and marked Number IV. The flask was rinsed with 50 c.c. dilute acid and the rinsing added to the portion in the beaker. Not all the solutions were examined for recovery of total iron but only a sufficient number to prove that the iron originally weighed out could be recovered. The solutions used for the data of Table V had an acidity of pH 4.6.

We have stated above that a sufficient number of samples were analyzed to prove that full recovery of the iron weighed out can be made. The fact is that most of the others that are left blank were necessary to solve another problem. In the acid solutions used up to this time the dispersed precipitate had become granular and settled after heating on the water bath. When NH_4OH was added to a nutrient solution, a colloidal dispersion was formed which would not settle. On the filter it formed an unmanageable gelatinous mass which filtered very slowly and was impossible to wash free from nitrates. It was observed that the Number IV samples in each case were less colloidal. The more crystalline condition of sample Number IV could result from two causes, the solid ferric salt, which induced further precipitation and the acidity caused by adding the 50 c.c. of dilute acid from washing the flasks. Both of these may be a factor but the addition of acid solved the problem. This is explained by Alexander Smith (83) by the theory of colloidal suspensions. The

Table V.

Iron Dissolved in Nutrient Solution After Sixty Days.

Sample	c.c. $KMnO_4$		Fe by Dotm.	Fe by Wt.	Error
1	(.11)				
2	(.08)				
3	.29				
1	.10				
2	.11				
3	.12				
1	.10				
2	.11				
3	.11				
1	.11				
2	.13				
3	.13				
4	3.00	2.94*	0.008422	0.00789 Gm.	+0.000532
1	(.12)				
2	(.13)				
1	.11				
2	.12				
3	.33				
4	3.86	3.97*	0.01133	0.0103 Gm.	+0.00103
1	(.12)				
2	(.09)				
3	(.13)				
4	6.29	6.17*	0.01768	0.0179 Gm.	-0.00022

*Gives the number of c.c. of $KMnO_4$ required to oxidize the iron in number IV when .11 c.c. is considered to be equal to the blank.

addition of 5 c.c. of concentrated sulphuric acid before precipitation caused the precipitate to settle as it had done in the earlier determinations.

The errors in Table V seem to be large but this is a comparison between quantities of ferric phosphate. The amount of iron determined is the equivalent of the sum of four titrations thus allowing four end point errors to enter. The percentage of iron in iron phosphate is less than 25%, hence the error is multiplied four fold. This multiplies the initial error sixteen times. The effect of an error of .01 c.c. is shown by the following calculation:

Iron equivalent of 1 c.c. N/80 $KMnO_4$ = 0.000698
" " " .01 c.c. " " " = 0.00000698
x 16
Multiplied sixteen times = 0.00011168

Only one titration can be made of Number IV. If the end point is overrun, no duplicate can be made to correct it. When it is not known where to expect the end point, it is easily overstepped, especially in titrating large quantities of iron in solution. Error may also result from incorrect weighing.

The results of the analysis of the ferric phosphate are given in Table VI. The first column gives the weight of the sample of iron phosphate weighed out; the second, the number of c.c. of permanganate required to oxidise this sample; the third, the iron equivalent of the permanganate, and column four, the per cent iron in the sample.

Table VI.

Determination of Iron in $FePO_4.4H_2O$.

Wt. of Sample	c.c. $KMnO_4$	Wt. of Fe	% Fe
0.00833 Gm.	2.90 c.c.	0.0020242	24.30%
0.009135 Gm.	3.20 c.c.	0.0022316	24.43%

Theoretically there should be 26.84% iron in $FePO_4.4H_2O$. The results of some preliminary analyses of the stock salt gave variable results when the phosphate was dissolved in acid, reduced, and titrated. The method of preparing ferric phosphate: $Fe(NO_3)_3 + H_3PO_4 = FePO_4 + 3HNO_3$ suggested that nitrates might be present from incomplete washing. A qualitative test of an acid solution proved that they were present.

When ammonium hydroxide is added to an iron solution colloidal ferric hydroxide is formed. To prevent the colloidal formation, calcium acid phosphate was added to the acid solution before precipitation. This induced crystalline precipitation when ammonium hydroxide was added and the solution digested on the water bath.

On February 23 another series of solutions with ferric phosphate was set out and the iron in solution and suspension estimated. The results are given in Table VII. Column one gives the time of standing; two, the volume of permanganate used to titrate the sample

of decanted solution; three, the iron equivalent of column two; four, the weight of ferric phosphate dissolved; five, the amount of ferric phosphate derived by calculation of the iron recovered by determination; six, the pH value of the solution to which the phosphate was added, and seven, the iron present by colorimetric estimation in parts per million (p.p.m.). The colorimetric estimations were made on 50 c.c. portions in colorimeter tubes of this capacity. The standard method was used (53). The c.c. $KMnO_4$ are the average of two or three titrations. The pH values were made colorimetrically.

Table VII.

Solubility of Iron in Nutrient Solutions.

Period of standing	c.c. $KMnO_4$	Fe by detm. of decanted soln.	$FePO_4$ by (Wt.)	$FePO_4$ Detm.	pH	Fe colorimetrically
3 Days	.03	0.00209 Mgs.	0.0114 Gm.	0.0117 Gm.	3.75	-
3 "	.03	0.00209	0.0281	0.02755	3.75	-
6 Days	.00	-	0.0284 Gm.	0.0292 Gm.	4.00	.03 p.p.m.
8 Days	.00	-	0.0237 Gm.	0.02402 Gm.	5.00	.01 "
14 Days	.00	-	0.0350 Gm.	0.0309 Gm.	4.60	.005 "

Due to the difference in hydrogen-ion concentration, these values are not comparable, nevertheless they show that even at a low pH most of the iron is out of solution and suspension at the end of three days. This agrees with the work of Gile and Carrero (28) and is less than that reported by Marsh (52). The results of the

different investigations are not comparable because the same iron compounds and solutions were not used in each. The other investigators filtered their solutions. It was thought that the precipitate might be a suspension of such small dimensions that it passed through the filter paper.

To test this point, a solution of pH 5.0, containing .022 gm. ferric phosphate which had been standing for four days, was decanted with the siphon apparatus. Part of this solution was filtered. Both the unfiltered and filtered solutions were analyzed for iron colorimetrically. As there was no difference in iron content between the filtered and unfiltered solutions, the solution of four days standing was shaken so that the precipitate was thoroughly dispersed. After ten hours standing another portion was filtered. The colorimetric estimations gave the same results as those of the first filtration. Assuming that the size of the particle had already been determined by the previous four days standing, so that the shaking and ten hours standing after agitation did not effect the size, it was further proved that the particles are of such dimensions that they do not pass through an ordinary No. 30 quantitative Whatman filter paper. Ferric phosphate was suspended in a solution which was decanted after eight hours standing. The iron was estimated in the decanted solution after it was filtered through one filter paper, through three filter papers folded within each other, through one hard filter

and through an iron-free asbestos filter. The results are recorded in Table VIII. Column one gives the method of treatment; two, the time of standing; three, the c.c. of standard iron added to a check to match the color of the sample; four, the acidity and column five, the iron in solution estimated colorimetrically.

Table VIII.

Iron in Filtered Solutions.

$FePO_4$ dissolved per liter - 0.0220 Gm.
Iron in standard solution per c.c. - 0.00001404 Gm.
Iron in 1 c.c. per 50 c.c. of sample - 0.3 p.p.m.

Method of treatment	Period of standing	c.c. Fe soln. used	pH	p.p.m.
Decanted	4 Days	.05 c.c.	5.00	0.015
Decanted and filtered	4 "	.03		0.009
Filtered after shaking	4 "	.05		0.015
Decanted	10 Hrs.	8.00		2.4
Decanted and filtered	10 "	.05		0.015
Filtered after shaking	10 "	.05		0.015
One filter paper	8 "	0.00		Beyond
Three filter papers	8 "	0.00		colori-
One hard filter	8 "	0.00		metric
Asbestos filter	8 "	0.00		range

The data goes to prove that no colloidal iron passes through the filter when a suspension of ferric phosphate is filtered after standing at least eight hours.

The value of ferric phosphate as a source of iron for plants does not seem to lie in the amount that goes into solution because after three days, as much or more remained in solution than after eight hours. After three days it is unavailable to plants, hence one may conclude that its dispersion through the medium in colloidal form may be an important factor in availability.

Attention was turned to the rate of settling, thinking that this might be of practical use. A series of two-liter flasks containing about .01 gm. ferric phosphate per liter was prepared. Three samples were decanted at the intervals of time stated in the first column of Table IX. Colorimetric estimations were made for comparison. The turbidity of the solution was also noted. The solutions were not filtered. The data is recorded in Table IX.

Table IX.

Time of standing	c.c. $KMnO_4$	Fe in soln. or suspension Fe/L		Appearance	pH	Colorimetric c.c. of Fe soln.	p.p.m.
8 Hrs.	.15 c.c.	0.1047 Mg.	0.4188Mg.	Turbid	4.0	4.00 c.c.	45.28
18	.12	0.08376	0.3340	Slightly turbid	4.0	3.00	33.90
24	.07	0.0048	0.0192	" "	4.0	2.50	27.50
38	Not taken			Clear	4.0	1.40	15.84
56	Too small to be detm.			"	4.0	1.20	13.58
72				"	4.0	1.20	13.58
96				"	4.0	1.00	11.30

The quantities in suspension are very much higher than those shown in Table VIII where a decanted solution contained 0.015 p.p.m. after four days standing. This seemed paradoxical and the reason is not known. The only apparent reason is the difference in $(\overset{+}{H})$ concentration. Thus far our results show that ferric phosphate settles less rapidly at pH 4.0 than at pH 5.0. The rate of settling is related to the size of the particle which in turn is related to the solubility. Solubility varies with the acidity of the solutions.

To further test the effect of acidity upon the rate of settling, ferric phosphate suspensions were made of solutions of different pH values. The results are given in Table X. They are arranged as in the previous table. The turbidity was not noted and the colorimetric estimations were not made.

Table X.

Time of standing	c.c. $KMnO_4$	Fe in suspension in sample	Fe/L	pH
8 Hrs.	.17 c.c.	0.000118	0.000472	4.00
8	.19	0.000132	0.000528	4.30
8	.10	0.0000698	0.0002792	5.25
24	.10	0.0000698	0.0002792	4.00
24	.10	0.0000698	0.0002792	4.30
24	.06	0.00004188	0.000167	5.25
48	.04	0.00002792	0.0001116	4.00
48	.02	0.0000139	0.000055	4.30
48	.00			5.25

The results of this table show solution R2S1 at pH 4.0 has a tendency to keep more ferric phosphate in solution and suspension than at pH 5.25.

Marsh (52) found ferric glycero-phosphate, ferric tartrate, and soluble ferric phosphate more soluble at pH 5.5 in a modified Tottingham solution $T_1R_1C_5$ than at either pH 4.6 or 6.2. This was also true in this three salt solution for ferric glycero-phosphate and soluble ferric phosphate but not for ferric tartrate. Tottingham's unmodified solution $T_1R_1C_5$ at pH 5.5 dissolved as much or more ferric glycero-phosphate,

ferric tartrate, $FeSO_4$ and soluble ferric phosphate. His solubilities are given in the table below.

Solubility of Iron.

Solution	pH	Amounts of Iron in Mgs. per Liter			
		Ferric glycero-phosphate	Ferric tartrate	Soluble ferric phosphate	$FeSO_4$
Tottingham's $T_1R_1C_5$ modified by Jones and Shive (50)	4.6	0.29	0.47	0.37	0.78
	5.5	.35	.55	.44	.38
	6.2	.26	.48	.34	.66
3 Salt R_2S_1	4.6	0.30	0,49	0.43	0.57
	5.5	.50	.41	.47	.44
	6.2	.29	.37	.17	.13
Tottingham $T_1R_1C_5$	4.6	0.20	0.70	0.40	0.70
	5.5	.35	.73	.40	.84
	6.2	.20	.40	.00	.00

All phosphate compounds are most soluble at pH 5.5 except soluble ferric phosphate in Tottingham's unmodified solution $T_1R_1C_5$ in which it is equally soluble at pH 4.6. Reference has been made to the work of Atkins (4) in which it is indicated that phosphates of iron are more soluble in almost neutral solutions than those which are slightly acid or basic.

The amounts of soluble iron reported by Marsh are very much higher than those reported in our work. This is perhaps due to the fact that the solutions and the forms of iron are different. The quantities that Marsh reports could be very accurately determined by the method of determination outlined in this work.

Colorimetric Estimations. Problem. Before the experimental work was begun the colorimetric method had been rejected because of its limitations. The chief objection, current in the literature, is the interference of phosphates in making accurate color comparisons. There are other objections; Scott (82) says, "Nitric acid gives a color with sulphocyanates that may easily be mistaken for iron." Further that the chlorides of alkaline earths prevent or retard the sulphocyanate reaction. In nutrient solutions the alkaline earth calcium is always present, and an ammonium salt is often present.

Deso, in measuring known quantities of iron in the presence of interfering phosphates finds that the solution in which the iron is dissolved should contain enough hydrochloric acid to be about one-half normal. Above or below this acidity greater negative errors result than at this acidity. When the acid phosphate of sodium was present the quantity of iron by estimation was less than the quantity actually present.

Procedure and Apparatus. After decanting or filtering the sample, 50 or 100 cc. of the culture solution are treated by the standard method (53) which is as follows: To a 100 cc. sample in a Nessler tube add 5 cc. of strong hydrochloric acid, enough N/10 permanganate to make a tinge last for two minutes, and then 5 cc. potassium thiocyanate (KCNS 20 gm. per liter). The color produced by a complex salt of ferric iron and potassium thiocyanate is matched with a sample containing a known amount of iron similarly treated. The matching can be done in several ways; one by varying the length of the column through which the light passes as in the Schreiner

colorimeter and another by keeping the length of the column the same but varying the standards to which the unknown are compared. A Schreiner colorimeter was used in the studies on interference and later the varying standard method was used in obtaining the data in this work. In the Schreiner apparatus to secure uniformity and equality of illumination the light from a 50 watt, 110 volt desk lamp was thrown on white paper which reflected the light through columns while in the varying standard method light was used from the same lamp with a white paper background. This gives a more sensitive reading than daylight.

Studies in Interference. In the colorimetric work the first step in the investigation was a study of the error produced by the component salts of the nutrient solutions. The limit where interference of the component salts begins depends on several factors; the intensity of the light, the concentration of the interfering substances and the concentration of the iron in the sample. This takes for granted that enough of the potassium thiocyanate is present to react with all the iron. The limit of interference was approached in several ways. With the iron constant various quantities of the single salts were added, and with constant quantities of salt various amounts of iron were added. These samples with known amounts of iron and nutrient salts were estimated for iron. Interference has always been claimed for phosphates. This was the first salt to be tested. The results are given in Table XII.

Table XI

Effect of Phosphates

C.c. N/10 $Ca(H_2PO_4)_2$ per 100 c.c.	Fe in P.P.M.	Fe P.P.M. + Blank	Remarks
5	.28	.28	No error
5	.566	.566	" "
5	.70	.70	" "
5	.84	.84	" "
5	.91	.91	" "
6	.28	.28	No error
7	.28	.28	" "
10	.294	.28	Error of -.014 P.P.M.
10	.56	.52	" .04 "
10+	.84	.76	" .08 "
10	.56	.52	" .04 "
15+	.56	.52	Error of -.04 "
15+	.84	.71	" -.13 "
15+	.84	.76	" .08 "
15+	1.11	1.02	" -.09
15	1.41	1.30	" -.11
15	1.67	1.56	" -.11
10	2.20	1.60	" -.60 "
15	2.20	1.50	" -.70 "
25	2.20	1.20	" -1.0 "
3	.84	.84	No error
5	.84	.78	Error of -.06
10	.96	.85	" -.11
15+	.84	.78	" -.06

+ The olive green color from the phosphates distinctly interfere with the estimation masking the red color of the iron compound.

The results from Table XI bring out the fact that the magnitude of the minus error from the interference of calcium acid phosphate in culture solutions depends upon the concentrations of the acid salt and the iron. With the quantities of iron given in the above table the concentration of acid phosphate used in nutrient solutions is too low to cause significant error. The greatest volume that is used in any of the Tottingham-Livingston solutions is 11.3 cc. per liter in solution III, R_1S_6. To prove that the minus error was due to the acid phosphate and not to the calcium ion, known quantities of iron added to calcium hydroxide were estimated. The results are given in Table XII.

Table XII

Effect of Calcium Hydroxide

.14	P.P.M.	iron	in	saturated	$Ca(OH)_2$	=	.14	P.P.M.	in	blank
.42	"	"	"	"	"	=	.42	"	"	"
.70	"	"	"	"	"	=	.70	"	"	"
1.40	"	"	"	"	"	=	1.40	"	"	"

The results show that the calcium ion has no effect on the colorimetric estimation of iron.

To positively prove that the minus error was due to the phosphate ion, phosphoric acid was added to iron solutions. The data are given in Table XIII.

Table XIII

Effect of Phosphoric Acid

1.4 cc.	H_3PO_4	(85%)	+ .84	P.P.M.	Fe	=	.70	P.P.M.	iron in	blank
1.4 "	"	"	+ .42	"	"	=	.98	"	"	"
1.4 "	"	"	+ 1.40	"	"	=	.98	"	"	"

The results show that 1.4 cc. of 85 per cent phosphoric acid upsets the determination; the errors generally being minus. The shades of color are so different that they cannot be matched. The phosphoric acid gives an olive tint while the iron complex is red. When phosphoric acid is present in the above quantity the red is obscured by the green. The same shade of green is produced when 15 cc. or more of tenth molar calcium acid phosphate are present in the sample.

The effect of potassium nitrate was also tested. It produces a deeper color indicating more iron than is actually present. This is shown by the results given in Table XIV. A molar solution of potassium nitrate was added.

Table XIV

Effect of Potassium Nitrate

2 cc.	KNO_3	+ .14	P.P.M.	Fe	=	Blank	+ .14	P.P.M	iron
2 "	"	+ 1.4	"	"	=	"	+ 1.4	"	"
2 "	"	+ 2.1	"	"	=	"	+ 2.32	"	"
10 "	"	+ .14	"	"	=	"	+ .14	"	"
10 "	"	+ .70	"	"	=	"	+ .90	"	"
10 "	"	+ 1.4	"	"	=	"	+ 2.1	"	"

The error produced by the presence of potassium nitrate is positive and greater with larger amounts of iron.

As the phosphate error is negative and the potassium nitrate error is positive in nutrient solutions containing both these salts, it is probable that one offsets the other so that the net error is very small.

Moreover the quantities of iron and salts present in the nutrient solutions tested is so small that no noticable error was observed in colorimetric estimations. The amounts of interfering salts in the culture solution R_2S_1 are potassium nitrate, 5.4 cc. molar solution, and calcium acid phosphate, 2.7 cc. molar solution. From the data in Tables XIII and XIV it is clear that large quantities of iron would have to be present before interference from the nutrient salts can be detected.

Data A series of culture solutions were set out and the iron estimated at various intervals. This series was set out to gain information on three points, viz., (1) the effect of hydrogen ion concentration on the solubility of iron; (2) the effect of hydrogen ion concentration on the rate of settling of the suspension, and (3) the solubilities of the iron compounds, ferric phosphate or citrate, in different solutions. The partial volume molecular concentrations and the pH of the six solutions used in this series are given in the table below.

Table XV

Composition of Solutions Tested

Solution	pH	Partial volume molecular concentration KNO_3	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	$(NH_4)_2SO_4$	$Ca(H_2PO_4)_2$
R_2S_1	4.1	0.0054			0.01350		0.0027
R_2C_1	4.1	0.0288			0.00500		0.0026
R_2C_2	4.5		0.01800	0.0052	0.01500		
$T_1R_1C_2$	4.9	0.0020	0.00211	0.0073	0.00711		
$T_1R_1C_1$	4.9	0.0020	0.00211	0.00146	0.00166		
$T_1R_1C_1$ modified	4.9		0.00211	0.00146	0.00166	0.0014	

The ($\overset{+}{H}$) concentrations were varied by adding phosphoric acid to lower the pH, and by adding the hydroxides of potassium or calcium to raise the pH. When the acid phosphate was the potassium salt, potassium hydroxide was added and when the acid salt was calcium, calcium hydroxide was added. The composition of the solutions with their original pH values are given because these values are changed to test the solubility and suspension of iron at different ($\overset{+}{H}$)concentrations. When the acidity of a culture solution has been changed we cannot expect results like those obtained in the original because the equilibrium of the system has been altered. The ratios of the ions are changed with the change in acidity. Plant growth is undoubtedly affected by such changes.

Suspensions of ferric citrate and ferric phosphate were added to the solutions given above so that 0.3 mg. iron was present in a liter of nutrient solution. The solutions to which ferric phosphate was added had a different composition than those to which ferric citrate was added.

At the eighth and twenty-sixth hour intervals 10 cc. aliquots were taken and diluted to 50 cc. in Nessler tubes. The

suspensions taken after longer intervals and the samples filtered were 50 cc. each. These estimations were not made with the Schreiner colorimeter but by the varying standard method as described . The results are given in Tables XVI and XVII. In each table column one gives the pH and column two the time at which samples were decanted. Column three gives the iron in a decanted sample, column four a decanted sample filtered and column five the iron in suspension. Column five gives the difference between columns three and four.

Table XVII

Effect of pH on the solubility and suspension of iron when ferric citrate is added to a nutrient solution.

		Culture Solution R_2S_1			Culture Solution R_2C_1		
		Iron in p.p.m.			Iron in p.p.m.		
pH	Time	Suspension & Solution	Solution	Suspension	Suspension & Solution	Solution	Suspension
4	8	37.8	.084	37.7	25.2	0.98	24.1
5		25.9	.064	25.8	16.8	.28	16.5
6.7		14.0	.084	13.9	-	.38	-
4	26	3.36	3.5	-	5.32	3.92	1.40
5		2.52	.056	2.46	3.09	.17	2.82
6.7		2.8	.056	2.74	2.04	.20	1.84
4	50	6.72	2.24	4.48	10.64	5.04	5.60
5		2.80	-	2.80	1.82	.28	1.54
6.7		1.82	.112	1.71	1.06	.084	.97
4	72	6.16	3.36	2.80	5.60	2.80	2.80
5		1.40	-	1.40	1.96	1.40	1.56
6.7		1.96	-	1.96	1.23	0.34	.89
		R_2C_2					
3.9	8	12.6	.84	11.76			
5		16.8	.064	16.74			
6.6		6.30	.112	6.18			
3.9	26	5.04	2.80	2.24			
5		1.26	.11	1.15			
6.6		.84	.20	.64			
3.9	50	6.02	2.24	3.78			
5		.39	.06	.33			
6.6		.73	.112	.62			
3.9	72	4.48	2.8	1.68			
5		.34	-	.34			
6.6		.34	.06	.26			

Table XVI

Effect of pH on the solubility and suspension of iron when ferric phosphate is added to a nutrient solution.

		Culture Solution $T_1R_1C_5$			Culture Solution $T_1R_1C_1$ modified		
		Iron in p.p.m.			Iron in p.p.m.		
pH	Time	Suspension & Solution	Solution	Suspension	Suspension & Solution	Solution	Suspension
4	8 hrs.	12.6	.112	12.5	20.4	--	--
5		14.0	.112	13.9	16.8	.064	16.5
6.7		5.9	.028	5.9	4.48	--	--
4	26	.98	.28	.7	2.38	.84	1.54
5		.98	.28	.7	.56	.17	.39
6.7		1.12	.14	.98	.56	.20	.36
4	50	.84	.84	--	1.18	.78	.40
5		1.12	.056	1.11	.28	.112	.27
6.7		.40	.112	1.39	.62	--	--
4	72	.84	.78	.06	2.52	1.68	1.84
5		.28	.056	.26	.90	.28	.78
6.7		.28	.112	.27	.64	--	.64
$T_1R_1C_1$							
4.1	8 hr.	15.4	.112	15.3			
5		14.0	.064	13.9			
6.8		4.2	--	4.2			
4.1	26	.56	.67	--			
5		.42	.17	.25			
6.8		.56	.14	.42			
4.1	50	.67	.50	.17			
5		.28	.056	.22			
6.8		.95	.084	.87			
4.1	72	.56	.34	.22			
5		.39	.28	.11			
6.8		.39	.00	.39			

The results are very irregular and only a very general interpretation can be made. The iron in suspension is greatest in the most acid solutions at all times. The quantity of iron in solution also increases with increase of acidity.

Many other interesting facts are suggested but need further experimental confirmation before they can be accepted as true. Nutrient solutions are individualistic in their capacity to dissolve and disperse iron. More iron was held in suspension, initially and at the end of three days when ferric citrate was added than when ferric phosphate was added. This fact points to what has been suggested before, that is, in the reaction in which ferric citrate is changed to the phosphate the particles of colloidal phosphate first formed increase in size gradually, but when ferric phosphate is added, the stage, when the colloidal particles are smallest, is not passed through.

The time at which most iron is in solution is not reached in eight hours, but it requires a day or more to reach the point of maximum solubility. At pH 4 as much is in solution after three days as at any other period. In solution with a pH 5 the iron in solution and suspension is very much less after standing a day or more than in solutions of pH 4. This difference is more marked with ferric citrate than with ferric phosphate. There is not much difference in the quantity of iron in suspension and dissolved in solutions of pH 5 and pH 6.7 when ferric citrate is the source of iron. When ferric phosphate is the source of iron there is more iron in suspension in solutions with the lower acidity. This cannot be said of the iron in solution.

The outstanding differences between ferric citrate and ferric phosphate is the greater quantities of iron in suspension in all the solutions to which ferric citrate was added. Greater solubility was found for the citrate in solutions of pH 4.0 than of the phosphate. It is true that the solutions to which each form of iron was added were composed of different salts as shown in Table XV, but this difference does not seem adequate to account for the uniform differences in all solutions where the iron phosphate and citrate were used.

The estimation of iron in such quantities as those found in the results given is very difficult. The Nessler tubes are not made of glass of the same thickness and color. The eye is unable to see differences in tint after looking at the samples for a few minutes. Where the amounts found in suspension are larger at the 50-hr. interval than at the 26-hr interval the error may be due to the fact that at the 8-hr. and 26-hr. intervals 10 cc. aliquot parts were diluted to 50 cc. so that an error of dilution is introduced.

SUMMARY

The significance of iron in culture solutions was presented by a study of the literature. It seems to be more active physiologically in the ferrous form than in the ferric condition. The solubility of ferric iron varies with the change in acidity and composition of the solution in which it is present.

Ferrous iron combines with oxygen and water or bicarbonates to form an active complex compound which is able to reduce nitrates to nitrites and oxygen. This complex compound can both reduce by absorbing oxygen by coordinative linkage and oxidize or dehydrogenate by yielding the linked oxygen.

A volumetric method was developed for the determination of iron in nutrient solutions when the quantities present are of the magnitude reported by some workers in this field. The iron is precipitated as ferric hydroxide, the precipitate washed free from nitrates with cold water, and dissolved in dilute sulfuric acid. The iron in the acid solution is reduced with a Jones reductor and titrated with eightieth normal potassium permanganate.

A method for taking samples of suspensions without disturbing the precipitate at the bottom of the flask was developed. Studies were made on the interference of the component salts of nutrient solutions on the colorimetric estimation of iron. The interference when small quantities of iron are present is negligible.

Colloidal phenomena seem to be very important when ferric salts are used as a source of iron in culture solutions. Sufficient

ferric phosphate is not available to plants in a nutrient solution even though more is in solution at that period of time than after eight hours. The rate of settling depends primarily upon the acidity. The greater the acidity the slower the rate of settling and the greater the amount of iron in solution. The form of iron used in a solution also affects its solution and rate of settling. Ferric citrate is more soluble and settles less rapidly than ferric phosphate. The composition of the solutions, when acidity is not considered, also affects the solubility and rate of settling of the iron suspended in the solution. It seems that nutrient solutions are so different in their capacity to supply available iron to the plant that each solution must be studied separately.

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