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The determination of iron and its relation to the hydrogen-ion concentration in nutrient solutions

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OUTLINE OF THESIS

I. PURPOSE

Since the earliest investigations concerning plant nutrition, iron has been recognized as an essential element. To control nutritive conditions in experimental work, nutrient solutions are used for the growth of plants, and iron must be supplied in these solutions. The amount of iron in solution must be nicely adjusted, for too much is toxic to the plant, while a deficiency causes chlorosis. In the best nutrient solutions now in use, it is customary to add iron at frequent intervals, for it is a well recognized fact that insoluble products are formed which make the iron unavailable for plant use.

The purpose of the present investigation is not only to devise means by which the amount of dissolved iron may be measured, but also to determine the factors influencing the solubility of iron in plant nutrient solutions.

Note: -- In the following pages of review of literature comments made by the present writer are introduced by the phrase: "The writer's comment is ..... "

II. REVIEW OF LITERATURE

A. Importance of Iron in Metabolism

1. Role of Iron in Animal Metabolism:---Much more is known about the role of iron in animal metabolism than about the part taken by iron in the metabolism of plants. The writer's comment is that the close similarity of the fundamental metabolic processes of the two kingdoms indicates a common role for iron; hence the points brought forward concerning the role of iron in animal metabolism will doubtless be found to be also the important points of iron metabolism in the plant kingdom. Particular emphasis has, therefore, been placed upon this phase of the subject, inasmuch as it is probable that the unraveling of the metabolic history of iron in plants will follow the course already opened by investigations in animal metabolism.

(a) Iron as an Oxidation-reduction Agent.---The latest work and best theory concerning iron as an oxidation-reduction agent is presented first; the older and incomplete theories follow for purposes of comparison.

Cannon (19, 20), from whose reviews much of the following material is drawn, states that in the blood of all animals iron occurs in the hemoglobins, which are considered by Anson and Mirsky (1) to arise by combination of different globins with a common iron-containing substance which they call hem, which is capable of existing in two reversible states of oxidation. The relations of the hemoglobin derivatives are usually considered as follows:

Hem +	globin --->	hemoglobin
Hemoglobin	+ O_2 (acid) --->	oxyhemoglobin
Oxyhemoglobin	------>	globin + hemochromogen
Hemochromogen	------->	hematin
Hematin	------->	hematin-hydrochloride or hemin.

Anson and Mirsky (1) find that with many nitrogenous substances hem gives spectra allied to that usually attributed to "hemochromogen"; such nitrogenous substances include ammonia, glycine, pyridine, nicotine, albumin and globin. The globin compound is noteworthy in that it has the highest solubility and furthermore, by adjusting the pH, the hemochromogen spectrum passes into the hemoglobin spectrum, while at the same time the solution becomes capable of forming a dissociable compound with oxygen. It would, therefore, appear that it is this particular compound of hem and globin which acts as an oxygen-carrier in the blood, and the catalytic property would appear to be due to the atomic structure of hem. Hill and Holden (43) report the preparation of the globin of oxyhemoglobin.

Cytochrome, of Keilin, 1925, (55) is a mixture of two or three hemochromogens; it occurs in yeast, bacteria, some higher plants and the tissues of various lower and higher animals. It is probably the same as the myohematin of MacMunn, 1887. It is easily oxidized by air and reduced by the cell, where it exists chiefly in the oxidized state. Its concentration in the tissue runs parallel with the intensity of the peroxidase reaction of the tissue, and it occurs most abundantly in such cells and tissues as are suspected

of having greatest peroxidase activity; from such facts it may be concluded that cytochrome has a definite catalytic function. So far all work on it has been spectroscopic. If the oxygen compound is of the type of hemoglobin, it would not be expected to have catalytic properties, since hemoglobin yields up only molecular oxygen. Cytochrome may, however, be of the type of Baudisch's iron-oxygen compounds, which will even cause either oxidation or reduction, depending on the compounds in the system. Thus Pfaltz and Baudisch (86) give the oxidation of sodium pentacyanoaquoferroate as

$$Na_3\left[Fe\begin{matrix}H_2O\\(CN)_5\end{matrix}\right] + O_2 \longrightarrow Na_3\left[Fe\begin{matrix}O_2\\(CN)_5\end{matrix}\right],$$

following Manchot, and the activation of oxygen by ferrous hydroxide as

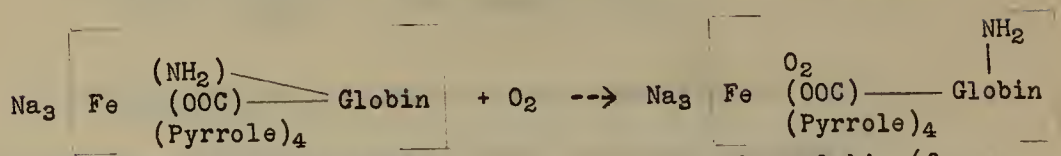
$$\left[Fe(H_2O)_6\right](OH)_2 + O_2 \longrightarrow \left[Fe\begin{matrix}O_2\\(H_2O)_5\end{matrix}\right](OH)_2 .$$

On the other hand, cytochrome may be essentially an electrochemical system not specifically involving oxygen; on this assumption Conant (22) would symbolize the reaction as $Fe^{++} \rightleftharpoons Fe^{+++} + e$. In such a case the compound would act as an oxygen or hydrogen acceptor, just as does methylene blue. (Note: -- A hydrogen acceptor is any element or compound which will receive hydrogen from another compound; such an action involves a difference of potential, the compound with the lower potential acting as the hydrogen acceptor.) This would involve two equivalents of hydrogen in the reaction, and the system would be quite different from that of methemoglobin. From this it would appear that the seat of oxidation-reduction in hematin is not the iron atom. The contrast of the two reactions might then be shown by the equations,

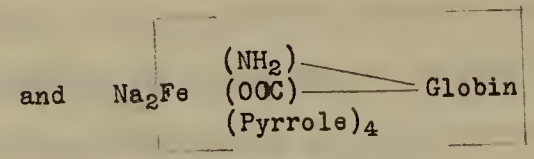
Hemoglobin (Fe^{++}) $\rightleftharpoons$ methemoglobin (Fe^{+++}) + $\underline{e}$

Reduced hematin (hem ?) $\rightleftharpoons$ hematin + $\underline{\underline{2e}}$.

Following Baudisch's assumption Cannon further assumes



Hemoglobin (ferro-compound) Oxy-hemoglobin (ferro-compound)

and 

Methemoglobin (ferri-compound)

Speaking of the mechanism of the catalytic action of iron salts, Baudisch and Welo (11) say that "The divalent iron has quite distinct properties from the trivalent. For example, it is able to absorb oxygen coördinatively. The auxiliary valence forces are usually stronger than in the trivalent form and the iron nucleus is, therefore, able to draw the various inorganic and organic radicals or compounds into the inner sphere selectively." They further give evidence to show that it is probable "that intimate relations exist between the coördination forces and magnetic forces, if they are not indeed one and the same thing".

It would appear from the evidence given above that the function of iron in the body system might be that of a catalyst in the oxidation-reduction processes.

Wieland (121, 122, 123, 124) has shown that oxygen is not always essential for the process of oxidation. For example he conclusively demonstrates that the oxidation of carbon monoxid proceeds as follows:

(1) $CO + H_2O \longrightarrow HCOOH$

(2) $HCOOH \longrightarrow CO_2 + H_2$

(3) $H_2 + O_2 \longrightarrow H_2O_2$

(4) $H_2O_2 \longrightarrow H_2O + 1/2\ O_2$

Wieland has actually isolated HCOOH, H_2 and H_2O_2 in the process. The extraordinary thing is that while water is essential in this oxidation, oxygen is not; so that carbon monoxide changes to carbon dioxide without the presence of any oxygen in the free state if the hydrogen in the second equation above (or the CO_2) can be removed. Wieland removes the hydrogen by means of palladium, and carries out the oxidation of carbon monoxide to carbon dioxide at room temperature in the absence of free oxygen; thus the essential step in this oxidation is the removal of the hydrogen in the second equation above. This forms the foundation of Wieland's "dehydrogenation theory", which postulates an activation of hydrogen rather than activation of oxygen to bring about oxidation; it has proven of valuable aid in explaining physiological oxidations.

Glucose and gluconic acid are easily oxidized in the absence of oxygen, as is also ethyl alcohol.

$$C_2H_5OH \xrightarrow[+H_2\text{-acceptor}]{+H_2O} CH_3COOH$$

It has also been shown that lactic acid is easily oxidized in the presence of a hydrogen acceptor, and this assumes great physiological significance when it is realized that lactic acid is an important intermediate product in the metabolism of both glucose and the proteins. The reaction is assumed to take place thus:

$CH_3.CHOH.COOH \longrightarrow CH_3.CO.COOH + H_2$ (oxidation)

$CH_3.CO.COOH \longrightarrow CH_3.CHO. + CO_2$ (removal of carbon dioxide)

$CH_3.CHO + H_2O \longrightarrow CH_3 . C(OH)(OH)H$ (addition of water)

$CH_3 . C(OH)(O H)H \longrightarrow CH_3.COOH + H_2$ (oxidation)

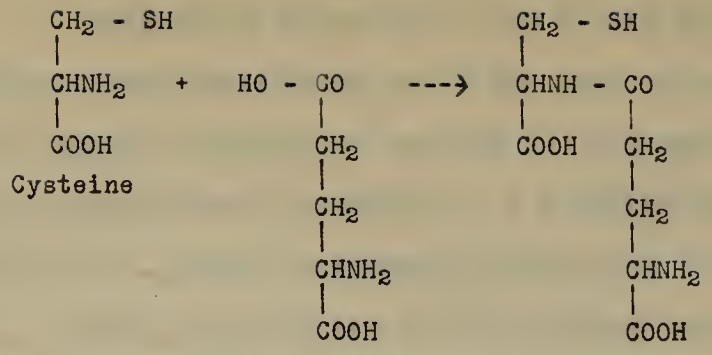
Wieland does not, however, claim that the dehydrogenation theory offers an explanation for all instances of oxidation, nor does he assume it to be the only oxidation mechanism. For some cases there are intermediate peroxides formed. Baeyer and Villiger (7) first isolated a peroxide (a per-acid) formed in the oxidation of benzaldehyde:

$C_6H_5.CHO + O_2 \longrightarrow C_6H_5.CO.O.OH$

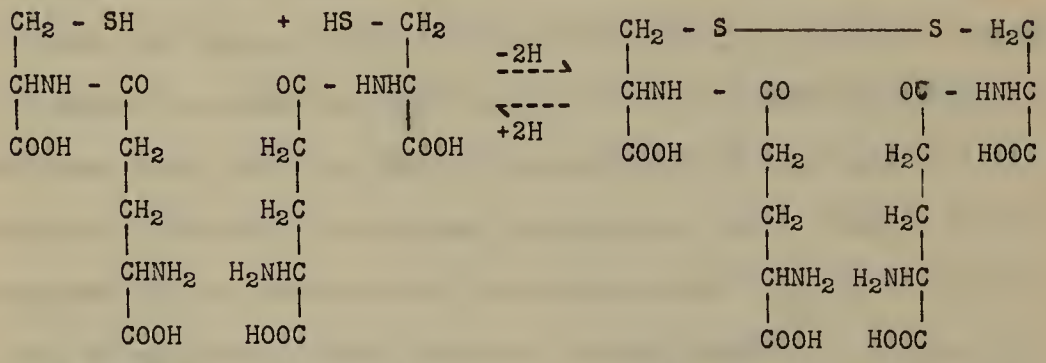
$C_6H_5.CO.O.OH + C_6H_5CHO \longrightarrow 2C_6H_5COOH$

or benzaldehyde ---> perbenzoic acid ---> benzoic acid.

As further support of Wieland's theory of dehydrogenation appears the discovery of glutathione by F. A. Hopkins (47) in 1921. Glutathione is a readily oxidizable substance, occurring in most, if not all, living tissues, tho in very small amount, usually 0.01 to 0.02%. It is thermostabile; i.e., it is unchanged by subjection to heat. Hopkins shows that it is a dipeptide compound of one molecule of cysteine and one of glutamic acid.



The reduced form is present in tissues to a much larger degree than the oxidized form which readily results from it.



The reaction is easily reversible; reduced glutathione readily combines with any available oxygen, and the hydrogen lost is converted into hydrogen peroxide. The oxidized glutathione is reduced by tissues; here it acts as a hydrogen acceptor, and many of the intermediate products of metabolism will give up hydrogen easily in this manner.

Thunberg (108), as well as Hopkins, has performed experiments to show that glutathione can and does bring about oxidation in vitro, in the laboratory, under precisely the same conditions as prevail in living tissues, especially under anaerobic circumstances.

Mathews and Walker (66) found in 1909 that a neutral solution of cysteineprovides one of the most delicate tests for iron; a violet or pink color develops in the presence of a ferric salt, even in slight concentration. A 6/1000000 molar concentration of iron was found sufficient to double the speed of oxidation of the cysteine. They explain the mechanism of acceleration by iron salts as follows: "The oxygen of the air oxidizes the iron to a ferric salt; this ferric salt then unites with the cystein to form a violet or blue colored compound which at once breaks up, the iron passing a positive charge of electricity to the cystein and becoming ferrous iron again. It is then reoxidized and the process is repeated." The writer's comment is, that it is obvious that another hydrogen acceptor could take the place of the atmospheric oxygen, when iron would still continue to oxidize cysteine, especially since by further experiments the investigators just quoted prove that the violet compound to which they refer "does not involve atmospheric oxygen, but only ferric iron, probably hydroxylions and cystein".

D. C. Harrison (39) refers to Warburg and Sakuma (117), who find that the oxidation of pure cysteineis markedly increased in velocity by the addition of a few hundred thousandths of a milligram of iron to the solution. Representing the catalytic activity of iron as (increase of oxygen uptake in c.m.m.) ÷ (mg. of added iron) x (time in minutes) = ^{n}Fe , they find the average value of the quotient to be 1700. Harrison finds results which agree well with those of Warburg and Sakuma.

Harrison further finds 0.6% Fe in crude glutathione prepared by him, and shows "that the oxidation of purified glutathione

is strongly catalyzed by minute quantities of iron, the rate of uptake of oxygen being increased from 42 cmm. to 57 cmm. in the first hour, by 0.0004 mg. of iron". In experimenting with another sample of impure glutathione under the same conditions, he finds the initial velocity of oxygen uptake to be 760 cmm. per hour, which is reduced to 42 cmm. per hour by removal of the iron.

Mathews and Walker (66) show that hydrogen cyanide inhibits the oxidation of both cysteineand glutathione. Harrison (39) also notes this, and concludes that the effect is due to the formation of a non-catalytic cyanide complex with iron.

Harrison finds, however, that the iron in hematin is capable of catalyzing the oxidation of cysteineor of glutathione,and remarks: "It is not difficult to understand why the iron in the haematin compound should be active as a catalyst while the cyanide compound with iron is inactive. In cyanide-iron compounds, in presence of oxygen, the ferrocyanide form is known to be more stable than the ferricyanide form, and hence the ferrocyanide compound, when once formed, would show no tendency to take up oxygen. In haematin, however, the ferric compound is the more stable in presence of oxygen, and consequently as soon as this is reduced to the ferrous compound by the sulphydryl group, it becomes oxidized again by the air, the iron in haematin thus acting as an oxygen carrier." The writer's comment is that it should be pointed out that in this case oxygen is, according to the dehydrogenation theory of Wieland, acting as a hydrogen acceptor, and "oxygen carrier" is synonomous with "hydrogen transportase".

Dhar (24) finds that many substances not easily oxidized by atmospheric oxygen at ordinary temperatures are readily oxidized by

that agent when mixed with sodium sulfite or with freshly precipitated ferrous hydroxide, which are themselves easily oxidized in air. "Thus oxidation has been induced in the following substances in the presence of Na_2SO_3 or freshly precipitated $Fe(OH)_2$; urea, starch, grape sugar, cane sugar, $K_2C_2O_4$, CH_3COONa, sodium potassium tartrate, sodium formate, sodium citrate, acetone, chloral hydrate, chloroform, glycerol, quinine sulfate, sodium succinate, methyl alcohol, ethyl alcohol, phenol, glutaric acid, maltose, potassium stearate, cholesterol, anthraquinone, acetanilide, brucine, phenolphthalein, gum arabic, etc."

He further points out that "It is impossible to ignore the importance of these reactions in their relation to the phenonenon of oxidation and reduction in the animal body. It is well known that a molecule of stearic acid taken into the body in the form of fat undergoes combustion so that eventually each of its 18 carbon atoms will become converted into carbon dioxide. But no one imagines that such a change is immediate or direct, or that every carbon atom simultaneously parts with its attached H-atoms, and by combining with oxygen yields CO_2 and H_2O. We have brought about the same change in the laboratory with potassium stearate by inducing its oxidation through the oxidation of Na_2SO_3 or $Fe(OH)_2$ by passing oxygen through the mixture at the ordinary temperature. In the animal body, acetic acid is oxidized with great ease into CO_2 and H_2O, though it is resistant to strong oxidizing agents such as chromic acid, potassium permanganate, etc. Its oxidation in the laboratory has been effected by us with the help of sodium sulphite or ferrous hydroxide when it is being oxidized by passing oxygen through it. Oxalic acid, although

very readily oxidized by many laboratory reagents is oxidized with great difficulty in the animal body; we have also observed that oxalic acid is only very slowly oxidized by passing air through a solution of oxalic acid or an oxalate containing a sulphite. The substances undergoing active metabolism in the animal body, comprising the proteins, carbohydrates, fats and their derivatives are practically resistant to oxidation by oxygen under ordinary conditions. Yet in the animal body the carbon of these compounds is readily oxidized to carbon dioxide. It is generally conceded that the same process of activation of the atmospheric oxygen must take place in the body in order to account for the observed chemical changes. It is remarkable that a very large number of biochemical oxidations have been imitated by us in the laboratory by the simple process of induced oxidation as already mentioned."

"It has been shown in a previous paper that the oxidizing power of hydrogen peroxide at the ordinary temperatures is greatly accelerated in presence of ferrous and ferric salts. Thus, if tartaric acid or starch or sugar and hydrogen peroxide be brought together at the ordinary temperature, hardly any chemical reaction takes place; but, as soon as a ferrous or ferric salt is added, oxidation of tartaric acid or starch takes place rapidly. Reactions of this type are of great importance in explaining the oxidation in the human body. The food in the animal body is oxidized by the atmospheric oxygen, giving us heat and energy. In the animal body there is evidence with regard to the formation of the peroxide from the oxygen taken up by the animal and this peroxide oxidizes the

food taken up in the body. We have shown in the laboratory that the activity of H_2O_2 at the ordinary temperatures is accelerated markedly by the presence of ferrous or ferric salts. Similarly, in the animal body, iron in the haemoglobin present in the blood accelerates catalytically the oxidation of the food stuff by the peroxide formed in the body from the inhaled oxygen. When there is a deficiency of iron in the blood, the animal body suffers from anemia because the amount of catalyst necessary for regular oxidation falls short. At this stage any iron salt taken into the system will supply the natural deficiency and the necessary amount of oxidation will take place. This is the probable mechanism of the internal use of iron salts, whether ferrous or ferric, in medicine."

(b) Presence of Iron in Organs.---Nonnenbruch (84) confirms the conclusions of V. Noorden and Morawitz by showing that the action of iron is not a direct stimulation but is more complex in nature. The administration of 2 g. ferrum reductum daily over a period of 10 days leads to no effect on metabolism. This points to action of iron thru the endocrine system.

Nakayama (2) working under Leon Asher at the University of Bern, finds the spleen to be an organ important in iron metabolism; and Scheinfinkel (101) in the same laboratory confirms these views, and finds that the liver also acquires this function very quickly by a process of compensation.

Elvehjem and Peterson (26) have examined a number of animal tissues for iron content and find such tissues as heart, muscle, intestines, hide and bone marrow to be low in iron, while tissues such

as spleen, liver, lung and kidney are found to contain relatively high amounts of iron.

The general conclusion that the endocrine organs are the seat of iron metabolism is further confirmed by the work of Williamson and Ets (125), who show that the addition of inorganic iron to the diet of rats is followed by no increase in hemoglobin content. However, the livers and spleens of iron-fed animals show storage of iron.

(c) Iron as a Cure for Anemia.---Considerable question has been raised concerning the therapeutic value of iron in anemia. Whereas Dhar (24) postulates that any ferrous or ferric salt may be used for the treatment of anemia, Mitchell and Schmidt (77) find that only such compounds as are soluble give enough available iron to be of therapeutic value. Fiessinger (27) believes that the iron, even though insoluble in nature, as metallic iron or ferrum reductum, may be absorbed by the leucocytes and transferred by them to the lipoids, and so to the active centers of organic oxidation. The leucocyte passes through the epithelium of the intestine with the absorbed iron, and, in this way, renders available iron which is naturally insoluble. It has previously been mentioned, however, that Nonnenbruch (84) finds ferrum reductum to have little, if any, influence on metabolism.

The idea was held for some time that only organic iron compounds were available to the animal organism. This would make the animal solely dependent upon iron from plant or animal sources. Experiments conducted by Williamson and Ets (125) seem to substantiate this view. Although iron is stored in the spleens and livers of rats

receiving inorganic iron added to the standard casein diet of Osborne and Mendel, this does not increase the hemoglobin content of the blood, nor hasten the return to normal after bleeding. Dogs also have been used, on a diet of bread and milk; after reduction of the hemoglobin content of the blood by repeated bleedings, the return of hemoglobin values to normal is not hastened by subcutaneous or intravenous injection of iron and ammonium citrate, but is hastened by the substitution of an isodynamic amount of meat for some of the bread. From these results Williamson and Ets conclude that only organic compounds of iron are suitable for therapeutic use in anemia.

There is much evidence contrary to this view, however. Mitchell and Schmidt (77), working with anemic rats, find that the criterion of the availability of iron for the organism seems to be solubility vs. insolubility rather than organic vs. inorganic. Thus they find molasses, meat, ovoferrin, ferric chlorid and ferric ammonium citrate to be very good sources of iron, while ferric oxide and ferrous carbonate are poor.

In conducting further work along the same lines, Mitchell and Vaughn (78) classify ferric acetate, ferric albuminate, ferric chloride and ferric citrate as good; peptonized ferric oxide, saccharated ferric oxide, saccharated ferrous carbonate and ferrous iodide as fair; and ferric oxide, ferrous carbonate, ferric potassium tartrate, ferrous lactate, ferrum reductum and ferrous sulfate as poor, as regards availability of iron. Here again one may note that the more soluble compounds, either organic or inorganic, are available, or "good", while those that are insoluble are unavailable, or "poor".

Hart and his co-workers (40, 41) show that certain plant constituents are capable of making available some naturally unavailable forms of iron. For example, ferric oxide alone will not prevent hemoglobin deficiency of rabbits, leading finally to anemia; addition of an alcoholic extract of desiccated cabbage or or yellow corn meal to the ferric oxide, however, prevents the development of anemia. Purified chlorophyll is found to be effective in place of the alcoholic extracts. This action of chlorophyll is denied by Mitchell and Schmidt (77). Hart (40) finds the ash of lettuce or of cabbage, or even the ash of an alcoholic extract of cabbage, to be potent in the correction of anemia on a whole milk-ferric oxide diet. The same investigators find impure (C.P.) ferrous sulfate, ($FeSO_4.7H_2O$), to be much more effective in the prevention or cure of nutritional anemia than ferric oxide, doubtless due to difference in solubility, but the purified preparations of this C.P. salt are less effective, though still more effective than ferric oxide.

Hart (41) suggests that the substance essential for hemoglobin formation, which is thus obviously found in alcoholic extracts of green plants and in their ash, may be identical with vitamin E. This follows the work of Yoshiue (127), who finds vitamines B and C to be important factors in iron retention in the animal body. This agrees also with the work of Haramaki (37), who shows that lack of vitamines in the diet of a growing dog leads to deficiency of iron, while sufficient iron is readily taken up by the body when vitamines are fed.

Roessingh (95) believes the therapeutic action of iron to be in the form of stimulation of the cells of the bone marrow to the

production of erythrocytes, which are released into the blood stream and are especially active while in the newly-formed state.

(d) Relation of Iron to Diseases Other Than Anemia.--- Anemia is not the only disease caused in animals by a deficiency of iron in the diet. In the Patetere Plateau of New Zealand, Aston (3) finds that the prevalent bush-sickness of cattle is due to the deficiency of iron in the forage plants, which in turn is due to deficiency of available iron in the soil. This establishes the important relationship between the iron metabolism of plants and herbivorous animals.

Furthermore, deficiency of iron in the diet seems no more dangerous than an excess of iron, for recent investigations carried out by K. Waltner (115) on rats and rabbits show that an addition of 2% ferrum reductum to an otherwise suitable diet gives, within four weeks, the pathological picture of rickets, viz., normal blood-calcium content but a lowered serum-phosphate value. Ferrous phosphate, ferric phosphate and ferric citrate also show toxicity; the ferric phosphate is especially toxic, and animals receiving it lose weight noticeably. Rickets of either the calcium-deficient or phosphorus-deficient type is increased by the addition of ferrum reductum to the diet.

2. Role of Iron in Plant Metabolism.

(a) Mechanism of Iron Assimilation.---Although Morison and Dupre (82) doubt the existence of either ferrous or ferric iron in normal soil solutions in any appreciable amount, yet it seems certain that iron, as an element essential for plant life, must be furnished by the nutrient medium. It is equally certain that there must be some mode or mechanism of assimilation of iron by the plant.

Brown and Corson (15) cite Halligan (35) as believing "that enough iron is soluble in the weak soil acids to supply the needs of plants so that this material need not be considered when applying fertilizers". To quote further from the same article: "Russell and Hutchinson (99) assume likewise that iron salts are unnecessary as manure. Hall (34) is even more emphatic, in declaring the use of iron sulfate either for farm or garden crops as unnecessary. He insists that no direct evidence has yet been adduced for a beneficial effect of iron salts either on color or yield, and that experiments have never been conducted in a manner to raise the supposed increase due to the iron, beyond the range of experimental error.

"While it must be admitted that the experiments along this line are rather unsatisfactory and broad conclusions should not be drawn from them, it seems that the importance of iron is too great and the possibility of its absence, at least of its absence in the proper form, is too real, to dismiss the question of iron fertilization with an unqualified statement that it is never necessary." The need of iron fertilization in certain areas is well shown by Aston (3) whose work on the Patetere Plateau of New Zealand has already been mentioned.

By experimentation, Brown and Corson (15) find that the common soil organisms, in addition to the iron bacteria, are capable of bringing about ferrification (i.e., the process of converting iron from an insoluble and hence unavailable form to a soluble, available form), and that not only bacteria, but also molds are able to oxidize ferrous iron. These facts seem to point to the existence of iron salts in the soil solution, and to the mechanism of assimilation developed by lower plant forms.

If iron be furnished by the nutrient medium, it is obvious that the roots must be the chief, if not, indeed, the sole agents of assimilation. The common practice of adding some form of iron to the nutrient solution depends upon this fact. Sidorin (104, 106) states that the absorption of iron salt solutions by the roots, and the subsequent passage of these solutions through the plant body, depends upon the vascular structure of the plant (corn), certain peculiarities of the circulation of solutions in the vascular system, and certain peculiarities of iron assimilation. The writer's comment is that with so many variants it seems dangerous to draw any definite conclusions about iron assimilation from this evidence.

It must not be assumed, moreover, that the roots are the only plant organs capable of assimilating iron in solution. Gris (32) shows that the detached stem, or even the leaf itself, may absorb dilute solutions of iron salts. Molisch (79) and Sidorin (105) as well as numerous other investigators confirm these findings.

We may conclude, therefore, that the plant is capable of assimilating iron, from dilute iron solutions, by various organs; but we can scarcely go farther than this on the rather meager evidence offered.

(b) Iron in Relation to Chlorophyll.---From existing evidence it seems safe to assume that iron is intimately related to the phenomenon of photosynthesis, and is necessary for the formation of chlorophyll.

The relationship between the presence of iron and that of chlorophyll in the plant cell has long been recognized. Any standard text on plant physiology states that in the absence of iron no chlorophyll is developed in the plant, and that the condition known as chlorosis results.

Pfeffer, in his "Physiology of Plants" (87), states that iron is essential not only for the normal growth of chlorophyllous plants, but also for that of fungi. Moore (81) cites various sources of evidence for the presence of iron in algae and lichens, and refers to Macallum as having established the fact that the chromatin of nuclei always contains iron.

An influence on both respiration and fermentation of baker's yeast is noted by Harpuder (38) when the yeast is subjected to varying concentrations of an iron salt solution; but in the case of beer yeast he finds only fermentation to be affected by the concentrations of iron used.

Bortels (13) finds that iron is essential for the existence of Aspergillus niger, and is related to the formation of humin; B. prodigiosus also requires iron and no prodigiosin is formed in its absence; the leuco compound formed gives the pigment only in the presence of ferrous salts.

While iron may be essential to all plant life, and indeed to all life, we may consider that at least a part of the iron of

chlorophyllous plants has a definite functional relation to the chlorophyll. It is well known that deficiency of iron leads to a chlorotic condition in plants. That such chlorosis, or blanching of the green parts, may be cured in young organs by the administration of dilute iron solutions, either through the nutrient medium or by direct application to the effected parts, is shown by Gris (32). Sidorin (105) shows that the assimilation of iron through the stem or leaves is less effective than assimilation through the root system.

Raulin (90) shows that fungi possessing no chlorophyll cannot do without iron, and his work is confirmed by Molisch (79). In agreement with the conclusions arrived at by Molisch (79), Raulin (90), Benecke (12) and others, Jost (54) remarks, "It seems probable that, like potassium and magnesium, iron is necessary to the formation of protoplasm, and that its absence is followed by chlorosis in the higher plants as a secondary effect".

This seems the more probable in view of the fact that Rippel (93) finds that excessive amounts of manganese will cause chlorosis, which may, however, be cured by administration of iron; he concludes that the manganese-induced chlorosis is due to the unfavorable effect of manganese in preventing normal action of iron in the tissues.

Johnson (49) finds that pineapples will stand excessive amounts of manganese if correspondingly large amounts of soluble iron salts are supplied. He further states that the manganese dioxide formed doubtless keeps the iron oxidized to the more difficultly

soluble ferric form, which would be nearly wholly precipitated, even in the acid solutions in which manganese-induced chlorosis often occurs.

Mazé, Ruot and Lemoigne (67, 68) find that large amounts of calcium also cause a chlorotic condition. They (69) attribute this to the increased concentration of calcium carbonate in the root secretions, which thereby lose a portion of their diesolving power toward iron compounds.

But calcium chlorosis may be observed in plants with normal or high iron content. Thus, Gilbert, McLean and Hardin (29) find the iron content of chlorotic plants grown on heavily limed eoils to be higher than that of normal plants. Gile and Carrero (30) explain such circumstances by showing that lime-induced chlorosis is the reeult of inavailability of iron.

Ruprecht (98) makes use of this action of calcium when he treats with calcium carbonate to relieve iron toxicity. He states that the calcium carbonate removee the iron as iron hydroxide; he also shows that calcium carbonate is not so effective in this reepect in high concentratione, due to the solubility of the iron hydroxide at such concentratione.

Hoffer and Carr (46) find a chlorotic condition in corn plants to be due to such a low hydrogen-ion concentration that immobile iron compounds form deposits at the nodes and prevent the paesage of water, or of iron in soluble form, to the leaves. McCall and Haag (72) find immobility of iron in wheat plante in which the pH is greater than 4.02 or 4.06.

Leroux and Leroux (61) find iron very unequally distributed in the various plant organs, and only in woody plants are the leavee the organs richest in iron.

Noack (83) finde evidence that iron ie a participant in the assimilation of carbon dioxide by chlorophylloue plants.

Moore (80, 81) summarizes the conclusions of his experiments by saying,

"1. Inorganic iron salts and iron or aluminum hydrates in colloidal solution possess the power of transforming the energy of the sunlight into chemical energy of organic compounds.

"2. Inorganic iron, in crystalloidal or colloidal form, is present in the colorless part of the chloroplaet of the green plant cell in many plants.

"3. In the abeence of iron the green colouring matter cannot develop in the leaf, although the green coloring matter itself contains no iron.

"4. In the presence of sunshine, the iron-containing substance of the chloroplast develops the colouring matter, eo that this iteelf is a product of photo-synthesis induced by the iron-containing compound.

"5. The facts afford an explanation of chlorosis, and its cure by inorganic iron salts, and demonstrate that iron is a primary essential in photo-syethesie and the production of chlorophyll.

"6. The iron-containing substances of the colourless portion of the chloroplaet, and the chlorophyll produced by them, then become associated in the functions of photo-syntheeie as a complete mechanism for the energy transformation."

Kostytschew (60) also emphasizes the importance of iron in oxidation and reduction processes, and refers to Gola (31) as finding organic iron salts to have an important role in biological oxidations.

This brings us back to the work of Keilin (55), who finds an intracellular respiratory catalyst, which he terms cytochrome, present in animals, bacteria, yeast and the higher plants. It is composed of three hemochromogen-compounds, two of which contain an iron-pyrrole nucleus, and it yields three hemochromogens which form compounds with oxygen and carbon monoxide. It is easily oxidized by air and reduced by the cell, and is doubtless of great biological significance in the oxidation-reduction processes. Bach and Kultjugin (6) point out the fact that the peroxidase of plants corresponds to the oxyhemoglobin of animals in function.

The writer's comment is, that while cytochrome may not be the only important iron compound in the plant cell, yet it is undoubtedly of great importance, and the function of iron is well illustrated by this example: - Photosynthesis is essentially an energy transformation. Oxidation-reduction processes are also energy transformations, and photosynthesis is a special phase of these processes. Hence, evidence leading to the establishment of iron as a necessary catalyst in physiological oxidation-reduction is also evidence for the necessity of iron as a photosynthetic agent. These conclusions are further supported by the direct relations between chlorophyll and the process of photosynthesis, and between the presence of iron and the production of chlorophyll.

(c) Iron in Relation to Nitrate Reduction.---Another biological reduction process in which iron has an important role is that of nitrate reduction. Baudisch (10) finds a direct relationship between iron absorption and assimilation by the cholera bacillus and the ability of the bacillus to reduce nitrates to nitrites and ammonia. In vitro, he also finds, oxygen will reduce nitrates to nitrites instantaneously, even in the cold, in the presence of ferrous salts, due to formation of complexes between oxygen and the ferrous salt.

Baudisch and Welo (11) believe in a definite relation between the ferromagnetic metals such as iron, nickel, cobalt, etc., and the paramagnetic gases such as oxygen and nitric oxide. This relationship shows itself in the particular affinity between these two types. They suggest that the 3_s orbits of Bohr, responsible for paramagnetism, may be capable of interpenetration with the orbits of another atom, or even that the orbits of the two atoms may coalesce and become coplanar, thus giving an approach much closer than atomic distances. When this relationship is more fully cleared up, Baudisch and Welo believe that the nature of the complex salts formed in nitrate will be better understood.

(d) Relation of Iron to Crop Yield.---Since deficiency of iron leads to disease, it is readily seen that the crop yield is intimately related to the iron supply.

Thus, Wolf (126) finds that barley not only develops chlorosis when the iron supply is diminished to below normal, but also becomes stunted. And Toole and Tottingham (109) show that increased application of iron, as ferric hydrate, leads to over

50% increase in barley tops; but in experimenting by the same methods with peas, they find no particular modification, except in the deeper green of the leaves.

Marsh (63) finds that different forms of iron have different effects; iron toxicity is produced in cultures of soy beans treated with ferric phosphate, while the addition of ferric glycerophosphate in the same concentration gives rise to normal plants. Jones and Shive (51) get similar results when using ferric phosphate and ferrous sulphate as a source of iron for wheat grown in nutrient solution. When they supply iron in these two forms, in doses ranging from 0.01 to 5.0 mg. per liter, the lower members of the ferric phosphate series show chlorosis, and only the higher amounts produce plants with heads; while in the case of the ferrous sulfate only the very lowest members of the series are unhealthy, and the rest produce large, well formed heads. But in both series the amounts above 2 mg. iron per liter show slight toxicity and corresponding decline in yields.

Brioux (14), considering phosphates, finds that iron phosphate has little stimulation on the production of dry matter.

The increase in crop yield is, of course, related to the availability of the iron supplied, which in turn is in some degree dependent upon the hydrogen-ion concentration of the nutrient medium. These relationships will be more fully discussed in the following sections.

B. The Plant Nutrient Solution and its Relation to Iron

1. The Nutrient Solution.

(a) Composition of Solution.---The plant nutrient solution is prepared to provide all elements necessary for the growth and maturity of the plant, and to supply those elements in such forms as are most readily used by the plant. Such solutions are especially adapted for experimental purposes since they simulate the natural nutrient medium of the plant, yet allow control of nutrient factors, such as could never be exercised with the use of soil or sand cultures.

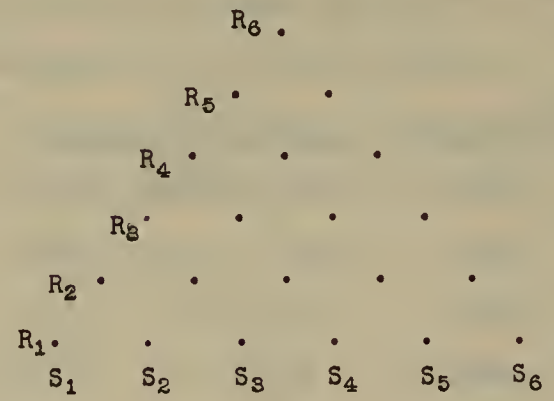
Livingston (62), in his Plan for Cooperative Research on the Salt Requirements of Representative Agricultural Plants, gives a comprehensive summary of the character of nutrient solutions of varying types. Much of the following information is drawn from this source.

The three-salt solutions are made to contain the ions K, Ca, Mg, H_2PO_4, NO_3 and SO_4. Since all six ions must occur in any solution, the following combinations are possible.

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
$MgSO_4$	$Mg(H_2PO_4)_2$	$MgSO_4$	$Mg(NO_3)_2$	$Mg(H_2PO_4)_2$	$Mg(NO_3)_2$

If an osmotic value of one atmosphere at 25°C. be taken as standard, a large number of solutions of the type given above may be formed by simply varying the partial concentration of the individual components.

If the increment of volume-molecular partial concentration from solution to solution is one-eighth of the total volume-molecular concentration, we may diagram all the possible sets of salt proportions with increments of one-eighth, and arrive at the well known triangular diagram:



"Let the base line for the potassium salt be the base of the triangle, and let that for the calcium salt be the left side. The right side will then be the base line for the magnesium salt. Each of these three base lines represents a row of solutions each having one-eighth of its total volume-molecular concentration due to the salt for which the line is named. The apex opposite this line represents a solution in which six-eighths are due to that salt. The volume-molecular proportions of all three salts are quickly determined for any solution represented on the diagram. ..... To designate the solutions, the rows on the diagram are numbered from below upward, and the solutions are numbered in each row from left to right. To refer to a solution we first write the Roman numeral denoting the type (what three salts are used), then we write the row number (preceded by the letter R), then we write the solution number in the row (preceded by S), and finally we state (in parentheses) the total concentration in terms of atmospheres

of osmotic pressure representing the calculated osmotic value of the solution in question. Examples are: IR2 S3(1.00 atm.), IIIR1 S2 (1.50 atm.), V R6 S1 (1.65 atm.), etc."

Livingston says that the solutions are controlled by Shive's R5C2(1.75 atm.). The relation of this solution to some others may be best seen by comparing it with a few taken from Livingston and given in the following table.

	Molecular Proportions			Partial Volume-molecular Concentrations		
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
Shive's R5C2	3.77	1.09	3.14	.0180	.0052	.0150
I R2S1	2	1	5	.0053	.0027	.0132
S2	2	2	4	.0049	.0049	.0099
S3	2	3	3	.0047	.0071	.0071
S4	2	4	2	.0045	.0090	.0045
S5	2	5	1	.0041	.0104	.0021
R4S1	4	1	3	.0099	.0025	.0074
S2	4	2	2	.0094	.0047	.0047
S3	4	3	1	.0090	.0068	.0022
R6S1	6	1	1	.0145	.0024	.0024

To explain this table more clearly, let us consider the solution IR2S1. The figures under the heading "Molecular Proportions" mean that for every two gram-molecular weights of potassium dihydrogen phosphate in solution there will be one gram-molecular weight of calcium nitrate and five gram-molecular weights of magnesium sulfate.

Now let us consider the figures under the heading "Partial Volume-molecular Concentrations". For potassium dihydrogen phosphate the value .0053 is given. This means that potassium dihydrogen phosphate has a molar concentration of .0053 in the solution. Then this factor multiplied by the molecular weight of potassium dihydrogen phosphate, 136.2, gives us the number of grams of potassium dihydrogen phosphate in one liter (1000 cc.) of the nutrient solution: 136.2 x .0053 = .7209 gms. per liter. But it should be remembered that the component salts are often added as so many cubic centimeters of the molar solution. Then, to find the number of cubic centimeters of molar potassium dihydrogen phosphate to be used for one liter of the nutrient solution we have the proportion:

$$\frac{.7209}{136.2} = \frac{x}{1000}$$

when $x = 5.3$ cc. molar solution.

But now we may note that our factor was .0053, meaning that the solution is fifty-three ten-thousandths molar in terms of potassium dihydrogen phosphate, and we may read that directly to mean 5.3 cc. diluted to one liter without passing through these intermediate steps. In the same way the value .0027 for calcium nitrate shows us that we should use .4430 gms. or 2.7 cc. molar solution in one liter of normal solution; and, from the value .0132 for magnesium sulfate, we find we shall need 1.5890 gms. or 13.2 cc. normal solution per liter of nutrient solution. To summarize: - If we wish to make a liter of the nutrient solution IR2S1 we may start with the three component salts themselves and weigh out .7209 gms. potassium dihydrogen phosphate, .4430 gms. calcium nitrate, and 1.5890 gms. magnesium sulfate, and put these all

into a liter measuring-flask and make the solution up to 1000 cc. with distilled water; or we may start with molar solutions of the salts already made up, when we shall use 5.3 cc. of molar potassium dihydrogen phosphate, 2.7 cc. molar calcium nitrate and 13.2 cc. molar magnesium sulfate, and make these up to one liter with distilled water. It might be well to note here that it is well to add the salts or their solutions to about 200 to 300 cc. of distilled water, and then make up to the liter mark with more distilled water, since in greater concentrations the salts often react to form insoluble compounds.

McCall (70), using potassium dihydrogen phosphate, calcium nitrate and magnesium sulfate, with a total initial concentration of 1.75 atmospheres maximum osmotic pressure, makes 36 different proportions of the three components in solution, and determines optimum pressure and correlates ratio of salts with growth.

(b) Hydrogen-ion Concentration of the Nutrient Solution.--- Hydrogen-ion concentration may be considered either in terms of hydrogen-ion concentration itself, or in terms of pH. The pH of a solution is the value for $\log \frac{1}{\text{H-ion conc.}}$. It will be noted that as the pH increases, the H-ion concentration decreases. Neutrality is expressed by pH 7.0; hence pH greater than 7.0 is alkaline, while pH less than 7.0 is acidic.

The pH of the nutrient solution is most important, since it must be comparable with the pH of the natural nutrient medium, the soil.

The pH of a nutrient solution is usually kept fairly constant by means of "buffers". These may be carbonates or phosphates; phosphates are more commonly used. Thus, if potassium dihydrogen phosphate be used

as the buffer, the solution will stay at the pH of that salt under ordinary circumstances, and cannot become more basic than the pH of potassium monohydrogen phosphate until all of the monobasic salt has been converted into the dibasic form. Under ordinary conditions the influence toward basicity is so feeble compared with the amount of buffer present that the pH never does rise above that of the dibasic salt.

Wiegner and Gessner (120) point out that most soils have a pH between 4 and 8. They mention carbonic acid, humus, clay and the hydrolysis of salts as the chief sources of buffering action. Dirks (25) speaks of humates as exerting a definite alkaline reaction, which is kept below the toxic concentration by the action of zeolitic material.

For the nutrient solution the pH is maintained at a fairly constant value by the use of phosphates for buffers. Buytendijk and Brinkman (16) find that HCl solutions may be buffered to pH 8.5 to 6.5 by the use of Na_2HPO_4, and that the calculated pH is established immediately and remains constant. Atkins (5) finds varying solubility of $CaH_4(PO_4)_2$ with change of pH, the phosphate becoming more soluble as the acidity increases; but the amounts in nutrient solutions are too small for this to be observed.

McCall and Haag (71), working on the solutions recommended by Livingston (62), find that "the hydrogen-ion concentration of the solution is a function of the volume-molecular proportion of the dihydrogen phosphate salt present". It is not, they point out, possible thus to compare solutions containing different dihydrogen

phosphate salts; but such comparison holds within any one type of solution. They also find that the solutions buffered with potassium dihydrogen phosphate have a higher pH (i.e., lower H-ion concentration) than those buffered with either magnesium dihydrogen phosphate or calcium dihydrogen phosphate, and that these salts have a predominating part in the determination of the hydrogen-ion concentration over that of the sulfates and nitrates present.

Jones and Shive (50) show the inter-relation between the number of plants grown in solution, total osmotic-concentration of solution and volume of nutrient solution in the summary of their work:

"1. With each number of plants used in a given volume of nutrient solution the rate of change of the hydrogen-ion concentration decreased with an increase in the total osmotic-concentration value of the solution.

"2. With each number of plants given in the nutrient solution having a given total osmotic-concentration value the rate of change of the hydrogen-ion concentration decreased with increase in the volume of the solution.

"3. With each volume of nutrient solution and with each total osmotic-concentration the rate of change of the hydrogen-ion concentration increased with each increase in the number of plants grown in the solution."

Barnette and Shive (8) also note that the rate of reaction change is proportional to the volume of the culture solution.

These are not the only workers to recognize the fact that growing plants will change the pH of the nutrient solution. Meier and Halstead (75) show that acid cultures used for the growth of wheat tend

to become neutral, and suggest that this change may be due to the selective absorption of ions from the solution by the plants. Jones and Shive (52), also working with wheat seedlings, find that Tottingham's $T_3R_1C_4$ is changed from pH 4.6 to pH 5.5 in 52 hours, and Shive's R_5C_2 is changed from pH 4.5 to pH 5.3 in the same time. The minimum change in any solution used by them was 0.6 pH unit.

Mevius (76) believes that the permeability of the plant cell depends upon the pH of the nutrient medium, and that the kind, number and proportions of ions in the solution, as well as temperature, influence the processes of absorption. Robbins (94) shows work to support this view as regards the pH. Hoagland (45), using barley as the test plant, finds that the absorption of several ions is greater with a pH 5 to 5.5 than from a solution of pH 6.8.

Prince, Jones and Shive (89) find that nitrate-ion is taken up more rapidly by plants than calcium-ion, and that potassium-ion is taken up more rapidly than dihydrogen phosphate-ion. The first difference has a greater tendency to decrease the hydrogen-ion concentration than the second has to increase it, so the change of reaction is always toward the neutral point. In the case of magnesium-ion and sulfate-ion both are taken up slowly and at about the same rate; when ammonium sulfate is used, however, the ammonium-ion is absorbed much more rapidly than the sulfate-ion or any other ions present, and a shift to the acid range results.

Rudolfs (96, 97) finds that seeds soaked in a solution will change the pH of that solution to a definite value which depends upon the species of the seed, and is constant for any given species. In his earlier work (96) he attributes this to selective ion absorption

by the seeds, but later concludes (97) that this change is due to the chemical properties of the chief protein constituent of the seed.

Meier and Halstead (75) agree with McCall and Haag (71) that there seems to be no direct relation between differences in pH of solutions, or in change of pH within the solution and crop yield.

There is, however, conflicting evidence upon this point, and the writer's comment is that doubtless the range of pH was not great enough to satisfactorily demonstrate such relations.

Hopkins and Wann (48, 116) find that Chorella will grow well between pH 3.4 and pH 8.4, with no definite optimum pH. However, the more alkaline portion of the range is complicated by the question of availability of iron, which will be discussed later. Haas (33) finds that alfalfa seedlings have average growth at pH 5 and optimum at pH 8. Powers (88) finds evidence that all plants grow best in a slightly acid medium, and gives the following pH values of maximum growth:

Alfalfa,	pH 5.6 - 6.0
Hungarian vetch,	pH 5.3
Spearmint,	pH 6.0
Alsike clover,	pH 5.5 - 6.0
Rhododendrons,	pH 5.0

Hoagland (44) finds toxicity for barley seedlings with a hydrogen-ion concentration of 0.3×10^{-3} (pH 3.5) and with hydroxyl-ion concentration greater than 1.8×10^{-6} (pH 8.25), while extreme toxicity is shown when the hydroxyl-ion concentration is greater than 2.5×10^{-5} (pH 9.4). But with a hydrogen-ion concentration of approximately 0.7×10^{-5} (pH 5.2) results favorable for growth are obtained.

Wherry (118) finds that plants as highly epecialized ae the saprophytic orchide, as for example Corallorhiza and Hexalectrie, have very definite soil reaction preference, and narrow limite of tolerance. It ie exceedingly interesting to note that the tolerance of plants so closely related as the species of one genus may be widely different.

Wherry (119) has also done extensive work considering the soil reaction preferences of many of the plant orders. He classifies pH 4.1 to 5.0 as mediacid, pH 5.1 to 6.0 as subacid, and pH 6.1 to 7.9 as circumneutral, and finde that of the orders within the range of "Gray'e Manual", three have a mediacid optimum, 11 a subacid optimum and 30 a circumneutral optimum . There ie no evident correlation between eoil reaction preference and relation between ordere.

The writer's comment on this data is that it appears that the average nutrient solution, with its phosphate buffer ealt, is well adapted for general culture use, but that changes toward a pronounced acidic or alkaline condition are more to be avoided, ae injurioue, than thoee toward neutrality.

2. Iron Supply of the Nutrient Solution.

(a) Supply of Iron.---As early as 1860 Sachs (100) reports that iron must be supplied in nutrient solutions, but in very small amounts. Wollf (126) finds that neither chromium nor nickel can be made to take the place of iron. He also finds that either the citrate or the cyanide may be used as a source of iron for barley.

Richter (92) finds that Mohr's salt, ferrous ammonium sulfate, furnishes a satisfactory source of iron for the rice plant. Jones and Shive (51) find that ferrous sulfate is a better source of iron for wheat than is ferric phosphate, but either is toxic in amounts exceeding 2 mg. iron per liter. Marsh and Shive (65), experimenting with soy beans, find that iron may be added to the culture solution as ferric glycerophosphate, soluble ferric phosphate, ferric tartrate or ferrous sulfate with equally good results, so long as the supply is renewed daily, and the total amount in solution is not in excess of the nutritive needs of the plant. Marsh (63) finds ferric glycerophosphate to be a better source of iron than ferric phosphate, for the growth of soy beans.

Deuber (23) finds that ferric citrate is four to thirteen times more efficient than ferrous sulfate as a source of iron for plants.

Mazé, Ruot and Lemoigne (68) advocate the use of iron nitrate as a remedy in case of chlorosis, and the work of Sherwin (103) also points to this salt as a readily available form of iron.

Toole and Tottingham (109) find great increase of growth of barley tops when ferric hydroxide is added, but attribute this

beneficial effect to the neutralization of acids rather than to any direct action of the iron. Udluft (111) finds that ferric hydroxide sol is not soluble or transportable in carbonate or bicarbonate unless stabilized by a protective colloid, and a definite concentration of bicarbonate-ion will precipitate ferric hydroxide sol and the humus sol which has stabilized it.

It is generally conceded that plants can use either organic or inorganic iron if it be soluble. The question of solubility and availability will be more fully considered in the next section.

(b) Availability of Iron.---Barnette and Shive (8) find a direct correlation between the decrease in hydrogen-ion concentration of the culture solution and the appearance of chlorosis in the plants grown in the solution.

Patten and Mains (85) obtain a clear solution of ferric chloride when ammonium hydroxide is added at pH 3.3, but at pH 3.5 there is obtained a cloudy solution due to the formation of a colloidal precipitate of ferric hydroxide, and this cloudiness increases with increasing pH until at pH 6.0 a heavy flocculent precipitate is obtained. By adding hydrochloric acid, the pH is lowered with increasing disappearance of the precipitate until a clear solution is again obtained at pH 3.3. Sodium hydroxide may be used in place of ammonium hydroxide with the same results. These workers also find that pH 3.3 is the lowest acidity at which iron will remain in solution when hydrogen sulfide gas is passed through the solution.

Working with Hawaiian soils, McGeorge (73) finds that soluble crystalloid salts of iron may be formed in soils more acid than pH 5.8.

Reed and Haas (91) have worked with ferric tartrate as a source of iron in nutrient solutions. They find that iron supplied in this form rapidly becomes converted into insoluble compounds when added to nutrient solutions, and the higher the pH of the solution, the faster is the rate of change. When the hydrogen-ion concentration of the nutrient solution is increased by the introduction of carbon dioxide, the solubility of the iron compounds is not increased; but certain organic compounds, as the ammonium salts of organic acids, may be used to lower the pH (increase the hydrogen-ion concentration), and in this case the iron becomes more soluble.

Halvorson and Starkey (36) find evidence to show "that at reactions more alkaline than pH 5.0 very small amounts of ferrous iron will occur in solution under atmospheric conditions and even smaller amounts of ferric iron are soluble".

With either organic or inorganic iron salts, Meehan and Baas-Becking (74) find that while ferrous iron is soluble below (approximately) pH 6.2, ferric iron becomes soluble only at a concentration more acid than pH 5.0.

There seems to be a general feeling throughout much of the literature that ferrous iron may be more available to plants than ferric iron, but Kostytschew (60) cites both Knop (58) and Wagner (114) as believing that "ferrous and ferric salts may be equally well assimilated".

The writer's comment is that no doubt the greater solubility of ferrous salts in solution of weak hydrogen-ion concentration, such as commonly used for nutrient solutions, has led to the

belief that ferrous salts are more readily assimilated by the plant, than ferric salts. We must also remember that the opinions of Knop and Wagner, cited in the preceding paragraph, are dated 1869 and 1870 respectively, years before the present ideas of acidity were established, so that their remarks about the equal assimilation of ferric and ferrous salts have no weight when considered under the refinement of pH measurements.

Atkins (4) shows that the gardan hydrangea, H. Hortensis, produces blue flowers when grown in soils at pH 5.7 to pH 6.0, and pink flowers, or pink and blue flowers on the same plant, when grown in soils with pH 6.0 to pH 7.5, while above pH 7.5 only pink flowers occur. He finds blue or pink flowers from the same plant to have the same pH value, 4.2; and he further shows that, calculated on dry weight of the flowers, the blue contain about 140 parts per million of iron, while the pink contain only about 60 parts per million. The blue color he attributes to an iron-anthocyanin complex, which cannot be formed when the iron is rendered insoluble by the higher pH. The writer's comment is that this diminution in the amount of iron in the flowers of the plants grown in soils of a high pH seems quite natural, since the high alkalinity of the soil would prevent solubility, and hence availability of the iron.

Using ferric phosphate as a source of iron for soy beans and Japanese buckwheat in nutrient solutions, VanAlstine (112) finds that the plants can withstand an acidity between pH 3.7 and 4.1 and obtain sufficient iron, but at a pH greater than 4.1 chlorosis results. When ferric sulfate is used as the source of iron, no chlorosis appears, even at pH 6.5 to 6.7. The reason for the chlorosis in the first case seems

to be that the semi-colloidal ferric phosphate used is not sufficiently soluble, in solutions more alkaline than pH 4.1, to furnish enough iron for normal growth.

The writer's comment is that inasmuch as legumes develop chlorosis due to iron deficiency at a hydrogen-ion concentration less than pH 4.1, while hydrangeas grow and flower at pH 7.5, there must evidently be some protection of the iron by colloids in the sap, or by some buffering action in the sap which keeps the pH at such a value as to render the iron soluble. Nor does either of these hypotheses explain how the hydrangea gets any iron from a soil of pH 7.5 unless we assume great modification of the pH of the soil solution by excretion from the root hairs, at least in the adsorbed layer. Whatever the mechanism involved, it appears to be a distinguishing mark of certain plant orders.

Zinzandze (128) shows that maize seedlings change the pH of the nutrient solution during growth, and suggests a model solution in which the reaction remains between 5.3 and 3.8 during the growth period; with ferric chloride or ferric sulfate as a source of iron, this is sufficiently acid.

Marsh (64) also notes greater solubility of iron compounds in solutions of lower pH. His work will be considered later in more detail.

III. RESEARCH

A. The Problem

The immediate problem of this research is to devise and use an accurate method for the determination of iron in plant nutrient solutions, and to establish, if possible, the rules governing the solubility of iron in such solutions.

That such a problem exists is shown by numerous references cited in the introduction; it seems clear that the supply of iron in a plant nutrient solution must be carefully adjusted and maintained within narrow limits.

In order that such control may be exercised over the iron supply, there must be some accurate means of determining the amount of iron in solution at any given time. Up to date no reliable method of estimating extremely small amounts of iron in such solutions has been shown. The work of Marsh (64) and Louwsma (62a) has opened this field, and it is as a continuation of Louwsma's work that this investigation has been undertaken.

B. Apparatus

Since the methods investigated are of two kinds, two distinct sets of apparatus are to be considered. The volumetric method described by Louwsma (62a) requires a modification of the apparatus used by Murray (82a). This consists of a Jones reductor for reducing the iron, and a small burette for titration of the iron with N/80 potassium permanganate, both of which enter a suction flask, so that the titration

is carried on in partial vacuum. A detailed description of the apparatus is given by Louwsma.

For the colorimetric work 50 cc. Nessler tubes, approximately 1.7 cm. in diameter, are found best. Use has also been made of a(Bosch and Lomb)Schreiner colorimeter; it was found however, that this piece of apparatus is not adapted to our range of colors.

The Pyrex tubes used for pH determinations are soaked for several days in a sulfuric acid solution, washed and dried, and then allowed to lie for an equal length of time in a solution adjusted to pH 6.8-7.0 to which Brom Thymol Blue has been added. If any change of color is shown by the solution, the tubes are again subjected to the same treatment until they no longer affect the solution.

Some of the earlier solutions were kept in flasks of glass other than Pyrex, but the work done on these was found to be inaccurate. Since it is practically impossible to season the glass used in volumetric flasks by the method outlined, such flasks are unsuitable for solutions that are to be tested for small changes in pH.

For sampling the solutions, use is made of the pressure siphon devised by Louwsma, which consists of a regular wash-bottle stopper arrangement, but has a curved-up inner end to the delivery tube to avoid stirring up any sediment, and a pinchcock on the rubber insert of the delivery tube to prevent backflow after sampling.

The iron compounds are weighed on a Troemner balance which weighs accurately to within two or three hundredths of a milligram.

C. Materials and Solutions

The potassium permanganate for the volumetric work is made up to approximately N/80 and standardized with pure iron wire as described by Louwsma.

A standard solution of iron is made by dissolving one gram of pure iron wire in as little 6-N hydrochloric acid as possible, oxidizing to the ferric form with hydrogen peroxide, and diluting to one liter total volume after boiling out the excess hydrogen peroxide. This gives one part in one thousand or one thousand parts per million. Ten cubic centimeters of this solution diluted to one liter give a solution containing one part iron in one hundred thousand, or ten parts per million. Greater dilutions are obtained by the same method.

The nutrient solutions studied are the same as those used by Louwsma. Their composition is given in Table I, which is copied from Louwsma's thesis.

TABLE I

Composition of the Nutrient Solutions Used.

Solution	pH	Partial volume-molecular concentration					
		KNO_3	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	$(NH_4)_2SO_4$	$CaH_4P_2O_8$
III R_2S_1	4.1	0.0054			0.01350		0.0027
R_3C_1	4.1	0.0288			0.00500		0.0026
R_5C_2	4.5		0.01800	0.0052	0.01500		
$T_1R_1C_5$	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 sources of the salts used in making these solutions is shown in Table II.

TABLE II

Sources of Salts Used for Nutrient Solutions

Baker & Adamson, C.P.	Baker's Analyzed
$MgSO_4$	
$Ca(H_2PO_4)_2$	
$Ca(NO_3)_2$	$Ca(NO_3)_2$
$(NH_4)_2SO_4$	$(NH_4)_2SO_4$
	KNO_3

Stock solutions of these salts have unmodified pH values as given in Table III. It is necessary to keep these solutions in Pyrex containers in order to avoid pH change.

TABLE III

Strength and pH of Stock Solutions

Salt	Normality of stock solution	pH value of stock solution
$Ca(NO_3)$,	N/1	7.6
$Ca(H_2PO_4/2$	N/10	3.2
KH_2PO_4	N/1	3.7
KNO_3	N/1	7.0
$MgSO_4$	N/1	7.5
$(NH_4)_2SO_4$	N/1	5.6+

The potassium thiocyanate used for the colorimetric test is "Baker's Analyzed". This is made into a solution of 20 grams per liter. The concentrated hydrochloric acid used in this test is also "Baker's Analyzed". The potassium permanganate used for oxidizing

is the N/80 solution standardized for the volumetric method. When hydrogen peroxide is used for this purpose, it is 3%, B and A 1780, from the General Chemical Company.

The ferric citrate used as the source of iron in a part of the earlier work is Eimer & Amend, U. S. P. VIII scales. For most of the work Baker & Adamson, C.P., ferric phosphate is used. Some of the ferric phosphate, however, is that made by the writer by a method given by Friend (28) which follows: A mixed solution of ferric chloride and disodium hydrogen phosphate in the molecular proportion of four to one is evaporated with an excess of nitric acid. As the liquid becomes syrupy, ferric phosphate ($FePO_4.2H_2O$) precipitates out in large amounts. Where no indication is made of the source of the ferric phosphate, the Baker & Adamson material is understood to be that used. The two samples were found to give the same results.

The pH determinations are run by the use of LaMotte indicators, and LaMotte standards of comparison. The indicators are bought either ready for use or as a 1% solution which must be diluted to 0.02% or 0.04% with carbon dioxide free water. This water is obtained by redistilling distilled water from alkaline permanganate in a carbon dioxide free system. All the indicators used except LaMotte Yellow and a portion of the Bromphenol Blue have been diluted from the 1% solution in this way. The pH standards supplied by the LaMotte Chemical Products Company consist of sealed tubes containing buffer solutions of the proper pH with the required amount of indicator. Consecutive tubes of the same indicator differ by 0.2 pH unit. In the middle of an indicator range it is possible to read accurately to 0.1 pH unit; either end of the range is less accurate. But these transition zones of the range are checked by

the use of indicators with overlapping ranges, By the use of two indicators it is possible to make any pH reading accurate to 0.1 pH unit.

The indicators used, with the pH range and color change of each, are given in Table IV.

TABLE IV

Range and Color Chagge of Indicators Used.

Indicator Name	pH Range	Color Change
Meta Cresol Purple	1.2-2.8	Red - Yellow
LaMotte Yellow	2.6-4.2	Red - Yellow
Bromphenol Blue	3.0-4.6	Yellow-Blue
Bromcresol Green	4.0-5.6	Yellow-Blue
+Bromcresol Purple	5.2-6.8	Yellow-Purple
Chlorophenol Red	5.2-6.8	Yellow-Red
Bromthymol Blue	6.0-7.6	Yellow-Blue
Phenol Red	6.8-8.4	Yellow-Red
Cresol Red	7.2-8.8	Yellow-Red

+ Bromcresol Purple gives trouble due to dichromatism, and is best replaced by Chlorphenol Red. For all colorimetric work a north light is used, and white paper placed under and behind the tubes.

D. Development of a Method for the Determination of Iron in Nutrient Solutions.

1. Work on Louwsma's Titration Method.

Louwsma (62a) describes his titration method, adapted from that of Murray (82a), in detail in his thesis. Briefly, it consists of the precipitation of the iron as ferric hydroxide, which is washed free of nitrates and dissolved in dilute sulfuric acid; this solution is passed through the Jones reductor to reduce the iron, which is then titrated, in partial vacuum, with N/80 potassium permanganate.

A number of samples have been run by this method in order to check Louwsma's work. In every case the results obtained agree with his within the limits of experimental error. We agree with Louwsma, however, that the amounts of iron usually found in nutrient solutions are too small to be accurately measured by this method.

2. Work on Louwsma's Colorimetric Method.

The colorimetric method as used by Louwsma is very simple. Fifty cubic centimeters of the sample are taken and to it, in a Nessler tube, is added 2.5 cc. concentrated hydrochloric acid, enough potassium permanganate to give a faint pink tinge, and 2.5 cc. of potassium thiocyanate solution (20 grams per liter). The amount of iron present is determined by comparing the color thus obtained with that developed in a distilled water-iron solution of known strength treated by the same method.

This method is found to be reliable if iron is present in comparatively high concentration with low concentration of the nitrate or phosphate ions, or if iron is present in low concentration with complete absence of the nitrate or phosphate ions. In other cases, the nitrate and phosphate ions seriously interfere with the test, as is shown in the following section. Nutrient solutions normally contain a very low concentration of iron, with a comparatively high concentration of both the nitrate and phosphate ions. For this reason we offer the compensation method, which is discussed in a subsequent section.

3. Studies in Interference.

(a) Interference of Component Salts.---The solutions tested by Louwsma's colorimetric method show varying shades of color, and are not always well matched by the distilled water-standard iron solutions used for comparison.

For this reason a study has been made of the interference of the various components used in the nutrient solutions. In preliminary tests with calcium and potassium nitrates (0.5 cc. each per 50 cc. solution), it is found that such a nitrate solution with 0.2 parts per million iron added is two to three times as intense in color as a blank of distilled water with the same amount of iron.

It may be important to observe here that all solutions containing nitrates not only have a more intense color than distilled water standards, containing the same amount of iron, but also gain

in intensity of color when allowed to stand, whereas the standard fades rapidly, usually within five to ten minutes.

Table V contains the data from a number of preliminary tests run with nitrates and phosphates to determine their interference.

TABLE V

Preliminary Tests in Interference, 50 cc. in Volume;

Louwsma's Colorimetric Test Used.

	Solution to be Tested		Standard		
No.	Amt. Iron Present p.p.m.	Salt Present	Amt. Iron Present p.p.m.	Salt Present	Remarks
1	0.00	0.5 cc. M/1 KNO_3	0.10	0.00	Not comparable.
2.	0.10	0.5 cc. M/1 KNO_3 5×10^{-4} M	0.10	0.00	Nitrate soln. more intense in color.
3.	0.20	0.5 cc. M/1 KNO_3	0.20	0.00	Nitrate soln. more intense in color.
4.	0.20	1.0 cc. M/1 KNO_3	0.20	0.00	Nitrate soln. more intense in color; requires less $KMnO_4$ to give pink color in test.
5.	0.20	1.0 cc. M/1 $Ca(NO_3)_2$	0.20	0.00	Nitrate soln. more intense, and more yellow than true iron color.
6.	0.20	1.0 cc. M/1 KH_2PO_4	0.20	0.00	Phosphate soln. about 4 times as intense, and more yellow than true iron color.

Each set of data in Table V represents the summary of a number of tests. The first four tests deal with potassium nitrate, and show that this salt intensifies the color of the test, even at a M/100 concentration. Hence in the presence of potassium nitrate more iron is apparently found than is known to be present.

The fifth test in Table V shows that calcium nitrate not only intensifies the color, but also changes the color from the true reddish-yellow of the iron test to a clearer yellow; these two colors are difficult to describe to those who have not seen them, but they are wholly incomparable.

The sixth test shows the effect of potasssium dihydrogen phosphate upon the iron color. The presence of this salt increases the intensity, and gives a more yellow color than the true reddish-yellow of the iron test. This color is incomparable with the iron color.

The tests recorded in Table V indicate that some interference, as that caused by potassium nitrate, may be measurable, while the interference produced by calcium nitrate or potassium dihydrogen phosphate is of such a quality that comparison of the tests and measurement of the interference is impossible. Further tests run to prove this are given in Table VI.

TABLE VI.

Studies in Interference 50 cc. Volume;

Louwsma's Colorimetric Test Used.

No.	Solution to be Tested		Standard		Error or Remarks
	Amt. Iron Present p.p.m.	Salt Present	Amt. Iron Present p.p.m.	Salt Present	
1.	0.2	0.5 cc. M/1 KNO_3 .01M	0.21	0.00	+.01 p.p.m.
2.	0.2	1.0 cc. M/1 KNO_3 .02	0.21	0.00	+.01 p.p.m.
3.	0.2	2.0 cc. M/1 KNO_3 .04	0.22	0.00	+.02 p.p.m.
4.	0.1	0.5 cc. M/1 $Ca(NO_3)_2$ .02N	0.1	0.00	Not comparable; nitrate solution more yellow.
5.	0.2	0.5 cc. M/1 $Ca(NO_3)_2$ .02N	0.2	0.00	Not comparable; nitrate solution more yellow.
6.	0.1	0.5 cc. M/10 $Ca(H_2PO_4)_2$ .002	0.1	0.00	Not comparable phosphate sol. more yellow; more error than in 4 or 5.
7.	0.2	1.0 cc. M/10 $Ca(H_2PO_4)_2$ .004N	0.2	0.00	Not comparable; phosphate sol. more yellow; more error than in 6.
8.	0.2	1.0 cc. M/1 KH_2PO_4 .02	0.27	0.00	+.07 p.p.m. for nearest match; not comparable; phosphate sol. much more yellow.
9.	0.2	1.0 cc. M/1 $(NH_4)_2SO_4$	0.2	0.00	0.00

The results of the tests recorded in Table VI substantiate those shown in Table V. The first three tests show that the positive error due to the interference of potassium nitrate can be measured. The error is shown to amount to 5% of the total iron present when potassium nitrate is present in a M/100 concentration; doubling this concentration shows no measurable increase in error, but when four times the initial amount of potassium nitrate is present, i.e., a M/25 concentration, the error becomes 10% of the total iron present.

Tests 4 and 5 confirm the finding of Table V, where we found that calcium nitrate interferes to such a degree that the color of the solution containing this salt and that of the distilled water-iron standard are not comparable. The yellow of the solution containing calcium nitrate is distinctly different from the reddish-yellow of the true iron test.

Tests 6 and 7 show that calcium dihydrogen phosphate gives even more interference than does calcium nitrate. The phosphate produces a paler yellow, wholly incomparable with the true iron color.

Test 8 shows an attempt to measure the interference of potassium dihydrogen phosphate. It is not possible to get a good match, since the phosphate gives a deep yellow that cannot be well matched in shade by the iron test; the nearest approach in intensity is measured, however, and a positive error of 35% of the total iron present is found when the concentration of potassium dihydrogen phosphate is M/50. This is seven times as great as the interference of potassium nitrate at the same concentration.

Test 9 is run with a M/50 concentration of ammonium sulfate. There is no interference.

After a careful consideration of the results given in Tables V and VI, it is seen that solutions containing the nitrate and phosphate ions show interference with the colorimetric test for iron used by Louwsma.

The nitrate ion intensifies the color of the test when present in amounts less than those often used in nutrient solutions. Louwsma finds serious interference caused by nitrates, but his work is carried on with concentrations of both iron and nitrates higher than those used in the present investigations.

Louwsma also shows that in higher concentrations than those used in the present investigation the phosphate-ion gives an olive-green tint which, when present in some intensity, obscures the red of the iron complex. He reads this as a negative error. In the present investigation this is encountered as the clear yellow already referred to, but by trying to match intensity rather than tint of color a positive error is obtained. This is merely a matter of personal technique and the results given do not disagree because of this with the work done by Louwsma. It should also be remembered that this work is done at much lower concentrations of both the interfering salts and iron than that done by Louwsma.

b. Oxidation by Hydrogen Peroxide. Whenever the tests recorded in Table V and VI are allowed to stand for several days, or even over night, it is observed that the tubes containing the nitrates have gained in intensity; but the standards fade rapidly, so that it is often impossible to read them with accuracy five minutes after they have been made up. It might be possible for the manganous salts, formed in the test, to catalyze a reaction which would destroy the

iron complex, thus causing a disappearance of the color.

Further, in making the tests it is very difficult to add the potassium permanganate, used to oxidize iron and any organic matter present, so that only the faintest tinge of pink is noticeable, since the power to observe the first tinge of pink varies with the intensity of the light. When too much permanganate is present, it lends a distinct reddish tinge to the test, which must then be discarded.

In Table VII are given the results of several tests run to determine the effect of the oxidizing agent upon the fading of the standard, and incidentally to check the studies in interference already made. No attempt was made to match the colors, as in the previous table.

In Table VII potassium permanganate and hydrogen peroxide are compared as oxidizing agents for the colorimetric method. The first test shows that the color fades rapidly even when no oxidizing agent is used. Tests 2, 7 and 10 show that the standards fade rapidly when potassium permanganate is used as an oxidizing agent; this fading is often so rapid that it is difficult to read the test with any degree of accuracy. Tests 3, 4, 5, 6, 8, 9, 11, 12 and 13 show that the color holds well in the standards when hydrogen peroxide is used as the oxidizing agent. It is true that even these will fade gradually, but the change is so slow that it is not appreciable for several hours, and even after two or three days the color has not entirely disappeared.

Test 13 shows the effect of alcohol. This is the record of an attempt to use alcohol for making up the standard iron solutions. The alcohol not only does not prevent the fading, but it also introduces other positive errors.

TABLE VII

Oxidation by Hydrogen Peroxide
50 cc. Solution Used

	Solution to be Tested		Standard			
No.	Amt. Iron Present p.p.m.	Salt Present	Amt. Iron Present p.p.m.	Salt Present	Oxidizing agent	Remarks
1.	0.2	2.0 cc. M/1 KNO_3	0.2	0.00	None	Standard fades fast; difficult to read.
2.	0.1	1.0 cc. M/1 KNO_3	0.1	0.00	$KMnO_4$	Standard fades fast; difficult to read.
3.	0.2	2.0 cc. M/1 KNO_3	0.2	0.00	12 drops H_2O_2	Standard holds color; slight positive error.
4.	0.2	2.0 cc. M/1 KNO_3	0.2	0.00	18 drops H_2O_2	Standard holds color, positive error.
5.	0.2	1.0 cc. 6-N HNO_3	0.2	0.00	12 drops H_2O_2	Standard holds color; slight positive error.
6.	0.2	2.0 cc. 6-N HNO_3	0.2	0.00	12 drops H_2O_2 before adding HNO_3	Same as last.
7.	0.2	1.0 cc. M/1 $Ca(NO_3)_2$	0.2	0.00	$KMnO_4$	Standard fades fast; slight positive error.
8.	0.2	1.0 cc. M/1 $Ca(NO_3)_2$	0.2	0.00	12 drops H_2O_2	Standard holds color; slight positive error.
9.	0.2	2.0 cc. M/1 $Ca(NO_3)_2$	0.2	0.00	12 drops H_2O_2	Standard holds color; positive error.
10.	0.2	1.0 cc. M/1 KH_2PO_4	0.2	0.00	$KMnO_4$	Standard fades fast; large positive error.
11.	0.2	1.0 cc. M/1 KH_2PO_4	0.2	0.00	12 drops H_2O_2	Standard holds color; large positive error.
12.	0.2	2.0 cc. M/1 KH_2PO_4	0.2	0.00	12 drops H_2O_2	Standard holds color; large positive error.
13.	0.2	25 cc. 95% CH_3CH_2OH	0.2	0.00	12 drops H_2O_2	Standard holds even; alcohol soln. shows much evolution of gas, and large positive error.

The tests in this table also confirm the interference results already discussed in the previous section.

4. Development of a Compsnsating Method.

By the use of hydrogen peroxide as an oxidizing agent in place of potassium permanganate, the difficulty of the rapidly fading standards can be overcome. But there is still serious interference with the test when nitrates or phosphates are present, as shown by the data in Table VII.

Since every nutrient solution differs in the proportion of salts, if not in the kind of salts, the error due to the interference of the ions given by the salts present will be different for every solution.

This difficulty should be overcome, however, by introducing the standard iron into a solution of the same composition as the solution to be tested, minus, of course, any iron other than the standard. In this manner, the same kind and degrees of errors are obtained in test solution and standard, which at once become comparable. Such a compensating method is now used, and gives colors comparable in both shade and intensity.

E. The Study of Iron in Nutrient Solutions

First, by the use of Louwsma's colorimetric method, and later, by the compensating method, studies have been made of the solubility of iron in nutrient solutions. The results of these studies are discussed in the following sections.

1. The Time-curve of Iron Solubility.

It has already been mentioned in the review of literature that it is common practice among those who use nutrient solutions to add iron every two to three days. This is accounted for when it is remembered that iron is known to become relatively insoluble, and hence unavailable for plant use, in a short time after its addition to the nutrient solution.

From these facts it would appear that the iron in solution might be measured at short intervals after its addition, the data plotted with the soluble iron as a function of time, and a graph obtained. Knowing the amount of iron added and the time elapsed since its addition, it should be possible to tell how much iron is still in solution. In order to establish such a curve the work in the following sections has been undertaken.

(a) By Louwsma's Colorimetric Method.---Louwsma shows that the soluble iron rises to a maximum in about a day and thence drops again. The present investigation shows similar results. Table VIII gives data from several tests run by Louwsma's method. Tests 1 to 8, inclusive, are preliminary studies; the rest are routine tests checked

by duplicate, and in part by triplicate sets.

The first test shows that 0.5 mg. of ferric phosphate in 500 cc. of distilled water adjusted to pH 5.0 with phosphoric acid gives a maximum iron content of 0.2 p.p.m. in 118 hours. The long intervals between tests allow some error, and the maximum iron content may be slightly higher, and may occur before or after the 118 hour period. Since the readings on either side are 0.1 and 0.13 p.p.m. and are at equal time intervals on either side of the 118 hour reading, it appears that the 118 hour reading must be nearly at the point of maximum solubility.

Test 2 shows a parallel to Test 1, but with ferric citrate used in place of ferric phosphate, and with the acidity adjusted to pH 5.0 with citric acid rather than with phosphoric acid. Here we find a maximum of 0.4 p.p.m. of iron in solution at the end of 100 hours. This corresponds in time with the maximum shown by the phosphate. Since the citrate is far more soluble than the phosphate the maximum is twice as high. At the 49 hour interval, by omitting oxidation of the ferrous iron by permanganate, the iron present in the ferric form is measured, and by the difference between that and the total iron in solution it is found that 0.1 p.p.m., or half of the iron present, is in the ferrous form.

In the third test are given the results of adding 0.9 mg. ferric phosphate to one liter of R_3C_1 nutrient solution. During the first part of this test accurate readings were not made below 0.1 p.p.m. But there is a maximum of 0.1 p.p.m. within one hour, and at the 55-hour interval the soluble iron has appreciably decreased in amount. However, a lapse of 54 hours gives time for a higher maximum

TABLE VIII

Data Obtained by Louwsma's Method

No.	Solution Tested	Iron added	Time since addition of iron. Hrs.	Iron found p.p.m.	Approximate pH
1.	.5 mg. $FePO_4$ in 500 cc. water + H_3PO_4 to give pH 5.0	($FePO_4$) .5 mg.	1	< .1	5.0
			20	< .1	5.0
			67	.1	5.0
			118	.2	5.0
			164	.13	5.0
			212	.1	5.4
			252	.1	5.5
			298	< .1	5.1
2.	.5 mg. iron citrate in 500 cc. water + citric acid to give pH 5.0	(Iron citrate)	1	< .1	5.0
			49	.2[1]	6.7
				.1	6.7
			100	.4	6.7
			146	.2	7.0
			194	.1	7.0
3.	R_3C_1	0.9 mg. $FePO_4$ per liter	1	.1	5.3
			55	< .1	5.2
			105	< .1	5.3
			150	< .1	5.1
			206	.05	5.1
			470	.05	5.1
			518	< .05	5.2
			638	< .05	5.2
4.	R_5C_2	1.2 mgs. Fe_2PO_4 per liter	1	.1	5.2
			49	.13	5.0
			93	.1	5.3
			213	.05	5.3
			333	< .05	5.3
			500	< .05	5.2
5.	.01 gms. per liter $FePO_4$	($FePO_4$)	1	.1	8.8
			53	.07	7.5
			149	.05	5.5
			205	< .05	5.7

[1] (ferrous)

TABLE VIII (Con't.)

No.	Solution Tested	Iron added	Time since addition of iron. Hrs.	Iron found p.p.m.	Approximate pH
6.	.01 gms $FeC_6H_5O_7$. $4H_2O$ per liter; regulated to pH 5.0 with citric acid.	($FeC_6H_5O_7$. $4H_2O$)	1	.1	5.0
			50	2.5	5.4
			98	2.2	5.5
			146[2]	2.0	5.5
				1.0[3]	5.5
				.94	5.5
			202	.55	5.5
				.5[3]	5.5
				.2[4]	5.5
			250	.66	5.5
				.13[3]	5.5
				.00[4]	5.5
			300	.4	5.4
				.1[3]	5.4
				.05[4]	5.4
			355	< .05	5.5
			383	< .05	5.5
7.	R_2S_1(pH 5.7) made to pH5.0 with H_3PO_4	0.01 gms. per liter iron citrate	1	< .05	5.0
			18	.25	5.1
			68	2.5	5.2
				2.4[1]	5.2
			118	2.0	5.1
				1.9[1]	5.1
			166	2.0	5.2
				1.9[1]	5.2
			222[2]	1.7	5.3
				1.6[1]	5.3
				1.3[3]	5.3
				1.2[4]	5.3
			275	1.7	5.3
				1.7[3]	5.3
				1.7[4]	5.3
			320	1.7	5.2
				1.7[3]	5.2
				1.7[4]	5.2
8.	R_2S_1	2.0 mgs.iron citrate per liter	1	--	5.5
			4	.08	5.4
			24	.2	5.4
			56	.28	5.3
			102	.17	5.1
			155	.2	5.5
				.06[3]	5.5
			208	< .05	5.4
			424	< .05	5.4

1 = ferrous
2 = beginning to appear colloidal.
3 = filtered
4 = filtered, ferrous

TABLE VIII (Con't.)

No.	Solution Tested	Iron added	Time since addition of iron. Hrs.	Iron found p.p.m.	Approximate pH
9.	R_3C_1	2.0 mgs.iron citrate per liter.	1	--	5.5
			4	.2	5.4
			24	.28	5.4
			50	.25	5.4
			102	.08	5.1
			155	< .05	5.5
			208	< .05	5.5
			424	< .05	5.4
10.	R_5C_2	2.0 mgs.iron citrate per litre.	1	--	5.1
			4	.22	5.1
			24	.25	5.1
			56	.25	5.1
			102	.20	5.3
			155	.12	5.3
			208	.06	5.1
			424	< .05	5.1
11.	$T_1R_1C_5$	2.0 mgs.iron citrate per liter.	1	--	6.3
			4	.25	5.1
			24	.25	5.2
			56	.25	5.3
			102	.14	5.4
			154	.06	5.4
			208	< .05	5.4
			424	< .05	5.4
12.	$T_1R_1C_1$	2.0 mgs.iron citrate per liter.	1	--	6.5
			4	.20	5.3
			24	.23	5.2
			56	.18	5.3
			102	.11	5.5
			154	.05	5.5
			208	< .05	5.5
			424	< .05	5.5
13.	$T_1R_1C_1$ modified	2.0 mgs.iron citrate per liter.	1	--	6.7
			4	.14	5.4
			24	.20	5.3
			56	.20	5.4
			102	.17	5.4
			154	.06	5.4
			208	< .05	5.4
			424	< .05	5.5
14.	R_2S_1	1.2 mgs.ferric phosphate per liter.	2	.11	5.3
			24	.05	5.3
			48	< .05	5.3

TABLE VIII (Con't.)

No.	Solution Tested	Iron added	Time since addition of iron, Hrs.	Iron found p.p.m.	Approximate pH
15.	R_3C_1	1.2 mgs. ferric phoephate per liter.	2	.14	5.3
			24	.05	5.3
			48	< .05	5.3
16.	R_5C_2	1.2 mgs. ferric phosphate per liter.	2	.18	5.1
			24	.06	5.1
			48	.05	5.1
17.	$T_1R_1C_5$	1.2 mgs. ferric phoephate per liter.	2	.11	5.3
			24	.05	5.2
			48	< .05	5.3
18.	$T_1R_1C_1$	1.2 mgs. ferric phosphate per liter.	2	.18	5.4
			24	.06	5.3
			48	< .05	5.3
19.	$T_1R_1C_1$ modified	1.2 mgs. ferric phosphate per liter.	2	.13	5.4
			24	< .05	5.3
			48	< .05	5.4

during this time, and subsequent decrease, and from data given in following tests it seems possible that such a maximum occurs within this time. We shall refer to this condition later, when considering tests 15 to 20.

Test 4 is a preliminary test with 1.2 mgs. ferric phosphate per liter of R_5C_2. This is included primarily for comparison with tests 15 to 20, to show the danger in reading the first sets for accuracy. It takes some time to accustom the eye to the faint tints of color shown by .05 p.p.m. or less. Test 5 is one run early in the work and the accuracy of the readings seems doubtful. We shall consider it later in connection with tests 15 to 20. It is a preliminary test with .01 gm. ferric phosphate per liter of distilled water. Here there is a quickly dropping solubility curve, which is later found to be characteristic of ferric phosphate. The highest reading is .1 p.p.m. of iron at the end of one hour; at the 53-hour interval the iron has dropped to .07 p.p.m. and steadily decreases to less than .05 p.p.m. at the end of 205 hours (8.5 days).

In test 6 (is) are data on a solution of .01 gm. ferric citrate per liter, adjusted to pH 5.0 with citric acid. At the end of one hour iron is in solution in a concentration of .1 p.p.m., and at the end of 50 hours has reached a concentration of 2.5 p.p.m. where it stays until the 98-hour period, at least, unless it rises to a higher maximum during this interval and again drops to 2.5 p.p.m. at the 98-hour period. After this time, however, the iron goes out of solution steadily, until at the 300-hour period we have only .4 p.p.m., and less than .05 p.p.m. at the 355-hour interval and subsequently.

Beginning with the 146 hour reading the solution begins to appear cloudy. At this time 2.0 p.p.m. iron is found in the unfiltered solution, but only 1.0 p.p.m. in the filtered solution, of which .9 p.p.m. is in the ferrous state. Half of the iron of the unfiltered portion is in suspension, therefore; and nearly all of the iron in solution is in the ferrous form. At the end of 202 hours the total iron in the unfiltered portion is .55 p.p.m., of which .5 p.p.m. remains in the filtered portion; hence very little is now in suspension. Only .2 p.p.m., or less than half, of the iron in the filtered portion is in the ferrous condition. The amount of iron in the unfiltered solution has increased slightly at the end of 250 hours, but this may be due to slight error in reading, since there is an appreciable decrease in the amount of iron (.13 p.p.m.) in the filtered solution, and none is left in the ferrous form. However, at the end of 300 hours the total iron has decreased to .4 p.p.m., only .1 p.p.m. of which remains after filtering, and .05 p.p.m. of this is in the ferrous form. The next reading, at the 355-hour period, shows less than .05 p.p.m. and no attempt is made to read below this, so no data is available as to solubility and state of oxidation of this iron.

Test 7 is the nutrient solution R_2S_1, with pH adjusted to 5.0 with phosphoric acid, and with 0.01 gm. per liter iron citrate added. At the end of one hour less than .05 p.p.m. of iron is in solution, but by the end of the 18-hour interval the amount has increased to .25 p.p.m., and at the end of 68 hours we find 2.5 p.p.m., of which 2.4 p.p.m. is in the ferrous condition. Either this is a maximum or the maximum occurs between this reading and one of the two

adjacent ones, for by the end of 118 hours the total iron has dropped to 2.0 p.p.m., with only 1.9 p.p.m. in the ferrous form. This condition still holds at the end of 166 hours. But by the end of the 222-hour period total iron has dropped to 1.7 p.p.m., of which 1.6 p.p.m. is ferrous iron. The solution is beginning to appear cloudy, also; after filtering, only 1.3 p.p.m. iron is found, and of this 1.2 p.p.m. is in the ferrous form. At the 275-hour interval, 1.7 p.p.m. total iron is still present, all in true solution, and all in the ferrous form, and this holds until the 320-hour period, at least, after which the test is discontinued.

Tests 8 to 13, inclusive, are a summary of duplicate tests on the six nutrient solutions under investigation, to each of which 2.0 mgms. per liter iron citrate is added.

Test 8 shows R_2S_1 with 2.0 mgms. iron citrate per liter. At the end of 4 hours only .08 p.p.m. iron is present, which is increased to .2 p.p.m. at the end of 24 hours, and .28 p.p.m. at the end of 56 hours. This is near the maximum for at the 102-hour interval we find only .17 p.p.m. iron in solution. There is either a negative error in reading or a peculiar secondary maximum near the 155-hour interval when .2 p.p.m. is found in solution. Of this, however, only .06 p.p.m. is in true solution, and remains after filtering. By the end of 208 hours the iron content has dropped to less than .05 p.p.m.

Test 9 shows R_5C_1 with 2.0 mgs. iron citrate per liter. The iron goes into solution slightly more rapidly than in the last test, and .2 p.p.m. and .28 p.p.m. iron is in solution at the end of 4 and 24 hours respectively. By the end of 56 hours the iron content has dropped to .25 p.p.m., and at 155 hours is less than .05 p.p.m.

Test 10 shows that R_5C_2 with 2.0 mgms. iron citrate per liter has a maximum iron content at some time between 24 and 56 hours, since at both these intervals .25 p.p.m. iron is found. From this time the amount of iron in solution drops, until at the 421-hour interval less than .05 p.p.m. is present.

Test 11 shows the result of adding 2.0 mgms. iron citrate to one liter of $T_1R_1C_5$. By the end of 4 hours there is .25 p.p.m. iron present and the iron appears to stay for some time at this concentration, for the same results are obtained at the end of 24 and 56 hours; but at the end of 102 hours only .14 p.p.m. is present, and the iron in solution drops rapidly to .06 p.p.m. at 154 hours, and to less than .05 p.p.m. at the end of 208 hours and subsequently.

Test 12 shows $T_1R_1C_1$ with 2.0 mgms. iron citrate per liter. At the end of 4 hours .2 p.p.m. iron is already in solution, and by the end of 24 hours the concentration of iron in solution has risen to .23 p.p.m. By the end of the 56-hour interval the iron drops to .13 p.p.m., and then steadily decreases to .05 p.p.m. at the 154-hour period, and finally to less than .05 p.p.m. after 208 hours.

Tests 14 to 19, inclusive, show the same solutions, but with iron supplied as ferric phosphate in place of iron citrate. 1.2 mgms. ferric phosphate contains an amount of iron equivalent to that contained in 2.0 mgms. iron citrate. Duplicate sets of these solutions show very close results.

Tests 14 shows R_2S_1 with 1.2 mgms. ferric phosphate per liter. A maximum value of .11 p.p.m. iron in solution is found within 2 hours, and the iron steadily decreases to .05 p.p.m. in 24 hours, and less than .05 p.p.m. at the end of 48 hours.

Test 15 shows R_3C_1 with 1.2 mgms. ferric phosphate per liter. This shows more soluble iron than R_2S_1, for at the end of two hours .14 p.p.m. iron is in solution, but by the end of 24 hours the soluble iron has decreased to .05 p.p.m., and at the end of 48 hours to less than .05 p.p.m.

Test 16 shows R_5C_2 with 1,2 mgms. ferric phosphate per liter. At the end of two hours .18 p.p.m. iron is in solution, but by the end of 24 hours this has decreased to .06 p.p.m. and by the end of the 48-hour period only .05 p.p.m. iron remains in solution.

Test 17 shows $T_1R_1C_5$ with 1.2 mgms. ferric phosphate per liter. At the end of two hours .11 p.p.m. iron is found in solution; the value then decreases rapidly until at the end of 48-hours less than .05 p.p.m. is found.

Test 18 shows $T_1R_1C_1$ with 1.2 mgms. ferric phosphate per liter. The two hour maximum is .18 p.p.m iron in solution. At the end of 24 hours only .06 p.p.m. remains in solution, and this decreases to less than .05 p.p.m. within 48 hours.

Test 19 shows modified $T_1R_1C_1$ with 1.2 mgms. ferric phosphate per liter. At the end of two hours a maximum of .13 p.p.m. soluble iron is obtained; but this value decreases to less than .05 p.p.m. at the end of 24 hours.

Let us now consider Tests 8 to 13 inclusive; these are the six nutrient solutions, with iron citrate as the source of iron. In every case there is a maximum amount of iron in solution with 24 to 56 hours, while after this time the amount of iron in solution decreases comparatively rapidly, and at the end of 424 hours (17.8 days) less than .05 p.p.m. iron is in solution.

Using either the iron citrate or ferric phosphate as a source of iron in the nutrient solutions tested, a maximum amount of iron in solution is observed, and from this maximum the amount decreases comparatively rapidly. However, the ferric phosphate shows a lower and earlier maximum, and a correspondingly earlier withdrawal of most of the iron from solution.

(b) By the Compensating Method.---The use of iron citrate as a source of iron is now discontinued, and ferric phosphate is used entirely. This is done in order to limit the field. At this time, also, a study of the separate components of the nutrient solutions is started; single salt solutions are under observation in order to determine the independent influence of each upon the solubility of iron. In order to study the effect of interaction of the components upon the amount of iron in solution, double salt solutions are also used. The molar ratio of the salts in solution is also varied, in an effort to determine the part played by either salt in the double salt solution. The data from these tests are presented in Tables IX and X.

If Table IX is considered, it is observed that the solution containing calcium dihydrogen phosphate shows the largest amount of iron in solution, both in the series with 2.4 mgms. ferric phosphate per liter and in the series with 0.6 mgms. ferric phosphate per liter. In neither case does the calcium dihydrogen phosphate solution show less than .05 p.p.m. iron in solution within 48 hours. This is perhaps most remarkable since the calcium dihydrogen phosphate solution is .001 molar whereas the other solutions are .01 molar, or ten times as concentrated. The probable explanation of this will be discussed in a later section.

TABLE IX

Data Obtained by Compensating Method

Single Salt Solutions.

No.	Solution Tested	Time since addition of iron Hrs.	24 mgm. Ferric phosphate per liter. Iron found p.p.m.	pH	0.6 mgm Ferric phosphate per liter. Iron found p.p.m.	pH	No iron added pH
1.	10 cc. M/1 KH_2PO_4 per liter.	4	< .05	4.0	< .05	4.0	4.0
		24	< .05	4.0	< .05	4.0	4.0
		48	< .05	4.0	m< .05	4.0	4.0
2.	10 cc. M/10 $CaH_4P_2O_8$ per liter.	3	.05	3.3+	.05	3.3+	3.3+
		24	.06⁻	3.3+	.05	3.3+	3.3+
		48	.06⁻	3.3+	.05	3.3+	3.3+
3.	10 cc. M/1 KNO_3 per liter.	3	.05	6.5	< .05	6.5	6.6
		24	< .05	6.1	m< .05	6.2	6.6
		48	m< .05	5.9	m< .05	6.0	6.6
4.	10 cc. M/1 $Ca(NO_3)_2$ per liter.	3	sl< .05	6.6	< .05	6.6	6.6
		24	< .05	6.1	m< .05	6.1	6.6
		48	m< .05	5.9	m< .05	5.9	6.6
5.	10 cc. M/1 $MgSO_4$ per liter.	3	< .05	6.6	< .05	6.6	6.6
		24	< .05	6.2	m< .05	6.2	6.6
		48	m< .05	6.0	m< .05	6.0	6.6
6.	10 cc. M/1 $(NH_4)_2SO_4$ per liter.	3	.05	6.0	< .05	6.0	6.0
		24	< .05	5.8	m< .05	5.8	6.0
		48	m< .05	5.6	m< .05	5.6	6.0

Note: sl.< means slightly less than.
< " less than.
m.< " much less than.
These indicate approximate degrees of intensity below the range of accurate readings.

TABLE X

Data Obtained by Compensating Method

Double Salt Solutions.

No.	Solution Tested	Time since addition of iron Hrs.	24 mgm. Ferric phosphate per liter. Iron found p.p.m.	pH	0.6 mgm. Ferric phosphate per liter. Iron found p.p.m.	pH	No iron added pH
1.	20 cc. M/1 KH_2PO_4 and 20 cc. M/10 $CaH_4P_2O_8$ per liter.	3	.05	3.5	.05	3.5	3.5
		24	.05+	3.5	.05	3.5	3.5
		48	.05	3.5	.05	3.5	3.5
2.	10 cc. M/1 KH_2PO_4 and 20 cc. M/10 $CaH_4P_2O_8$ per liter	3	.06	3.4	.06	3.4	3.4
		24	.07	3.4	.07	3.4	3.4
		48	.06	3.4	.05	3.4	3.4
3.	20 cc. M/1 KH_2PO_4 and 10 cc. M/10 $CaH_4P_2O_8$ per liter.	3	.05	3.5+	.05	3.5+	3.5+
		24	.05	3.5+	sl<.05	3.5+	3.5+
		48	< .05	3.5+	< .05	3.5+	3.5+
4.	20 cc. M/1 KH_2PO_4 and 20 cc. M/1 KNO_3 per liter.	3	.05	4.1-	sl<.05	4.1-	4.1-
		24	sl<.05	4.1-	<.05	4.1-	4.1-
		48	<.05	4.1-	m<.05	4.1-	4.1-
5.	10 cc. M/1 KH_2PO_4 and 20 cc. M/1 KNO_3 per liter.	3	<.05	4.1+	<.05	4.1+	4.1+
		24	<.05	4.1+	<.05	4.1+	4.1+
		48	m<.05	4.1+	m<.05	4.1+	4.1+
6.	20 cc. M/1 KH_2PO_4 and 10 cc. M/1 KNO_3 per liter.	3	.05	4.0-	.05	4.0-	4.0-
		24	sl<.05	4.0-	sl<.05	4.0-	4.0-
		48	sl<.05	4.0-	<.05	4.0-	4.0-
7.	20 cc. M/1 KH_2PO_4 and 20 cc. M/1 $Ca(NO_3)_2$ per liter.	3	.05	4.1⁻	.05	4.1⁻	4.1⁻
		24	sl<.05	4.1⁻	sl<.05	4.1⁻	4.1⁻
		48	sl<.05	4.1⁻	sl<.05	4.1⁻	4.1⁻

TABLE X (Con't.)

No.	Solution Tested	Time since addition of iron Hrs.	24 mgm. Ferric phosphate per liter. Iron found p.p.m.	pH	0.6 mgm. Ferric phosphate per liter. Iron found p.p.m.	pH	No iron added pH
8.	10 cc. M/1 KH_2PO_4 and 20 cc. M/1 $Ca(NO_3)_2$ per liter.	3	.05	4.2	sl.<.05	4.2	4.2
		24	sl.<.05	4.2	<.05	4.2	4.2
		48	<.05	4.2	<.05	4.2	4.2
9.	20 cc. M/1 KH_2PO_4 and 10 cc. M/1 $Ca(NO_3)_2$ per liter.	3	.05	4.1-	.05	4.1-	4.1-
		24	sl.<.05	4.1-	sl.<.05	4.1-	4.1-
		48	<.05	4.1-	<.05	4.1-	4.1-
10.	20 cc. M/1 KH_2PO_4 and 20 cc. M/1 $MgSO_4$ per liter.	3	.05	4.1	.05	4.1	4.1
		24	sl.<.05	4.1	sl.<.05	4.1	4.1
		48	<.05	4.1	<.05	4.1	4.1
11.	10 cc. M/1 KH_2PO_4 and 20 cc. M/1 $MgSO_4$ per liter.	3	.05	4.2+	sl.<.05	4.2+	4.2+
		24	sl.<.05	4.2+	<.05	4.2+	4.2+
		48	<.05	4.2+	<.05	4.2+	4.2+
12.	20 cc. M/1 KH_2PO_4 and 10 cc. M/1 $MgSO_4$ per liter.	3	sl.<.05	4.0+	sl.<.05	4.0+	4.0+
		24	<.05	4.0+	<.05	4.0+	4.0+
		48	<.05	4.0+	<.05	4.0+	4.0+
13.	20 cc. M/1 KH_2PO_4 and 20 cc. M/1 $(NH_4)_2SO_4$ per liter.	2	sl.<.05	4.1	sl.<.05	4.1	4.1
		24	sl.<.05	4.1	sl<.05	4.1	4.1
		48	<.05	4.1	<.05	4.1	4.1
14.	10 cc. M/1 KH_2PO_4 and 20 cc. M/1 $(NH_4)_2 SO_4$ per liter.	2	sl.<.05	4.3+	<.05	4.3+	4.3+
		24	<.05	4.3+	<.05	4.3+	4.3+
		48	<.05	4.3+	m<.05	4.3+	4.3+
15.	20 cc. M/1 KH_2PO_4 and 10 cc. M/1 $(NH_4)_2SO_4$ per liter.	2	.05	4.0+	.05	4.0+	4.0+
		24	.05	4.0+	sl.<.05	4.0+	4.0+
		48	sl.<.05	4.0+	sl.<.05	4.0+	4.0+

TABLE X (Con't)

No.	Solution Tested	Time since addition of iron Hrs.	24 mgm. Ferric phosphate per liter. Iron found p.p.m.	pH	0.6 mgm. Ferric phosphate per liter. Iron found p.p.m.	pH	No iron added pH
16.	20 cc. M/10 $CaH_4P_2O_8$ and 20 cc. M/1 KNO_3 per liter.	2	sl.<.05	3.2	sl.<.05	3.2	3.2
		24	sl<.05	3.2	sl<.05	3.2	3.2
		48	<.05	3.2	<.05	3.2	3.2
17.	10 cc. M/10 $CaH_4P_2O_8$ and 20 cc. M/1 KNO_3 per liter.	2	.05	3.2	sl.<.05	3.2	3.2
		24	sl.<.05	3.2	<.05	3.2	3.2
		48	<.05	3.2	<.05	3.2	3.2
18.	20 cc. M/10 $CaH_4P_2O_8$ and 10 cc. M/1 KNO_3 per liter.	2	.05+	3.2	.05	3.2	3.2
		24	.05	3.2	sl.<.05	3.2	3.2
		48	sl.<.05	3.2	<.05	3.2	3.2
19.	20 cc. M/10 $CaH_4P_2O_8$ and 20 cc. M/1 $Ca(NO_3)_2$ per liter.	4	.05	3.3 -	.05	3.3 -	3.3 -
		25	.10	3.3 -	.08	3.3 -	3.3 -
		48	.07	3.3 -	.07	3.3 -	3.3 -
20.	10 cc. M/10 $CaH_4P_2O_8$ and 20 cc. M/1 $Ca(NO_3)_2$ per liter.	4	.05	3.4	sl.<.05	3.4	3.4
		25	.07	3.4	.06	3.4	3.4
		48	.07	3.4	.08	3.4	3.4
21.	20 cc. M/10 $CaH_4P_2O_8$ and 10 cc. M/1 $Ca(NO_3)_2$ per liter.	4	.06	3.3	.05	3.3	3.3
		25	.06	3.3	.05	3.3	3.3
		48	.08	3.3	.08	3.3	3.3
22.	20 cc. M/10 $CaH_4P_2O_8$ and 20 cc. M/1 $MgSO_4$ per liter.	3	.05	3.2+	.05	3.2+	3.2+
		25	.07	3.2+	.06	3.2+	3.2+
		46	.09	3.2+	.05	3.2+	3.2+
23.	10 cc. M/10 $CaH_4P_2O_8$ and 20 cc. M/1 $MgSO_4$ per liter.	3	sl.<.05	3.2+	sl.<.05	3.2+	3.2+
		25	.05	3.2+	.05	3.2+	3.2+
		46	.05	3.2+	sl.<.05	3.2+	3.2+

TABLE X (Con't)

No.	Solution Tested	Time since addition of iron Hrs.	24 mgm. Ferric phosphate per liter. Iron found p.p.m.	pH	0.6 mgm. Ferric phosphate per liter. Iron found p.p.m.	pH	No iron added pH
24.	20 cc. M/10 $CaH_4P_2O_8$ and 10 cc. M/1 $MgSO_4$ per liter.	3	.05	3.3	sl.< .05	3.3	3.3
		25	.07	3.3	.05	3.3	3.3
		46	.08	3.3	sl.< .05	3.3	3.3
25.	20 cc. M/10 $CaH_4P_2O_8$ and 20 cc. M/1 $(NH_4)_2SO_4$ per liter.	3	.06	3.3+	.06	3.3+	3.3+
		24	.10	3.3+	.07	3.3+	3.3+
		48	.09	3.3+	.06	3.3+	3.3+
26.	10 cc. M/10 $CaH_4P_2O_8$ and 20 cc. M/1 $(NH_4)_2SO_4$ per liter.	3	.05	3.4+	sl.< .05	3.4+	3.4+
		24	.06	3.4+	.06	3.4+	3.4+
		48	.05	3.4+	.05	3.4+	3.4+
27.	20 cc. M/10 $CaH_4P_2O_8$ and 10 cc. M/1 $(NH_4)_2SO_4$ per liter.	3	.05	3.3+	.05	3.3+	3.3+
		24	.10	3.3+	.07	3.3+	3.3+
		48	.09	3.3+	.07	3.3+	3.3+
28.	20 cc. M/1 KNO_3 and 20 cc. M/1 $Ca(NO_3)_2$ per liter.	3	< .05	6.6+	< .05	6.6+	6.7
		24	< .05	6.3	< .05	6.3	6.7
		48	< .05	6.2	m.< .05	6.2	6.7
29.	10 cc. M/1 KNO_3 and 20 cc. M/1 $Ca(NO_3)_2$ per liter.	3	sl.< .05	6.7	< .05	6.7	6.7
		24	< .05	6.2	< .05	6.2	6.7
		48	m.< .05	6.2	m.< .05	6.2	6.7
30.	20 cc. M/1 KNO_3 and 10 cc. M/1 $Ca(NO_3)_2$ per liter.	3	< .05	6.6	< .05	6.6	6.7
		24	m.< .05	6.2	m.< .05	6.5	6.7
		48	m.< .05	6.2	m.< .05	6.2	6.7
31.	20 cc. M/1 KNO_3 and 20 cc. M/1 $MgSO_4$ per liter.	3	.05	6.6	sl.< .05	6.7	6.8
		24	< .05	6.4	< .05	6.4	6.8
		48	m.< .05	6.3	m.< .05	6.4	6.8

TABLE X (Con't)

No.	Solution Tested	Time since addition of iron Hrs.	24 mgm. Ferric phosphate per liter. Iron found p.p.m.	pH	0.6 mgm. Ferric phoephate per liter. Iron found p.p.m.	pH	No iron added pH
32.	10 cc. M/1 KNO_3 and 20 cc. M/1 $MgSO_4$ per liter.	3	sl.< .05	6.7	sl.< .05	6.7	6.7
		24	< .05	6.4	< .05	6.4	6.7
		48	m.< .05	6.3	m.< .05	6.3	6.7
33.	20 cc. M/1 KNO_3 and 10 cc. M/1 $MgSO_4$ per liter.	4	< .05	6.7	< .05	6.7	6.8
		24	< .05	6.4	< .05	6.4	6.8
		48	m.< .05	6.2	m.< .05	6.2	6.8
34.	20 cc. M/1 KNO_3 and 20 cc. M/1 $(NH_4)_2SO_4$ per liter.	3	< .05	6.0	< .05	6.0	6.0
		25	< .05	5.8	m.< .05	5.8	6.0
		49	m.< .05	5.7	m.< .05	5.7	6.0
35.	10 cc. M/1 KNO_3 and 20 cc. M/1 $(NH_4)_2SO_4$ per liter.	3	< .05	5.9	< .05	6.0	6.0
		25	< .05	5.8	m.< .05	5.9	6.0
		49	m.< .05	5.7	m.< .05	5.7	6.0
36.	20 cc. M/1 KNO_3 and 10 cc. M/1 $(NH_4)_2SO_4$ per liter.	3	sl.< .05	6.0	sl.< .05	6.0	6.0
		25	m.< .05	5.9	m.< .05	5.9	6.0
		49	m.< .05	5.7	m.< .05	5.8	6.0
37.	20 cc. M/1 $Ca(NO_3)_2$ and 20 cc. M/1 $MgSO_4$ per liter.	2	sl.< .05	6.8	sl.< .05	6.8	6.8
		24	< .05	6.5	< .05	6.5	6.8
		48	m.< .05	6.2	m.< .05	6.3	6.8
38.	10 cc. M/1 $Ca(NO_3)_2$ and 20 cc. M/1 $MgSO_4$ per liter.	2	sl.< .05	6.7	sl.< .05	6.7	6.8
		24	< .05	6.2	< .05	6.4	6.8
		48	m.< .05	6.0	m.< .05	6.1	6.8
39.	20 cc. M/1 $Ca(NO_3)_2$ and 10 cc. M/1 $MgSO_4$ per liter.	2	sl.< .05	6.7	< .05	6.7	6.7
		24	< .05	6.0	< .05	6.2	6.7
		48	m.< .05	6.0	m.< .05	6.0	6.7

TABLE X (Con't)

No.	Solution Tested	Time since addition of iron. Hrs.	24 mgm. Ferric phosphate per liter. Iron found p.p.m.	pH	0.6 mgm. Ferric phosphate per liter. Iron found p.p.m.	pH	No iron added pH
40.	20 cc. M/1 $Ca(NO_3)_2$ and 20 cc. M/1 $(NH_4)_2SO_4$ per liter.	3	sl.< .05	6.0	< .05	6.0	6.0
		24	< .05	5.9	< .05	5.9	6.0
		48	m.< .05	5.5+	m.< .05	5.8	6.0
41.	10 cc. M/1 $Ca(NO_3)_2$ and 20 cc. M/1 $(NH_4)_2SO_4$ per liter.	3	sl.< .05	6.0	< .05	6.0	6.0
		24	< .05	5.9	< .05	5.9	6.0
		48	m.< .05	5.6	m.< .05	5.8	6.0
42.	20 cc. M/1 $Ca(NO_3)_2$ and 10 cc. M/1 $(NH_4)_2SO_4$ per liter.	3	sl.< .05	6.0	sl.< .05	6.0	6.0
		24	sl.< .05	5.9	< .05	5.9	6.0
		48	< .05	5.8	m.< .05	5.8	6.0
43.	20 cc. M/1 $MgSO_4$ and 20 cc. M/1 $(NH_4)_2SO_4$ per liter.	3	.05	6.0	sl.< .05	6.0	6.0
		24	sl.< .05	5.8	< .05	5.9	6.0
		48	m.< .05	5.8	m.< .05	5.9	6.0
44.	10 cc. M/1 $MgSO_4$ and 20 cc. M/1 $(NH_4)_2SO_4$ per liter.	3	sl.< .05	6.0	< .05	6.0	6.0
		24	< .05	5.8+	< .05	5.9	6.0
		48	m.< .05	5.8	m.< .05	5.8	6.0
45.	20 cc. M/1 $MgSO_4$ and 10 cc. M/1 $(NH_4)_2SO_4$ per liter.	3	sl.< .05	6.0	< .05	6.0	6.0+
		24	< .05	5.9	< .05	5.9+	6.0+
		48	m.< .05	5.8	m.< .05	5.8^+	6.0^+

Potassium nitrate and ammonium sulfate ~~both~~ show an initial value of .05 p.p.m. iron in solution with 2.4 mgms. ferric phosphate per liter, but in both of these solutions the iron rapidly disappears, until at the end of two days there is practically none in solution. All other solutions tested, with either of the two amounts of ferric phosphate used, show less than .05 p.p.m. iron in solution at the time of the first test, and appear to lose quite rapidly even the small amount that may be in solution at that time.

If we turn now to consider the double salt solutions, recorded in Table X, we find similar results. The tests are arranged in groups of three; each group is a set of tests on two salts in the proportions 2 : 2, 1 : 2, 2 : 1. Solutions in these proportions are run with two amounts of iron: 2.4 mgms. ferric phosphate per liter, and 0.6 mgms. ferric phosphate per liter.

The first three sets are solutions of potassium dihydrogen phosphate and calcium dihydrogen phosphate. The difference in amount of ferric phosphate added appears to have no effect upon the amount of iron in solution. The only general observation one might make from this set of tests is that the greater the proportion of calcium dihydrogen phosphate to the potassium dihydrogen phosphate, the greater the amount of iron in solution.

Tests 4, 5 and 6 are carried out with solutions of potassium dihydrogen phosphate and potassium nitrate. Here, also, the amount of ferric phosphate added appears to have little influence on the amount of iron in solution. The potassium dihydrogen phosphate seems to increase the solubility of the iron more than does the potassium nitrate.

Solutions of potaseium dihydrogen phosphate and calcium nitrate are used in tests 7, 8 and 9. In all cases, with the exception of test 8, the initial amount of iron in solution is .05 p.p.m. The iron in solution decreases in amount, but not so rapidly as in the preceding tests. Varying the proportions of the two salts appears to have no effect upon the amount of iron in solution.

Tests, 10, 11 and 12 are carried out with solutions of potassium dihydrogen phosphate and magnesium sulfate. Equal amounts of the two salts in solution show an initial maximum value of .05 p.p.m. iron in solution with either amount of ferric phosphate added. The solutions containing twice as much magnesium sulfate as potassium dihydrogen phosphate show practically as much iron in solution ae the solutions of equal amounts of the two salts. However, when magneeium sulfate is present in only half the concentration of the potassium dihydrogen phoephate, there is less iron in solution. Hence, it appears that magnesium sulfate increases solubility of iron more than does potassium dihydrogen phosphate.

Solutions of potassium dihydrogen phosphate and ammonium sulfate are used in tests 13, 14 and 15. The amount of ferric phosphate added has no appreciable effect upon the amount of iron in solution. The iron in solution never amounts to more than .05 p.p.m., and this amount is present only in the initial stages of the solutione containing twice as much potassium dihydrogen phosphate as ammonium sulfate. From this it appears that potassium dihydrogen phosphate not only increaees solubility of iron more than does ammonium sulfate, but seems to keep the iron in solution for a longer period.

Tests 16, 17 and 18 are run on solutions of calcium dihydrogen phosphate and potassium nitrate. With the larger proportion of the phosphate, the iron in solution amounts to .05 p.p.m., and stays in solution longer than in other tests. There is no appreciable effect due to the different amounts of ferric phosphate added.

Solutions of calcium dihydrogen phosphate and calcium hitrate are used for tests 19, 20 and 21. These solutions show more iron in solution than any of those already discussed. The amount of iron in solution increases with time, but at the end of 48 hours in no case exceeds .08 p.p.m.; and in the solution with equal amounts of the salts, the amount of iron in solution has decreased from .10 p.p.m. at 25 hours to .07 p.p.m. at 48 hours, when 2.4 mgms. ferric phosphate is present, and from .08 p.p.m. at 25 hours to .07 p.p.m. at 48 hours in the solution containing 0.6 mgm. ferric phosphate.

Tests 22, 23 and 24 are made on solutions of calcium dihydrogen phosphate and magnesium sulfate. The maximum amount of iron in solution tends to occur at the 25 hour period, but with the full amount of the phosphate present, and with the larger amount of iron added, the maximum carries over to the 46-hour period. At no time, however, does the amount of iron in solution exceed .09 p.p.m.

Tests 25, 26 and 27 are made with solutions containing calcium dihydrogen phosphate and ammonium sulfate. In all cases the maximum occurs around the 24-hour period, and has a tendency to be slightly, although not proportionately, higher with the larger

amount of ferric phosphate present. Calcium dihydrogen phosphate appears to influence the solubility of iron more than does ammonium sulfate.

Tests 28, 29 and 30 are carried out with solutions containing potassium nitrate and calcium nitrate. There is little difference between the three proportions used; at all times less than .05 p.p.m. iron is in solution. The amount of ferric phosphate added does not appear to influence the amount of iron in solution.

Solutions containing potassium nitrate and magnesium sulfate are used in tests 31, 32 and 33. Equal amounts of the two salts in solution show an original maximum of .05 p.p.m. iron in solution, with 2.4 mgms. ferric phosphate added per liter; and only slightly less iron is in solution with the smaller amount of ferric phosphate added. The solution with twice as much magnesium sulfate as potassium nitrate shows less than .05 p.p.m. iron in solution at all times, but still contains more than the solution containing more potassium nitrate than magnesium sulfate.

Tests 34, 35 and 36 are made with solutions containing potassium nitrate and ammonium sulfate. The amount of iron in solution does not depend upon the amount of ferric phosphate added, or upon the proportions of the two salts used, and is always less than .05 p.p.m.

Solutions containing calcium nitrate and magnesium sulfate are used for tests 37, 38 and 39. Here, also, we find no apparent influence upon the solubility of iron shown by the proportions of the salts used,or by the amount of ferric phosphate added. The iron in solution is always less than .05 p.p.m.

Tests 40, 41 and 42 are made upon solutions containing calcium nitrate and ammonium sulfate. The one with the larger proportion of calcium nitrate appears to retain iron in solution for a longer time, but this is difficult to judge, since all the solutions show values less than .05 p.p.m. soluble iron. The amount of ferric phosphate present seems to show only a slight, if any, relation to the amount of iron in solution.

Tests 43, 44 and 45 are run on solutions containing magnesium sulfate and ammonium sulfate. The solutions containing the larger amount of ferric phosphate show a larger amount of iron in solution, but this difference is not proportional. Only tho 2 : 2 solution with the larger amount of ferric phosphate shows an initial maximum of .05 p.p.m. iron in solution. All other solutions show no value as high as .05 p.p.m. of iron.

If we consider collectivdly the tests recorded in Tables IX and X, we find that test 2 of Table IX and tests 2, 19, 20, 21, 22, 24, 25, 26 and 27 of Table X are the only ones showing any values higher than .05 p.p.m. iron in solution. Of these, test 2 of Table IX and, in Table X, tests 20, 21, 24 and the portion of 22 containing the larger amount of ferric phosphate, show at 48 houre an amount of iron in solution as high as, or higher than, any previous value; while tests 2, 19, 25, 26, 27 and the portion of 22 containing less ferric phosphate, show less iron in solution at 48 hours than at 24 hours. Even in these no amount of iron in solution greater than .10 p.p.m. is recorded. For the tests when no value greater than .05 p.p.m. iron in solution is obtained, the small amount of iron in solution a few hours after the addition of the ferric phosphate has diminished at the end of the 24-hour period and has ueually become negligible at the end of two days.

In general, the time curves shown by tests 14 to 19, inclusive, of Table XIII are very similar to the curves which are exhibited by the tests shown in Tables IX and X.

(c) Conclusions.---Louwsma (62a) notices that the maximum solubility of iron in nutrient solutions is reached in 8 hours, or slightly later, and may continue for three days or less, depending on the nature of the solution. He also observes that when iron citrate and ferric phosphate are added in equivalent amounts to two samples of the same solution, the sample containing the iron citrate shows more iron in solution, and for a longer period, than does the sample containing the ferric phosphate.

This investigation shows these same tendencies. The tests run with iron citrate show a high maximum, and a relatively large amount of iron in solution for several days. We also notice a tendency for the iron in these tests to go into a state of suspension.

The tendency of tests run with ferric phosphate as the source of iron is to exhibit a relatively low maximum, which may occur within a few hours or which may be delayed for a day or more, depending upon the nature of the solution, and upon the solubility of ferric phosphate in it.

Since only two salts have been used as the source of iron, no general statement can be made, but it seems probable from the evidence given that the time-curve of iron solubility depends upon two factors, (1) the nature of the compound used as a source of iron, and (2) the composition of the solution into which the iron is introduced.

2. The Relation Between pH and Iron Solubility in the Component Salts of Nutrient Solutions.

(a) Theory.---The work of Meehan and Baas-Becking (74) has already been mentioned. These workers show that ferric iron is soluble at pH less than 5.0, while ferrous iron becomes soluble at pH less than 6.2. At pH greater than 6.2, then, it might be expected that no iron would be found in solution, and at pH greater than 5.0 and less than 6.2 only ferrous iron might be expected. Below pH 5.0 both ferrous and ferric iron might be found.

Halvorson and Starkey (36) believe that "at reactions more alkaline than pH 5.0 very small amounts of ferrous iron will occur in solution under atmospheric conditions and even smaller amounts of ferric iron are soluble".

Patten and Mains (85) find the highest pH at which iron is held in solution when hydrogen sulfide gas is passed through it is pH 3.3. This indicates that the ferric condition cannot exist in solution above pH 3.3.

Marsh (64) finds some relation between the pH of the nutrient solution and the amount of iron in solution. For the cases where ferric glycerophosphate is used as the source of iron in solutions $T_1R_1C_5$, modified $T_1R_1C_5$, and R_5S_1, he finds more iron in solution at pH 5.5 than at pH 4.6 or 6.2, and the amount of iron in solution at pH 4.6 is equal to that in solution at pH 6.2. When soluble ferric phosphate, ferric tartrate or ferrous sulfate is used as the source of iron, in the same solutions the amount of iron in solution at pH 4.6 is less than at 5.5 but still greater than at 6.2. The writer's comment is that if the pH of the solution were the only factor there should be a larger

amount of iron in solution at pH 4.6 than at pH. 5.5; and, since this is not the case, there must be some other factor, which is doubtless inter-related with the pH. This idea will be more fully developed later.

It might be expected, however, that an increase in soluble iron content with decrease in pH would be observed. The decrease in pH may be brought about by addition of acid to increase the hydrogen ion concentration, or by interaction of the component salts of the nutrient solution.

One way to increase acidity by formation of acid within the system would be to maintain the solution in an atmosphere of carbon dioxide, which would form carbonic acid by combining with the water. Reed and Haas (91) find that the pH of slightly acid, neutral, or alkaline solutions may be lowered by the introduction of carbon dioxide, but they fail to find any effect upon the solubility of iron compounds in the solutions.

Truog (110) says that the addition of carbonic acid to water containing iron phosphate precipitate has little effect upon the solubility of the phosphate because ferric carbonate is not formed under ordinary conditions.

Buytendijk, Brinkman and Mook (17) refer to the work of Faurholt who finds two steps for the hydration of carbon dioxide:

$$CO_2 + H_2O \dashrightarrow H_2CO_3, \qquad \frac{d(CO_2)}{dt} = -K_1(CO_2)$$

$$CO_2 + OH \dashrightarrow H\bar{C}O_3, \qquad \frac{d(CO_2)}{dt} = K_2(\bar{O}H)(CO_2)$$

Since both processes are occurring simultaneously, the hydration is determined by

$$\frac{d(CO_2)}{dt} = -(K_1 + K_2(\bar{O}H))(CO_2)$$

If the pH is less than 8, $(\bar{O}H)$ is so small that the reaction has practically the type

$$CO_2 + H_2O \longrightarrow H_2CO_3 ,$$

but if the pH is greater than 10, the second reaction,

$$CO_2 + \bar{O}H \longrightarrow HCO_3^- , \text{ predominates.}$$

The dehydration of carbonic acid proceeds in two steps:

$$H_2CO_3 \longrightarrow H_2O + CO_2; \quad \frac{d(H_2CO_3)}{dt} = -K_3(H_2CO_3)$$

$$HCO_3 \longrightarrow CO_2 + OH; \quad \frac{d(HCO_3)}{dt} = -K_4(HCO_3)$$

Buytendijk and his co-workers find the velocity of hydration is proportional to the hydrogen-ion concentration of the system. At pH 6, they find the dehydration constant of H_2CO_3 to be 1.73.

The dissociation constants are derived from the equations representing equilibrium conditions:

$$(H_2CO_3) \longrightarrow K_{hydration}(CO_2)$$

$$(\dot{H}) \times (H\dot{C}O_3^-) \longrightarrow K_{H_2CO_3}(H_2CO_3)$$

$$(\dot{H}) \times (CO_3^{=}) \longrightarrow K_{HCO_3^-}(HCO_3^-)$$

Buytendijk, Brinkman and Mook give the second dissociation constant as 6×10^{-11} at 14°. Hastings and Sendroy (42) give it a value of 6.03×10^{-11} at 38°, and give 4.68×10^{-7} at 38° for the first dissociation constant. Klemenc and Herzog (57) find values for the first dissociation constant of 2.54×10^{-7} at 0° and 2.65×10^{-7} at 12.5°, and cite other values, 3.10×10^{-7} at 18° (Kendall), 3.04×10^{-7} at 18° (Walker and Cromack) and 3.50×10^{-7} at 25° (Kendall).

Klarmann (56) states that water containing carbon dioxide will dissolve iron to form a supersaturated solution of ferrous acid carbonate, $Fe(HCO_3)_2$, and that this salt then depresses the ionization of carbonic acid sufficiently to make the rate of solution of iron inappreciable, and hence a state of apparent, but not true, equilibrium results.

Phosphates are present in the nutrient solutions. By hydrolysis we may expect them to give phosphoric acid. Kolthoff (59) gives the first dissociation constant of orthophosphoric acid as $8.5 - 9.5 \times 10^{-3}$ at 18°, and the third as 5×10^{-13} at 18°. Cohn (21) gives 0.69×10^{-7} for the s second dissociation constant.

The influence of ammonium sulfate on hydrogen-ion concentration is discussed by Jones and Shive (53). They believe that the lowering of the pH of the solution by the ammonium sulfate added leads to increased solubility of iron.

Any other salt used in a nutrient solution which might be expected to lower the pH of that solution, might also be expected to lead to an increase in the amount of iron in solution.

(b) Data.---From the theoretical considerations it might be expected that a decrease of pH would be accompanied by an increase in the amount of iron in solution. An examination of the data, however, shows a surprising situation. In all the tests, the amount of iron in solution decreases as the pH decreases.

The results given in Table VIII are not important since the indicators used and the method of comparison for pH were not standard. Even here, however, it is noticed that while Tests 1, 2 and 11 show decrease in amount of iron in solution with practically no change in pH, and test 5 shows a decrease in amount of iron present with a decrease in pH.

The tests in Tables IX and X show accurate pH determinations, carried out with the LaMotte indicators and standards already described.

For Table IX, tests 1 and 2 show constant pH values; these are phosphate solutions, and therefore are buffered against small changes in pH. Test 3 shows a decrease of pH from 6.5 at 3 hours to 5.9 at 48 hours, with corresponding readings of .05 and much less than .05 p.p.m iron in solution. Tests 4, 5 and 6 show corresponding decrease in amount of iron in solution with decrease in pH value. However, it is evident that there is some relation between the decrease in pH and the presence of iron, since the control solution containing no iron maintains the original pH throughout the experiment. The ammonium sulfate, though, having pH 6.0, which is lower than the pH 6.6 of potassium nitrate, calcium nitrate and magnesium sulfate, does not appear to have a pH sufficiently low to bring the iron from ferric phosphate into solution, since with 2.4 mgms. ferric phosphate per liter the initial amount of iron in solution is only .05 p.p.m., while with 0.6 mgm. ferric phosphate per liter the initial concentration is less than .05 p.p.m.; and although the pH of both solutions containing ferric phosphate decreases to 5.6 within 48 hours, the iron in solution also decreases in amount during the same time, so that at the end of the 48-hour period both solutions contain much less than .05 p.p.m.

It is true that calcium dihydrogen phosphate, with pH 3.3^{+}, shows more iron in solution than any of the other single salt solutions. But it is also true that ammonium sulfate, pH 6.0, and potassium nitrate, pH 6.6, show more iron in solution than potassium dihydrogen phosphate, pH 4.0. The solubility of iron in the calcium dihydrogen phosphate solution cannot, therefore, be entirely attributed to the low pH value.

In Table X it is noticed that tests 1 to 27, inclusive, show no evidence of any change in pH; all these tests are run on eolutions containing either potassium dihydrogen phosphate or calcium dihydrogen phosphate, and either of these salts has a strong buffer action.

Tests 28, 29 and 30 are made on solutions containing potassium nitrate and calcium nitrate. The controls maintain pH 6.7 throughout the experiment, but all solutions containing ferric phosphate show a decrease in pH, and reach pH 6.2 within 48 hours; at the same time there is a decrease in the amount of iron in solution.

Tests 31, 32 and 33, made with solutions containing potassium nitrate and magnesium sulfate, show no change in the pH of the controls, but do show decrease in pH accompanied by decrease in amount of iron in solution when ferric phosphate is present.

Tests 34, 35 and 36 are carried out on solutions containing potassium nitrate and ammonium sulfate. Here, also, is found no change in pH when iron is not present; but when iron is present as ferric phosphate, the solution shows decrease in pH from 6.0 to 5.7 within 48 hours, with decrease in the amount of iron present in solution during the same time.

Tests 37, 38 and 39, using solutions containing calcium nitrate and magnesium sulfate, tests 40, 41 and 42, using solutions containing calcium nitrate and ammonium sulfate, and tests 43, 44 and 45, made on solutions containing magnesium sulfate and ammonium sulfate show the same results. In all cases the control solutions maintain a constant pH, while the solutions containing ferric phosphate show a decrease in pH accompanied by a decrease in the amount of iron in solution.

In the last section the possibility of an increased amount of iron in solution, due to the lowering of the pH by carbon dioxide from the air, was mentioned.

Table XI gives data on two portions of R_2S_1, each containing 1.2 mgms. ferric phosphate per liter, where one was kept stoppered and the other exposed to the air.

TABLE XI

Effect of CO_2 in Air on a Nutrient Solution

R_2S_1, with 1.2 mgms. $FePO_4$ per liter

Time since addition of iron Hrs.	Stoppered solution Iron found p.p.m.	Approx. pH	Exposed solution Iron found p.p.m.	Approx. pH
1	.05	5.1	.05	5.1
49	.06	5.3	.08	5.2
94	.07	5.3	.10	5.3
150	.05	5.1	.05	5.2

Although the exposed solution shows .03 p.p.m. more iron in solution at the end of 94 hours, at the close of the 150-hour (6 1/4 days) period both solutions show less than .05 p.p.m. iron in solution. Neither solution shows any significant change in pH during the entire time. (This work was not done with standardized indicators, and is accurate only to within .2 to .3 pH unit on subsequent days, though the two tests on the same day are accurate to within .1 pH unit for purposes of comparison.)

Table XII shows data obtained on a solution of .01 gms. ferric phosphate made up to one liter, regulated to pH 5.0 with ortho phosphoric acid.

TABLE XII

Effect of CO_2 on Solubility of $FePO_4$

.01 gms. $FePO_4$ made up to 1 liter; pH modified to 5.0 with H_3PO_4

Time	CO_2 Pressure	Iron found p.p.m.	Approx. pH	Remarks
4 hrs.	---	.10	5.0	
3 days	---	.15	5.2	
5 "	---	.10	5.3	
7 "	---	.10	5.1	
10 "	---	.05	5.0	
12-19 "	---	.05	5.1	
19 "	108 cm. Hg			CO_2 tank attached.
22 "	108 cm. Hg	.05	5.1	
28 "	"	.10	5.1	
35				H_2S passed to reduce iron. Washed through (1) water, (2) brom cresol green, pH 5.5, (3) brom cresol purple, pH 6.0, and (4) brom thymol blue, pH 7.0. Pressure renewed directly.
39 "		.20	5.0	

From the data in Table XII it is observed that it takes a carbon dioxide pressure of 108 cm. Hg three days to bring .05 p.p.m. iron into solution, and this is done without any change in pH. Ten days of this pressure brings .20 p.p.m. iron into solution, but there is still

no change in hydrogen-ion concentration. It is obvious that the atmosphere will neger exert a carbon dioxide pressure of this magnitude upon any solution; hence even the slight change in amount of iron in solution after three days of this treatment would never come about due to atmospheric conditions.

(c) Conclusions.---From the data given in the preceding section, it is clear that atmospheric carbon dioxide cannot be regarded as a cause of decrease in pH in solution with dependent increase in amount of iron in solution.

Furthermore, from all the experimental data it is evident that decrease in pH is not accompanied by increase in the amount of iron in solution, but rather by a decrease. And that this decrease in amount of iron in solution is not directly due to the decrease in pH of the solution is demonstrated by the fact that the buffered solutions, whose pH does not change,also show the decrease of soluble iron. That is, independent of the change of pH of the solution, but coincident with it where such a change occurs, there is a characteristic decrease in the amount of iron in solution in all solutions containing ferric phosphate as a source of iron.

It appears, therefore, that the pH value and the iron solubility are not related in the way commonly assumed, but that there must be a common cause for the change in both. Iron does not become more soluble because of the lowering of the pH value in nutrient solutions, and it is equally obvious that iron does not become insoluble for this reason. Yet there is a large amount of experimental data to show that iron disappears from solution as the pH of the solution decreases. Since the two processes are not related as cause and effect, it may be assumed that they are both effects of some third process which is the

cause. What the nature of this third process is, and the mechanism of its causation, will be discussed in the following section.

3. The Interaction of Component Salts of Nutrient Solutions.

(a) Theory.---For theoretical discussion of the interaction of the component salts of nutrient solutions only those salts will be considered with which experimental work has been done, namely potassium dihydrogen phosphate, calcium dihydrogen phosphate, potassium nitrate, calcium nitrate, magnesium sulfate and ammonium sulfate. The relation of these salts to ferric phosphate will also be discussed.

In a solution of a single salt the only possible reaction is hydrolysis. With any appreciable amount of hydrolysis, the formation of a strong acid, as sulfuric acid from magnesium or ammonium sulfate, should make itself evident by a lowering of the pH.

Where two salts are together in solution, the hydrogen-ion concentration of the mixture may be changed. It may be changed by the influence of one salt. Thus a dihydrogen phosphate will buffer a solution, and keep the pH at a different value than would be given by the second salt and another non-buffering salt of the same original pH as the dihydrogen phosphate. On the other hand, the two-salt solutions may show the influence of both component salts, and have a pH value between that of the two, due either to pH of the salts themselves or to the pH of their reaction products.

Stollenwerk (107) finds that calcium dihydrogen phosphate in water solution shows hydration isomerism, and that the decomposition

of the salt proceeds as a unimolecular reaction. This may point to a reaction of the single salt solution, which is veiled because of the buffering effect of the part remaining unhydrolyzed.

Ungerer (11a) finds that ferric phosphate can decompose salts of strong acids and bases and so liberate free mineral acids. This should be shown in the solutions used by a lowered pH value in unbuffered salt solutions containing ferric phosphate. There are, however, other possibilities.

Complex salts might be formed by combination of ferric phosphate with other ions in solution. Or ferric phosphate might hydrolyze to a slight degree and liberate free phosphoric acid which, in turn, might react with other ions to give phosphates, acid phosphates or basic phosphates.

Interaction between the component salts themselves is not inconceivable. It is known that in higher concentrations calcium nitrate and magnesium sulfate react to give a precipitate of calcium sulfate, and magnesium nitrate, which remains in solution; there is no reason why this reaction should not take place in weaker concentrations where it would produce so little calcium sulfate that the solubility product constant would not be exceeded and therefore no precipitate would form. In fact, it is only logical to assume that this is what does take place. It would be expected, moreover, that other reactions would take place between the components, even though they may not be heralded by change in pH or by precipitation, due to the low concentration of the reacting substances.

(b) Data.---Diagram I shows graphically the final pH values of all tests in Tables IX and X. Every point on a line between two solutions represents a solution containing those two salts. Solutions

have been tested having the composition denoted by the points indicated by the arrows, i.e., 1 : 2, 2 : 2 and 2 : 1. When no arrow is present the point is at the nearest intersection of lines. Thus, on the line between calcium nitrate and magnesium sulfate there are three sets of figures. The set nearest calcium nitrate contains two parts calcium nitrate to one of magnesium sulfate; the central set contains equal parts of the two salts; and the set nearest magnesium sulfate contains two parts magnesium sulfate to one of calcium nitrate. The three values given at each of these points represent respectively the final pH of the solution containing 2.4 mgms. ferric phosphate per liter, the final pH of the solution containing 0.6 mgm. ferric phosphate per liter, and the final pH of the control solution, containing no iron. In all cases the final value of the control is the same as the initial value of all three solutions.

From Diagram I it is readily seen that potassium dihydrogen phosphate and calcium dihydrogen phosphate greatly modify the pH of any solution in which they occur, and by their buffering action keep the pH of such solutions constant. Sometimes solutions of two salts, however, show the dominance of one component, as in the case of combinations of ammonium sulfate and potassium nitrate, where the control, without ferric phosphate, shows the pH 6.0 of the ammonium sulfate and never the pH 6.6 of the potassium nitrate; and sometimes such solutions show the influence of both components, as in the case of combinations of calcium nitrate and calcium dihydrogen phosphate, where the solution containing two parts of the nitrate to one of the phosphate shows pH 3.4 which is nearer the pH 6.6 of the calcium nitrate than the pH 3.3 of the solution containing two parts of the phosphate to one of the nitrate, which shows the influence

pH OF NUTRIENT SOLUTIONS CONTAINING FERRIC PHOSPHATE

of the pH 3.3+ of the calcium dihydrogen phosphate.

Although a double salt solution shows no change in pH at the concentrations used for these tests, when ferric phosphate is added to these solutions a change in pH does occur. In every double salt solution which is not buffered by the action of a dihydrogen phosphate, the solutions containing ferric phosphate show a distinct lowering of the pH, while the control, with no ferric phosphate, shows no change in pH. Therefore, the ferric phosphate is concerned in some reaction which lowers the pH, or brings more acid into activity in the solution.

The reactions of salts in dilute solutions are difficult to follow. Tables XIII and XIV give data on single and double salt solutions run at much higher concentrations, and with more ferric phosphate added.

No controls are run parallel with these solutions. The control solutions are freshly prepared as needed for the tests; this may introduce a small error. However, since none of the more dilute solutions show a change in pH when no ferric phosphate is present, it seems probable that the error in the concentrated solutions due to change of the solution in the absence of ferric phosphate is negligible.

The tests for iron are hard to read when these concentrations of nitrates and phosphates are present. The test is read immediately, but in a few cases the interference of the salts present is very difficult to overcome, even by the compensating method, since the color given by the salts is far stronger than that given by the iron test.

Test 1 of Table XIII shows data obtained from 200 cc. of molar potassium dihydrogen phosphate with 0.5 gms. ferric phosphate added. The solution with ferric phosphate shows pH 3.7; with ferric phosphate

there is pH 4.0 within 4 days, and pH 3.9 at the 6 and 8-day periods. At the end of 8 days, with pH 3.9 only .05 p.p.m. iron is in solution; a microscopic examination shows only ferric phosphate.

Test 2 is run with 200 cc. of molar calcium dihydrogen phoephate, with 0.5 gms. ferric phosphate added. This shows a decrease in pH from 3.2 at 4 days, which is the same as that of the solution without ferric phosphate, to 3.1 at the 6 and 8-day intervals. At the end of 8 days .40 p.p.m. iron is found in solution. A microscopic examination shows only ferric phosphate in the undissolved state.

Test 3 is run with 200 cc. of molar potassium nitrate, with 0.5 gms. ferric phosphate added. The pH of the solution alone is 7.0, but with ferric phoephate present it drops to 3.2 within 4 days, and is at pH 3.0 at the 6 and 8-day periods. Only .05 p.p.m. iron is found in solution at the end of 8 days. The microscopic examination shows only undissolved ferric phosphate.

Test 4 gives data on 200 cc. of molar calcium nitrate. With no ferric phosphate, pH 7.5 is found, but on addition of 0.5 gms. ferric phosphate the pH changes to 3.4 with 4 days, is still at 3.4 at 6 days, and drops to 3.2 at 8 days. At the end of 8 days much less than .05 p.p.m. iron is in solution. The microscopic examination shows only undissolved ferric phosphate.

Test 5 is run with 200 cc. molar magnesium sulfate. The solution alone gives pH 7.5; when 0.5 gms. ferric phosphate is added the pH changes to 4.4 within 4 days and drops to 4.3 within 6 days, where it remains until the 8-day period. At the end of 8 days .08 p.p.m. iron is in solution. The microscopic examination shows only undissolved ferric phosphate.

TABLE XIII

Single Salt Solutions

Run at High Concentrations

No.	Solution Tested	Iron added gms.	Time since addition of iron Days	Iron found p.p.m.	pH	Soln. without iron, freshly prepared. pH	Remarks
1.	200 cc M/1 KH_2PO_4	0.5 $FePO_4$	4 6 8	 .05	4.0 3.9 3.9	3.7	Microscopic exam; $FePO_4$ only.
2.	200 cc. M/10 $CaH_4P_2O_8$	0.5 $FePO_4$	4 6 8	 .40	3.2 3.1 3.1	3.2	Microscopic exam; $FePO_4$ only.
3.	200 cc. M/1 KNO_3	0.5 $FePO_4$	4 6 8	 .05	3.2 3.0 3.0	7.0	Microscopic exam; $FePO_4$ only.
4.	200 cc. M/1 $Ca(NO_3)_2$	0.5 $FePO_4$	4 6 8	 m. < .05	3.4 3.4 3.2	7.6	Microscopic exam; $FePO_4$ only.
5.	200 cc. M/1 $MgSO_4$	0.5 $FePO_4$	4 6 8	 .08	4.4 4.3 4.3	7.5	Microscopic exam; $FePO_4$ only.
6.	200 cc. M/1 $(NH_4)_2SO_4$	0.5 $FePO_4$	4 6 8	 .25	4.0 4.2^- 4.2^-	5.6+	Microscopic exam; $FePO_4$ only.

TABLE XIV

Double Salt Solutions

Run at High Concentrations

No.	Solution Tested	Iron added Gms.	Time since addition of iron Days	Iron found p.p.m.	pH	Soln.without iron, freshly prepared. pH	Remarks
1.	100 cc. M/1 KH_2PO_4	.05 $FePO_4$	3		-	3.6	Microscopic exam.
			4		3.6		$FePO_4$ only.
	100 cc. M/10 $CaH_4P_2O_8$		6		3.6		
			8	.05	3.6		Microscopic exam; $FePO_4$ only.
2.	100 cc. M/1 KH_2PO_4	0.5 $FePO_4$	4		3.8	3.8	
			6		3.8		
	100 cc. M/1 KNO_3		8	.05	3.8		Microscopic exam; $FePO_4$ only.
3.	100 cc. M/1 KH_2PO_4	0.5 $FePO_4$	1		-	3.4	Flaky ppt.microscopic exam; rosettes of crystals.
	100 cc. M/1 $Ca(NO_3)_2$		3		-		Microscopic exam; rosettes of crystals.
			4		2.8		
			6		2.8		
			8	.15	2.8		Microscopic exam; $FePO_4$ and rosetts of crystals, shown to be $CaH_4P_2O_8$ crys.
4.	100 cc. M/1 KH_2PO_4	0.5 $FePO_4$	4		3.6	3.8-	
			6		3.7		
	100 cc. M/1 $MgSO_4$		8	.10	3.7		Microscopic exam; $FePO_4$ only.
5.	100 cc. M/1 KH_2PO_4	0.5 $FePO_4$	4		4.0	3.6	
			6		4.0		
	100 cc. M/1 $(NH_4)_2SO_4$		8	.10	4.0		Microscopic exam; $FePO_4$ only.
6.	100 cc. M/10 $CaH_4P_2O_8$	0.5 $FePO_4$	4		3.0	2.9	
			6		2.9-		
	100 cc. M/1 KNO_3		8	.15	2.9-		Microscopic exam; $FePO_4$ only.
7.	100 cc. M/10 $CaH_4P_2O_8$	0.5 $FePO_4$	4		2.9	2.8+	
			6		2.8		
	100 cc. M/1 $Ca(NO_3)_2$		8	.10	2.8		Microscopic exam; $FePO_4$ only
8.	100 cc. M/10 $CaH_4P_2O_8$	0.5 $FePO_4$	1		-	3.2	Flaky ppt.;microscopic exam; branching crystals, rising from $FePO_4$ base.
	100 cc. M/1 $MgSO_4$						
			4		3.4		
			6		3.4		

TABLE XIV (Con't)

No.	Solution Tested	Iron added Gms.	Time since addition of iron Days	Iron found p.p.m.	pH	Soln. without iron, freshly prepared. pH	Remarks
8.			8	4.0	3.4		Iron test doubtful; color not true iron color. Microscopic exam; $CaSO_4$ crystals and $FePO_4$.
9.	100 cc. M/10 $CaH_4P_2O_8$ 100 cc. M/1 $(NH_4)_2SO_4$	0.5 $FePO_4$	1	-		3.6	Flaky ppt. Microscopic exam; branching crystals from $FePO_4$ base.
			4		3.5		
			6		3.4		
			8	.3	3.4		Microscopic exam; $FePO_4$ and $CaSO_4$ crys.
10.	100 cc. M/1 KNO_3	0.5 $FePO_4$	3		-	7.4	Microscopic exam; $FePO_4$ only.
	100 cc. M/1 $Ca(NO_3)_2$		4		3.2		
			6		3.2		
			8	< .05	3.2		Microscopic exam; $FePO_4$ only.
11.	100 cc. M/1 KNO_3	0.5 $FePO_4$	4		3.8	7.0	
	100 cc. M/1 $MgSO_4$		6		3.8		
			8	.05	3.8		Microscopic exam; $FePO_4$ only.
12.	100 cc. M/1 KNO_3	0.5 $FePO_4$	4		4.0	5.8	
			6		3.9		
	100 cc. M/1 $(NH_4)_2SO_4$		8	.05	3.9		Microscopic exam; $FePO_4$ only.
13.	100 cc. M/1 $Ca(NO_3)_2$ 100 cc. M/1 $MgSO_4$	0.5 $FePO_4$	1			5.9	Immediate precipitation of $CaSO_4$. All tests filtered. Shows to be $CaSO_4$ on microscopic exam.
			3				Microscopic exam; $CaSO_4$ crys. collecting in bunches.
			4		4.4		
			6		4.5		
			8	.10	3.0		Microscopic exam., $CaSO_4$ crystals and $FePO_4$

TABLE XIV (Con't)

No.	Solution Tested	Iron added Gms.	Time since addition of iron Days	Iron found p.p.m.	pH	Soln. without iron, freshly prepared. pH	Remarks
14.	100 cc. M/1 $Ca(NO_4)_2$ 100 cc. M/1 $(NH_4)_2SO_4$	0.5 $FePO_4$	1			5.9	Immediate precipitation of $CaSO_4$. All tests filtered. Microscopic exam; $CaSO_4$
			4		4.4		
			6		5.0		
			8	.05	3.0		Microscopic exam; $FePO_4$ and $CaSO_4$ crys.
15.	100 cc. M/1 $MgSO_4$ 100 cc. M/1 $(NH_4)_2SO_4$	0.5 $FePO_4$	4		4.2	5.9	
			6		4.2		
			8	.10	4.2		Microscopic exam; $FePO_4$ only.

Test 6 is run with 200 cc. molar ammonium sulfate. The solution alone gives pH 5.6^{+}. With 0.5 gm. ferric phosphate added, pH 4.0 is found at the end of 4 days, and pH 4.2^{-} at the 6 and 8-day periods. At the end of 8 days, .25 p.p.m. iron is in solution. The microscopic examination shows only undissolved ferric phosphate.

Table XIV shows data on the double salt solutions run at higher concentration. For these tests 100 cc. each of two stock solutions (all molar, except the calcium dihydrogen phosphate which is tenth molar) are used to give a 200 cc. portion. Five tenths of a gram of ferric phosphate is used as the source of iron in each 200 cc. portion. The controls are freshly prepared and the pH may differ slightly from what it would be if the control had stood as long as the solution tested; however, from data already given in more dilute solutions, it appears that very little, if any, change in pH occurs in the double salt solutions to which no ferric phosphate is added.

Test 1 is run on a solution containing potassium dihydrogen phosphate and calcium dihydrogen phosphate. The pH remains constant for 8 days at 3.6, due doubtlessly to the buffer action of the two dihydrogen phosphates. The control also shows pH 3.6. At the end of 8 days .05 p.p.m. iron is in solution. Microscopic examinations show only undissolved ferric phosphate.

Test 2 is made with a solution containing potassium dihydrogen phosphate and potassium nitrate. Here, also, the pH 3.8 of the control is maintained by the test solution throughout the period of observation, due to the buffer action of potassium dihydrogen phosphate. At the end of 8 days .05 p.p.m. iron is in solution. The microscopic examination shows only undissolved ferric phosphate.

Test 3 is carried out with a solution containing potassium dihydrogen phosphate and calcium nitrate. The solution without ferric phosphate shows pH 3.4, but with ferric phosphate pH 2.8 is maintained throughout the experiment. The constancy of the pH may be due to the buffer action of the potassium dihydrogen phosphate. At the end of 8 days .15 p.p.m. iron is present. Within one day a flaky precipitate is noticed, and this shows itself, upon microscopic examination, to be composed of rosettes of crystals. These are identified as calcium dihydrogen phosphate crystals, since a control solution of the two salts also shows these crystals, and the same kind of crystals are obtained when a solution of calcium dihydrogen phosphate is partially evaporated. A final microscopic examination at the end of eight days shows undissolved ferric phosphate, and crystals of calcium dihydrogen phosphate.

Test 4 is run with a solution containing potassium dihydrogen phosphate and magnesium sulfate. The control shows pH 3.8; with ferric phosphate present, pH 3.6 is obtained at the 4-day interval and pH 3.7 at the 6 and 8-day periods. At the end of 8 days .10 p.p.m. iron is in solution. The microscopic examination shows only undissolved ferric phosphate.

Test 5 is run with a solution containing potassium dihydrogen phosphate and ammonium sulfate. Without ferric phosphate, pH 3.6 is obtained; with ferric phosphate, pH 4.0 is maintained throughout the period of observation. At the end of 8 days .10 p.p.m. iron is in solution. The microscopic examination shows only undissolved ferric phosphate.

Test 6 is run with a solution containing calcium dihydrogen phosphate and potassium nitrate. The control shows pH 2.9. With ferric phosphate added, the solution shows pH 3.0 at the end of 4 days, and pH 2.9^{-} during the remainder of the observation period. At the end of 8 days .15 p.p.m. iron is in solution. The microscopic examination shows only undissolved ferric phosphate.

Test 7 is run with a solution containing calcium dihydrogen phosphate and calcium nitrate. The control shows pH 2.8^{+}. At the end of 4 days the test solution shows pH 2.9, and shows pH 2.8 for the rest of the observation period. At the end of 8 days .10 p.p.m. iron is in solution. The microscopic examination shows only undissolved ferric phosphate.

Test 8 is made with a solution containing calcium dihydrogen phosphate and magnesium sulfate. The control shows pH 3.2. The solution with ferric phosphate added shows pH 3.4 at the end of 4 days; the pH appears to be slightly less at the 6 and 8-day intervals. At the end of 8 days 4.0 p.p.m. iron is found in solution, but the color does not seem to be the true iron color, and there is some question as to whether this is an accurate reading. A flaky white precipitate is noted in the solution within one day; a microscopic examination shows this to be composed of branching crystals, rising from undissolved ferric phosphate as a base. These are identified as calcium sulfate crystals by comparison with known crystals of calcium sulfate. The final microscopic examination shows undissolved ferric phosphate and crystals of calcium sulfate.

Test 9 is run with a solution containing calcium dihydrogen phosphate and ammonium sulfate. The control shows pH 3.6. The

solution containing ferric phosphate shows pH 3.5 at the end of 4 days, and pH 3.4 at the end of 6 and 8 days. At the end of 8 days .3 p. p.m. iron is in solution. Within one day a flaky precipitate is noted, which on microscopic examination is shown to be branching crystals of calcium sulfate like those of Test 8. The final microscopic examination shows undissolved ferric phosphate and crystals of calcium sulfate.

Test 10 is run with a solution containing potassium nitrate and calcium nitrate. The control shows pH 7.4, but the solution with ferric phosphate present shows pH 3.2 throughout the period of observation. At the end of 8 days less than .05 p.p.m. iron is in solution. Microscopic examinations show only undissolved ferric phosphate.

Test 11 is run with a solution containing potassium nitrate and magnesium sulfate. The control shows pH 7.0; the solution containing ferric phosphate shows pH 3.8 throughout the period of observation. At the end of 8 days .05 p.p.m. iron is in solution. The microscopic examination shows only undissolved ferric phosphate.

Test 12 is run with a solution containing potassium nitrate and ammonium sulfate. The control shows pH 5.8. At the end of 4 days the solution with ferric phosphate present shows pH 4.0, and at the end of the 6 and 8-day periods pH 3.9 is obtained. At the end of 8 days .05 p.p.m. iron is in solution. The microscopic examination shows only undissolved ferric phosphate.

Test 13 is carried out with a solution containing calcium nitrate and magnesium sulfate. The control shows pH 5.9; the solution containing ferric phosphate shows pH 4.4 at the end of 4 days, pH 4.5 at the end of 6 days, and pH 3.0 at the end of 8 days. At the end of

8 days .10 p.p.m. iron is in solution. When the two salts are mixed, there is immediate precipitation of calcium sulfate. All tests are made on the filtered solution. The microscopic examinations show undissolved ferric phosphate and crystals of calcium sulfate.

Test 14 is run with a solution containing calcium nitrate and ammonium sulfate. The control shows pH 5.9; the solution containing ferric phosphate shows pH 4.4 at the end of 4 days, pH 5.0 at the end of 6 days, and pH 3.0 at the end of 8 days. At the end of 8 days .05 p.p.m. iron is in solution. When the two salts are mixed there is immediate precipitation of calcium sulfate. All tests are made on the filtered solution. The microscopic examinations show undissolved ferric phosphate and crystals of calcium sulfate.

Test 15 is made with a solution containing magnesium sulfate and ammonium sulfate. The control shows pH 5.9; the solution containing ferric phosphate shows pH 4.2 throughout the period of observation. At the end of 8 days .10 p.p.m. iron is in solution. The microscopic examination shows only undissolved ferric phosphate.

When calcium dihydrogen phosphate is one of the components in a two-salt solution, it invariably shows a more pronounced tendency to increase the solubility of iron than does the other component. Magnesium sulfate exerts a less noticeable influence, and potassium dihydrogen phosphate shows even less tendency to render iron soluble. Potassium nitrate and ammonium sulfate show very little tendency to increase the solubility of iron. It is interesting to note that calcium nitrate appears to be equal in influence to whatever salt is with it in solution; this may be due to reaction with soluble ferric phosphate to form calcium phosphate. Such reaction would be governed by the amount of iron, and hence the amount of phosphate-ion, brought into solution by the action of the other salt present.

Since calcium dihydrogen phosphate shows most tendency to increase the solubility of iron, in both the single and double salt solutions, the accompanying lowering of the pH would hardly be expected to be due to liberation of phosphoric acid, since an excess of phosphate ions would tend to make the ferric phosphate more insoluble.

(c) Conclusions.---From the experimental data it appears that ferric phosphate interacts with the component salts of nutrient solutions to liberate acid, either from the salts or from the ferric phosphate.

In no single-salt solution, in the absence of ferric phosphate, is there any noticeable change in the hydrogen-ion concentration. From this it appears that no appreciable amount of hydrolysis takes place unless a second salt is present to react with one of the products of hydrolysis and so render the reaction irreversible.

In double salt solutions the interaction between the component salts is comparatively rapid, and equilibrium is reached within a few hours at the most, when it remains unchanged in the absence of ferric phosphate.

However, when ferric phosphate is added to a single or double salt solution there is a progressive lowering of the pH, unless the solution is buffered. The change in pH is not in proportion to the amount of ferric phosphate added, however, and most of the ferric phosphate remains insoluble. From this evidence it appears that only a very small amount of the ferric phosphate is concerned in bringing about the change in pH. The amount of iron in solution does not necessarily indicate the amount of iron taking part in such an action, since complexes may be formed which give very few ferric or ferrous ions.

If the increase in acidity is due to liberation of phosphoric acid by reaction of the ferric phosphate with the other salts present, the iron would be expected to be in soluble form, unless the hydrous oxide, $Fe_2O_3.3H_2O$, is formed. It seems more probable, however, that soluble and more readily ionized nitrates or sulfates might result. No reaction could be assumed between ferric phosphate and a dihydrogen phosphate to give free phosphoric acid, yet the same change in solubility of iron is found, and doubtless only the buffer action of the dihydrogen phosphate prevents the appearance of increased acidity.

Since iron is originally in solution in small amount, and decreases with decrease of pH, a common cause for these two effects has been assumed. The formation of a compound of iron which gives very few ferric or ferrous ions is accompanied by a lowering of the pH.

It is possible that there is an initial reaction that renders the iron soluble, followed by reprecipitation of ferric phosphate or precipitation of hydrous ferric oxide, but this does not explain the lowering of the pH, nor is there any plausible explanation for the reversion of such an action.

The most satisfactory explanation of the facts is the assumption of the formation of a complex of ferric phosphate with a component salt. It might be assumed that such a complex would give very few ferric ions, and would tie up the anion of the component salt, leaving the cation free to form free mineral acid in the presence of water. The resulting mineral acid would need to be present only in small quantities to produce a marked effect upon the pH of the solution.

IV. SUMMARY

The importance of iron in plant and animal metabolism has been presented by a study of the literature. It is essential in all known physiological processes of oxidation and reduction.

The significance of iron in nutrient solutions is shown, and the literature dealing with the supply and availability of iron in these solutions is considered.

The volumetric and colorimetric methods perfected by Louwsma are used, and results obtained that agree with his work.

The interference of the component salts of nutrient solutions with the colorimetric test is studied, and a compensating method is developed by which such interference is made negligible.

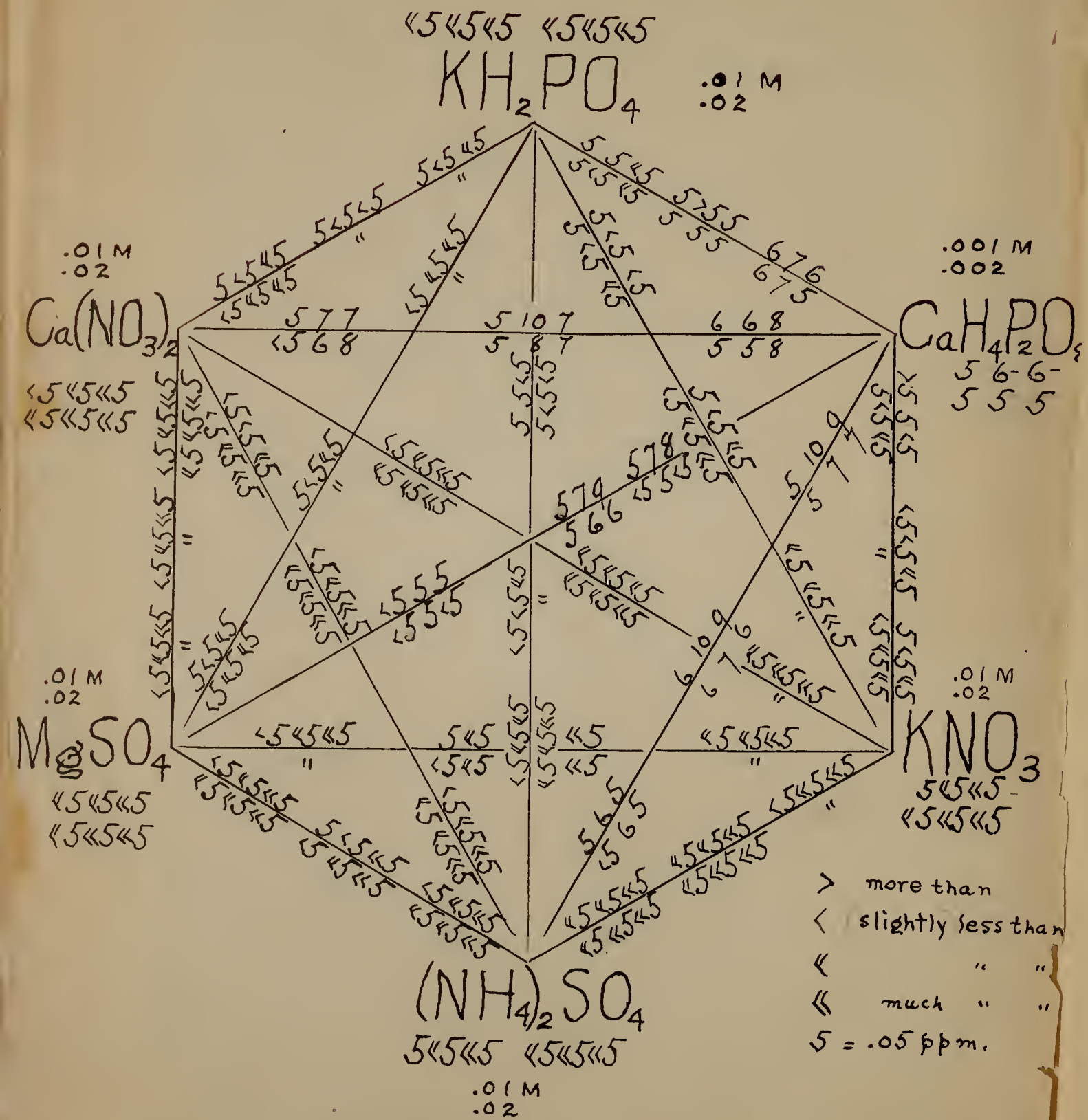
It is shown that the time-curve of iron solubility in a nutrient solution is dependent upon the type of nutrient solution and upon the compound used as a source of iron.

It is found that when ferric phosphate is used as a source of iron in solutions containing the component salts of nutrient solutions, or in the nutrient solutions themselves, the amount of iron in solution reaches a maximum value within a few hours, and then rapidly decreases, with an accompanying decrease in the pH of the solution.

It is shown that the decrease in pH does not occur in the absence of ferric phosphate; there is, therefore, some relation between decrease in amount of iron in solution and decrease in pH of the solution. Decrease in pH cannot be the cause of decrease in amount of iron in solution since, under normal circumstances, iron is more soluble in more acid solutions. Some common cause for the two actions is evident.

A theory is advanced which attributes these phenomena to the formation of complex salts of ferric phosphate with the component salts of the nutrient solutions. Such complexes would give very few ferrous or ferric ions, and, by liberating free mineral acids from the salts, would decrease the pH.

Iron In Nutrient Solutions $\frac{PPM}{100}$



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