On the modification of their environment by plant roots
by Willis B Johnston

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry
Montana State University

Abstract:
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The results obtained indicate that the majority of the dissolution can be attributed to absorption and adsorption of calcium by the plants. These phenomena are presumed to function by decreasing the concentration Of calcium- in the soil solution and thereby' shifting the solubility equilibria of calcium phosphates. Intimate contact between roots- and solid phase favored "feeding power". In view of the above findings, this is attributed to the formation of a closed system in the microenvironment of the root in which the calcium concentration is yery effectively decreased.

The effect of the secretion Of carbon dioxide and complexing agents by plant roots upon the solubilization of the fluorapatite used in this investigation was determined to be negligible.

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A THESIS

Submitted to the Graduate Faculty

in

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Doctor of Philosophy in Chemistry

at

Montana State College

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Bozeman, Montana
May, 1961

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BIOGRAPHICAL SKETCH

Willis B. Johnston was born December 2, 1922, in Butte, Montana, the youngest of three sons, to Einer C. and Gertrude E. Johnston. Mr. Johnston received his elementary education (1928-1936) in the Butte Public Schools. He graduated from Butte Public High School in 1940. After attending Montana State University for 1½ years, Mr. Johnston entered the United States Navy in January 1943. He was honorably discharged from the U. S. Navy in December 1945. Mr. Johnston received the B.S. and M.S. degrees in Chemistry in 1949 and 1952, respectively, from Montana State College. He was employed by Montana State College and the Montana Agricultural Experiment Station from September 1949 through May 1958. In June 1958, Mr. Johnston accepted a position with the U. S. Atomic Energy Commission, Idaho Operations Office, Idaho Falls, Idaho, a position which he still holds. Mr. Johnston is a member of Sigma Xi, Phi Kappa Phi, Alpha Chi Sigma, The Society of Nuclear Medicine, and The Eastern Idaho Chapter of the Health Physics Society.

ABSTRACT

Plant roots are known to chemically modify their environment. To the extent that they dissolve insoluble compounds in the substrate, they are credited with a corresponding amount of "feeding power" for the particular nutrient in question. The objective of this investigation was to determine the relative contributions, to phosphorus "feeding power" of four plant species, made by certain phenomena occurring at the root-substrate interface.

The results obtained indicate that the majority of the dissolution can be attributed to absorption and adsorption of calcium by the plants. These phenomena are presumed to function by decreasing the concentration of calcium in the soil solution and thereby shifting the solubility equilibria of calcium phosphates. Intimate contact between roots and solid phase favored "feeding power". In view of the above findings, this is attributed to the formation of a closed system in the micro-environment of the root in which the calcium concentration is very effectively decreased.

The effect of the secretion of carbon dioxide and complexing agents by plant roots upon the solubilization of the fluorapatite used in this investigation was determined to be negligible.

INTRODUCTION

Phosphorus ranks fifth in the order of abundance of the essential nutrient elements in the lithosphere. World reserves are estimated to be 26 billion tons of phosphate rock, and phosphorus comprises approximately 0.10 per cent of the soil by weight. A large proportion of the phosphate reserves in the lithosphere, however, are low grade minerals. In addition, the phosphate minerals of the soil are characteristically inert. Phosphorus reportedly occurs in the form of calcium, iron, and aluminum phosphates (including dicalcium phosphate, octocalcium phosphate, hydroxyapatite, fluorapatite, variscite, strengite, etc.), the most stable form, of course, depending upon prevailing chemical conditions. The inertness of these compounds is well exemplified by the fact that they have persisted in soil despite thousands of years of intermittent leaching. The quality of most phosphorus reserves is so poor, in fact, that some authorities consider phosphorus to constitute a major limiting factor to future production of food on this planet. This investigation deals with a mechanism by which certain plants have apparently become adapted to utilize existing low grade phosphate compounds of nature.

Minerals of the soil are more or less continually coated with aqueous films. From these films, plants obtain the phosphates to be utilized in their metabolic processes. In view of the relative abundance of phosphorus in soil, it appears rather paradoxical that native vegetation almost invariably responds favorably to phosphorus treatment. The concentration of phosphorus in the soil solution, however, seldom exceeds

0.5 ppm, and it is known that, unless a minimum concentration of approximately 0.05 ppm is maintained in the substrate, green plants may be expected to become phosphorus deficient.

Presumably the transfer of phosphates from soil solution through the aqueous films, extending through the cell wall, approximates conventional diffusion behavior. Thus, the rate of diffusion, dp/dt, might be expected to follow Fick's law:

$$dp/dt = \frac{DA}{L}(C-C_o)$$

where D is the diffusion coefficient of the phosphate ion, A is the effective area of the plant roots, L is the effective path length from soil to root surface, C is the concentration of phosphate in the soil solution, and C_o is the concentration of phosphate at the root surface. If C becomes less than 0.05 ppm, the concentration gradient $\frac{(C-C_o)}{L}$ could quite conceivably become too low to allow diffusion to occur at a rate adequate to meet plant requirements. The dissolution and diffusion processes are thereby seen to play vital roles in the phosphorus nutrition of plants.

Evidence at hand suggests that plants themselves play an active role in the dissolution and diffusion processes. Thus, plant roots reportedly lower the pH of the soil solution, secrete organic acids, and participate in ion exchange reactions, and thereby modify the dissolution process. Rate of diffusion of phosphate ions to the root surface is undoubtedly favored by an extensive root system (A in equation above) and by continual extension of new roots into unexploited soil.

It is the relative ability of the plant to absorb phosphorus from the substrate which is referred to as the phosphorus "feeding power" of the plant. Thus, the "feeding power" of a plant will be affected by modifications, by the plant, of its chemical environment and by physical phenomena such as extension of the root system into unexploited substrate and proximity of root surfaces to the nutrient source.

The relative abilities of four species of plants to absorb phosphorus from substrates containing Virginia Apatite as the only source of phosphorus have been studied. As a result of the investigation, an attempt has been made to postulate a more inclusive theory regarding "feeding power".

LITERATURE REVIEW

A great deal of experimental work has been done which indicates that plants modify their chemical environment, as suggested by the "feeding power" theories. It is well known that various types of plants have varying abilities to absorb nutrients, both from soil and nutrient solutions. For a rather complete review of the work (prior to 1930) on the subject of "feeding power", the reader is referred to Thomas (1930). Smith (1934), Lyness (1936), and Rabideau, et al (1950), showed that different varieties of corn exhibited differing abilities to absorb phosphorus from nutrient solutions. Rabideau suggested that part of the reason for these differing abilities was due to extensiveness of the root system. Davis, et al (1950), in a paper criticizing Truog's views on "feeding power", were also of the opinion that the size of the root system was a factor in "feeding power". Fried (1953) concluded that the "feeding power" of plants could not be accounted for by the extensiveness of the root system alone. He was able to show that, when the effect of root size had been separated from other possible effects, the plants still exhibited differences in ability to obtain phosphorus from the soil. Mehlich and Reed (1948) found that turnips, cotton, soybeans, cowpeas, oats, and wheat had differing abilities to extract calcium from a bentonite-sand mixture. They found that their abilities to extract calcium decreased as follows: turnips > cotton > soybeans > cowpeas > oats > wheat. Tyner (1935) showed that corn, rape, buckwheat, peas, sorghum, sudan grass, soybeans, and oats were poor feldspathic potassium

feeders, while alfalfa, alsike clover, red clover, and sweet clover were good feeders. The potassium content of the good feeders, receiving feldspathic potassium, was significantly increased over those not receiving potassium, whereas the potassium content of the poor feeders did not show a significant increase after receiving feldspathic potassium. Tyner also concluded that the relative "feeding power" of these plants for potassium was not related to the chemical form in which the potassium existed in the cytoplasm. Truog (1915, 1922), on the other hand, concluded that pH of the plant sap and the incorporation of potassium into compounds within the plant had a large effect upon the "feeding power" of plants for potassium. Newton (1928), studying the selective absorption of inorganic elements by various plants from both soils and nutrient solutions, found that the various plants had varying abilities to obtain nutrients from both of these media.

Many investigators have suggested that the "feeding power" of plants for phosphorus may be attributed to the cation exchange capacity of their roots. Presumably, plant roots, because of their ability to act as cation exchangers, are able to adsorb calcium and thereby affect a dissolution of insoluble calcium phosphates in the substrate, thus bringing phosphate ion into solution. If this be true, one would expect that plants having roots with high cation exchange capacities would be more efficient in this dissolution process than plants having roots with low cation exchange capacities, and could, therefore, be expected to be better "feeders". Drake, et al (1951), Williams and Coleman (1950), Smith and

Wallace (1956), and Dunham, et al (1956), determined the cation exchange capacities of various plant roots and found the roots of differing species to have widely differing cation exchange capacities. Drake, et al (1951), found that it was possible to get an indication of the root cation exchange capacity by determining the "ultimate pH" of root electrodyalizates. Drake and Steckel (1953) found that plants having high cation exchange capacity root systems are more effective in obtaining phosphorus from soil and rock phosphate than are plants having low cation exchange capacity root systems. Drake suggested that bonding of calcium by the plant root exchange sites causes the rock phosphate crystal to dissolve by the process of mass action. Truog (1927) claimed that the uptake of calcium by the plant results in a mobilization of phosphorus in rock phosphate systems. Burd (1948) has shown, by displacement studies, that when the calcium concentration of a soil solution is reduced by plant uptake of calcium there is a concurrent increase in phosphate concentration in the soil solution. McLean and Baker (1953) and McLean (1956) studied cationic activities in aqueous systems containing plant roots and determined the exchange capacities of alfalfa, soybeans, red top, red canary grass, corn, celery, oats, and lespedeza roots. They pointed out that, even though the roots of two plant species may have the same cation exchange capacity, a given ion may be held more tightly on one root than on the other, and, therefore, the feeding capacity of the plants for the ion in question may be quite different.

Investigators have long recognized that the pH of the growth medium

may affect the ability of plants to feed. It has been postulated that various root-soil interactions could affect the pH of the growth medium. Williams and Coleman (1950) have shown that plant roots, saturated with hydrogen ion, affect the pH of the growth medium. Burd (1948) has shown, on calcareous soils, that as the pH is decreased the phosphate solubility is increased. He noted, however, that in calcareous soils, containing $CaSO_4$, the pH had to be lowered almost one unit before the effect of the common ion, calcium, was overcome and an appreciable increase in the phosphate concentration of the soil solution occurred. Burd stated that in a soil containing gypsum it is difficult for the plant to shift the equilibrium in favor of dissolution of phosphorus. He suggested that in such a system the concentration of calcium is the dominant influence affecting phosphate concentration. Benne, et al (1936), in a study of the effect of calcium ions and reaction upon the solubility of phosphorus, treated H_3PO_4 solutions at varying pH's with CaO, $CaCO_3$, and $CaCl_2$. Large excesses of $CaCO_3$ failed to precipitate the phosphate from solution apparently because of the slight solubility of $CaCO_3$. Slight additions of CaO reduced phosphate concentration in solution to a minimum at pH 7.36 and maintained the low phosphate concentration at higher pH's. Large excesses of $CaCl_2$ precipitated no phosphate from solution until the pH was raised to 7.36. In all cases they found that calcium ion did not precipitate phosphate from solution below a pH of approximately 5.6. Teakle (1928) studied the solubility of phosphates in aqueous solutions and soils at various reactions. He concluded that calcium phosphate is insoluble under

alkaline conditions and that the main factor in the depression of phosphate solubility in alkaline soils is the presence of calcium ions. He also precipitated the calcium in one soil with $(NH_4)_2C_2O_4$ and caused a twenty-fold increase in phosphate concentration. He was able to reprecipitate this phosphorus by adding calcium ions and thereby reduce the phosphate concentration in the soil to its original value. Albrecht and Schroeder (1942) showed that far greater mobilization of nutrients occurred when the soil was acid. Mehlich and Reed (1948) stated that, irrespective of how plants absorb cations, hydrogen ions are involved in the mobilization of cations. Pratt and Thorne (1948) measured the concentration of phosphate between pH values of 4 and 10 in Na-clay and Ca-clay suspensions. The concentration of phosphate was greater over this pH range in the Na-clay system than in the Ca-clay system, again showing the effect of the common ion calcium on phosphate solubility. Lewis, et al (1952), found that salts of calcium having a common ion with fertilizer phosphate cause fixation of phosphate. Kittrick and Jackson (1955) demonstrated that, when kaolinite was added to suspensions of variscite ($AlPO_4 \cdot 2H_2O$), the concentration of phosphate was depressed because of the addition of the common ion, aluminum.

There is little doubt that CO_2 is excreted by plant roots and that this would have an effect on "feeding power" because of the ability of CO_2 to lower the pH of the nutrient media especially in the immediate vicinity of the root hair. Turpin (1920) showed that plant roots secrete CO_2. He showed that CO_2 production by plant roots is largely independent

of that produced by soil microorganisms. Newton (1924) measured the CO_2 evolved from the roots of various crop plants. He estimated that a good deal more CO_2 is produced by soil microorganisms than is produced by plants, but suggested that CO_2 from the plant roots may be much more important in plant nutrition than that produced in the soil by microorganisms. Parker (1924, 1925, 1927) studied the CO_2 production of plant roots in relation to "feeding power" of plants and found no correlation between CO_2 production and "feeding power". Burd (1947) suggested that the excretion of both CO_2 and organic acids by plant roots affects the efficiency with which plants feed. Truog (1915, 1922, 1927) contended that acids, other than carbonic, are not excreted by plant roots and that "feeding power" cannot be explained by carbonic acid excretion from plant roots. Hall (1906), in a study of the solvent action of roots upon soil particles, concluded that carbonic acid is the only acid excreted by plant roots and is solely responsible for the solvent action of roots. Islam (1955) claimed that there is no conclusive evidence for the excretion, by roots, of materials capable of causing the dissolution of calcium phosphates, other than CO_2. Sachs (1860) placed polished marble plates in soil with growing plants and found that where the roots contacted the plates the marble was etched. Davis, et al (1950), held that differences in the extent of the root system and CO_2 production by roots may be extremely important in "feeding power".

On the other hand, some investigators have found that materials, other than carbonic acid, are excreted from plant roots. Jackson and

Coleman (1959), in studying excised roots of snapbeans, found that pyruvate, malate, citrate, acetate, succinate, and fumarate ions were liberated from the excised roots. Katznelson, et al (1955), using aseptic cultures of peas, soybeans, wheat, barley, and tomato, demonstrated, by paper chromotography, that glutamic acid, aspartic acid, proline, leucine, alanine, cysteine, glycine, lysine, phenylalanine, and some sugars are either excreted from the intact roots of these plants or sloughed off from the roots during death and breakdown. Wheat and barley roots produced the greatest amounts of these materials. Nutman (1951) found evidence that roots of red clover, grown in aspetic agar cultures, excreted amino acids. Virtanen and Laine (1935), using sterile cultures, found that 87 - 98 per cent of the total nitrogen excreted from root nodules of legumes is excreted as amino acids. Virtanen and Hausen (1935) found that the excretion of amino acids by root nodules of legumes was highest when the plants were quite young. They concluded from this that the phenomenon observed was due to excretion by the nodule and not decomposition of the nodule. Using sterile culture techniques, Knudson (1920) found that plant roots excrete reducing sugars. Nance and Cunningham (1951) found that excised wheat roots excreted acetaldehyde when in the presence of nitrate and nitrite salts. For a review of work on root excretions, the reader is referred to Loehwing (1937).

The literature available on the effects of soil organic matter on phosphate solubility appears to indicate that one should expect the organic materials, excreted from plant roots, to be a factor in the

"feeding power" of plants for phosphate. Hines and Barber (1957) found that soil organic matter reacted with divalent metal cations in a manner similar to chelating agents. Swenson, et al (1949), showed that organic acids such as citric and tartaric are effective not only in preventing the formation of hydrated iron and aluminum phosphates but also in replacing the phosphate in the existing compounds. Struthers and Sieling (1950), using organic acids produced by plant decomposition, found that the most effective acids in mobilizing phosphate were citric, oxalic, tartaric, malonic, and malic. They stated that these acids are produced by the action of microorganisms on organic matter in the soil. Broadbent and Ott (1957) found that soil organic matter complexes with cations in very dilute solutions. They found that the amount of complex formed is a function of the type of cation, concentration of cation, pH, and time of contact. Stability of complexes studied was found to be in the order $Ca > Ba \geq Mg$.

It would seem reasonable to assume that, in the case of plants dissolving an insoluble compound, intimate contact between the root and nutrient source would be necessary for maximum rate of nutrient uptake. The plant would be expected to modify the immediate environment to a greater extent than the distant environment. That is, for example, due to the buffer capacity of the soil, CO_2 excreted from the roots would not be expected to appreciably lower the pH of the soil solution except in the very near vicinity of the root. Jenny and Overstreet (1939) and Jenny, et al (1940), emphasized the importance of the intimate contact

between soil colloid and root surface in their studies on "contact exchange". They maintained that it is not necessary for a cation to be in the soil solution for uptake by a plant root to take place, providing the "oscillation space" of an ion on the root exchange complex overlaps the "oscillation space" of an ion on the soil colloid exchange complex. Dean and Rubins (1945), Vlamis (1953), and Olsen and Peech (1960), could find no evidence to support Jenny's theory of "contact exchange". Lagerwerff (1958) found that, under equilibrium conditions, the composition of the soil solution fully characterizes the environment of the plant root. Islam (1955, 1956) agreed with Jenny's theory of "contact exchange" and purports to give evidence supporting this theory. It should be noted that, where the absorption of cations are concerned, the majority of the experimental evidence available supports the soil solution theory rather than the "contact exchange" theory. Where the absorption of anions are concerned, the author is unaware of any evidence in the literature which supports the "contact exchange" theory. Bray (1954) set forth a nutrient mobility concept of soil-plant relationships which proposes that there are two types of root sorption zones. One zone includes the whole volume of soil within the major part of the root system (where the roots absorb relatively mobile nutrients); the other includes only that zone in proximity to the roots (where relatively insoluble nutrients are absorbed). The first zone he called the root system sorption zone, and the second he called the root surface sorption zone.

Fried, et al (1957), in studying the kinetics of phosphate uptake

in the soil, have determined that the rate of formation of soil solution phosphorus, on an acre basis, in Bridger, Caribou, and Nibley soils, was greater than the rate of absorption of phosphorus by an acre of plants on the soils by a factor of at least 250. Tidmore (1930) showed that corn growing in a soil whose displaced solution contained only 0.02 to 0.03 ppm of phosphate grew better than that grown in a culture solution of 0.1 ppm phosphate. Burd (1947) stated that there is much experimental evidence suggesting that root surfaces must be in intimate contact with the soil particles to enable plants to get enough nutrients to satisfy their needs. Truog (1927) concluded that plant roots in contact with soil particles may obtain sufficient nutrients even if the concentration of nutrients in the soil solution is too low to support normal growth. Newton (1928) also suggested that intimate contact of the root with soil particles is necessary for nutrient uptake to take place. Comber (1922), emphasizing the importance of root-soil contact, suggested that "feeding power" might be explained on the basis of the absorption of colloids by roots and/or by the solvent action of various substances of the plant sap which may find their way into the growth medium. Marais (1922), from experiments involving the solvent action of roots on Plaster of Paris plates on which calcium phosphates had been precipitated, concluded that root-soil contact was extremely important to nutrient uptake. Broyer and Stout (1959) pointed out that the volume of soil explored by the roots is a very important factor in plant "feeding power" for phosphorus. Comber (1922) proposed that the root hairs form a mucilage which may encompass

soil particles, thus providing very intimate contact between the root hair and the soil. He then implied that the root hair excretes various organic materials which, either because of their acidity or their ability to complex cations, cause the solubilization of relatively insoluble compounds. Parker (1927) also proposed that the contact between root hair and soil colloid is so intimate that a closed system is formed. He further suggested that the organic acids, etc., in the root could have a solvent action on the colloid without actually being excreted by the root because of formation of this closed system. Gerretson (1948) found that the presence of microorganisms in the rhizosphere causes solubilization of phosphorus. Using a sterile culture technique, he found that oats, mustard, sunflower and rape took up much more phosphorus from apatite when the roots were inoculated with microorganisms than when the cultures remained sterile. In inoculated cultures, Plaster of Paris plates, upon which he had precipitated an insoluble calcium phosphate, showed solubilization zones at the points of root contact. These solubilization zones were absent in the uninoculated cultures. This led Gerretson to conclude: (1) that plant roots have very little solvent action, and (2) that intimate contact between root and insoluble nutrient source is necessary for nutrient uptake to take place.

In view of the fact that existing theories concerning the subject of "feeding power" are rather contradictory, it was deemed desirable to: (1) study some of the theories concerning the root-soil interactions purportedly contributing to the "feeding power" of plants, and (2) attempt

to measure the relative contributions of each of the contributing factors to the over-all "feeding power" of plants.

EXPERIMENTAL METHODS AND APPARATUS

Four species of plants (squash, soybeans, barley, and wheat), which reportedly differ quite widely in "feeding power", were chosen for this study. (See Table I.)

Method of Root Culture for the Determination of Root Cation Exchange Capacities. For the purpose of obtaining roots upon which to determine cation exchange capacities, a modification of the culture method of Epstein and Hagen (1952) was used. The seeds of the plants were treated with 12 per cent Purex solution for 20 minutes. Following this, barley and wheat seeds were continuously aerated in distilled water for 24 hours, at which time the seeds were sown on a piece of sterile cheese cloth supported by an aluminum screen, cut to fit a rectangular Pyrex dish. The screen was supported above the surface of the nutrient solution. After the seeds were sown, a second piece of sterile cheese cloth was placed on top of the seeds with the cloth extending beyond the ends of the screen so that when the screen was placed in the Pyrex dish the ends of the cheese cloth dipped into the $CaCl_2$ solution forming a wick which kept the seeds moist. The Pyrex dish was filled to within 1/4" of the screen with 10^{-4} molar $CaCl_2$. The Pyrex dishes were then placed in dark incubators maintained at a temperature of 26° C. The solutions were continuously aerated throughout the growth period. The top piece of cheese cloth was removed after about 48 hours, at which time the seedlings were developed to a point where the roots extended into the nutrient solution. The nutrient solution was replaced every two days, at which time the

roots of the seedlings were rinsed twice with distilled water. Wheat and barley roots were excised for use five days after planting.

Squash and soybeans could not be grown by this technique owing to putrefaction. Presumably the seeds were too moist. Therefore, the seeds of these plants, following the 12 per cent Purex treatment, were planted in acid-washed silica sand contained in the Pyrex dishes and placed in dark incubators maintained at 26° C. The plants were watered each day with 10^{-4} molar $CaCl_2$ and allowed to grow for seven days, at which time the sand was washed from the roots with distilled water and the roots excised. It was necessary to allow seven days growth period to obtain roots of size comparable to the five-day wheat and barley roots.

Determination of Root Cation Exchange Capacities. The cation exchange capacities of the roots of the four plant species used were determined in the following manner. The root exchange sites were saturated with hydrogen ion by placing the excised roots in distilled water and bubbling CO_2 through the system in a manner described by Williams and Coleman (1950). The roots were then centrifuged at 200 rpm in a basket centrifuge for five minutes to remove water adhering to the roots. Approximately four grams of fresh roots were weighed into 200 ml. of 1.0 N NaCl and titrated with 0.108 N NaOH to a pH of 7.0 using a glass-calomel electrode pair. The cation exchange capacities were calculated in milliequivalents per 100 grams of dry root tissue. The cation exchange capacities of the roots appear in Table I of the Appendix.

Method of Plant Culture. All experimental work, with the exception of

that described above, was carried out in a growth room having a 17-hour light period, a seven-hour dark period, and a temperature of 26° C. All plants were grown for a period of 16 days following transplantation of seedlings. Nutrient solutions were replaced on the eighth day of growth.

Seeds were germinated in pure silica sand previously washed with 6 N HCl. The sand was rinsed with distilled water until all traces of chloride ion were removed as evidenced by the silver nitrate test. Seedlings were watered with distilled water and allowed to grow until large enough for transplantation. Due to the fact that barley and wheat seeds germinated more rapidly than squash and soybeans, their seedlings were transplanted to the appropriate nutrient solution at the end of seven days, whereas squash and soybean seedlings were transplanted at the end of ten days.

Initially, the use of sand cultures was considered, but the difficulty of maintaining relatively constant nutrient concentrations in such cultures led to the adoption of the solution culture method. This, however, posed the problem of maintaining an insoluble nutrient in intimate contact with the plant roots and still having essentially a solution culture. This problem was solved by planting the seedlings in 150 mm. x 20 mm. soft glass test tubes having a hole blown in the bottom and containing coarsely ground du Pont cellulose sponge, which had previously been washed with 6 N HCl and rinsed with distilled water until all traces of chloride ion were removed. The test tubes containing the seedlings were placed in a square wooden rack which had been drilled to accommodate a certain

number of test tubes (depending upon plant used). The rack was of such size as to cover a No. 12 can allowing the test tubes to hang down into the container in contact with the nutrient solution which was contained in the No. 12 can (see Photograph 1). The use of sand rather than sponge was tried, but in such a restricted container the sand packed so tightly that the plant roots could not satisfactorily penetrate the substrate.

The nutrient solutions used in the growth room studies were all variations of Hoagland's No. 2 solution. The composition of this solution is as follows:

$Ca(NO_3)_2 \cdot 4H_2O$	0.95 g./l.
KNO_3	0.61 g./l.
$MgSO_4 \cdot 7H_2O$	0.49 g./l.
$NH_4H_2PO_4$	0.12 g./l.
Ferric tartrate	0.005 g./l.

In all experiments, the nutrient solution was diluted 1 to 2 with distilled water and two liters of this solution were used per No. 12 can. To each liter of the diluted solution was added one milliliter partial Reed and Haas solution, which was made up as follows:

H_3BO_3	0.60 g./l.
$MnCl_2 \cdot 4H_2O$	0.40 g./l.
$ZnSO_4$	0.05 g./l.
$CuSO_4 \cdot 5H_2O$	0.05 g./l.

Experiment No. 1. The purposes of this experiment were as follows:

a. To determine if the plants chosen would exhibit differing

abilities to obtain phosphorus from Virginia Apatite under the experimental conditions outlined below.

b. To determine the effect of three different calcium levels on phosphorus absorption by these plants.

c. To determine the effect of calcium absorption by the plants on phosphorus "feeding power".

In the instance of soybeans and squash, one plant was planted per test tube and each wooden rack was drilled to accommodate six test tubes. In the instance of wheat and barley, two plants were planted per test tube and each wooden rack drilled to accommodate eight test tubes. See Photographs 2, 3, 4, and 5.

The following nutrient solutions were used in this experiment:

Solution A

$Ca(NO_3)_2 \cdot 4H_2O$	0.95 g./l.
KNO_3	0.61 g./l.
$MgSO_4 \cdot 7H_2O$	0.49 g./l.
Ferric tartrate	0.005 g./l.

Solution B

KNO_3	0.71 g./l.
$MgSO_4 \cdot 7H_2O$	0.49 g./l.
Ferric tartrate	0.005 g./l.

Five treatments, each in duplicate, were carried out as follows:

Solution A - Apatite . . . . . 10 grams of Virginia Apatite (per test tube) mixed intimately with

ground sponge and Solution A used as nutrient.

Solution B - Apatite . . . . . 10 grams of Virginia Apatite (per test tube) mixed intimately with ground sponge and Solution B used as nutrient.

Solution A - No Apatite . . . . . No Virginia Apatite used; Solution A used as nutrient.

Solution B - No Apatite . . . . . No Virginia Apatite used; Solution B used as nutrient.

Solution B + 2 Hoag. Ca-Apatite . . . . 10 grams of Virginia Apatite (per test tube) mixed intimately with ground sponge; Solution B + 1.78 g./l. of $CaCl_2$ used as nutrient.

Experiment No. 2. The purposes of this experiment were as follows:

a. To determine the extent to which intimate contact between root and nutrient source contributes to phosphorus absorption.

b. To determine the relative abilities of the plants to obtain phosphorus under the experimental conditions outlined below.

Two treatments, each in duplicate, were carried out in this experiment. In one treatment, Virginia Apatite (20 g. placed in bottom of a No. 12 can) in conjunction with nutrient Solution A was used. In the other treatment, nutrient Solution A, alone, was used. These treatments were vigorously aerated at all times in an attempt to ensure suspension of the nutrient phosphorus source so that the solution would remain

relatively homogeneous with respect to soluble phosphorus. The number of plants per tube and the number of tubes per can were the same as in Experiment No. 1.

Experiment No. 3. The purpose of this experiment was to evaluate the effect of root excretions upon the dissolution of tricalcium phosphate.

Wheat and barley were allowed to germinate in the growth room according to the previously described technique (No. 12 cans were used in place of Pyrex dishes) with distilled water as the substrate. After one week, the water was replaced by 1/2 Hoagland's No. 2 solution. The plants were then allowed to grow in the nutrient solution for four days. On the fifth day, the nutrient solution was replaced with distilled water and the plants allowed to grow for another four days, at which time they were harvested and the roots excised. Soybeans and squash received the same treatment except that they were germinated and grown in a sand culture (watered with distilled water) for ten days, at which time they were then transferred to 1/2 Hoagland's nutrient solution. The plants were harvested and the substrate surrounding the plant roots during the last four days of growth was evaporated to approximately 75 ml. The substrate samples were brought to 100 ml. with distilled water and equilibrated with 0.2 grams of $Ca_3(PO_4)_2$ for 18 hours at 30° C. (It should be noted that $Ca_3(PO_4)_2$ was present in excess of its solubility so that some solid phase remained throughout the equilibration period.) The phosphorus concentration was determined in this system and in a similar system in which distilled water was used as the solvent. The phosphorus concentration in

the latter system was subtracted from that of the former and the phosphorus dissolved per gram of dry root tissue was calculated. Blanks for each plant species consisted of analyzing the solution from the last four days of growth, with no $Ca_3(PO_4)_2$ present, to determine how much phosphorus may have been transferred from the plants to the solution.

Experiment No. 4. The purpose of this experiment was to determine the effect of CO_2 excretion by roots on the absorption of phosphorus from Virginia Apatite by plants. The seedlings were planted as follows:

Four barley plants per test tube and six test tubes per rack.

Four wheat plants per test tube and six test tubes per rack.

Two soybean plants per test tube and six test tubes per rack.

One squash plant per test tube and six test tubes per rack.

All treatments were duplicated. There were two phosphorus treatments; one consisted of 1/2 Hoagland's No. 2 solution, and the other consisted of Solution B. As subtreatments, included in each phosphorus treatment, half of the plants in each can were aerated with CO_2-free air and half were not aerated. In order to at least partially rule out differences which might be attributed to aeration, the plants which were not aerated were planted in perforated soft glass test tubes. In those cases where Solution B was used, ten grams of Virginia Apatite was mixed with the sponge as in the other experiments.

Aerators were made by piercing 3/8" inside diameter Tygon plastic tubing, having a wall thickness of 1/16", with many fine holes. A pin-type flower vase frog was pressed through the walls of the tubing to make

these holes. The tubing was then cut into 7-1/2" lengths and one end of each tube was stoppered with a clear colorless glass marble. The Tygon tubes were then placed in the center of the glass test tubes, extending from the bottom past the top of the test tube, and sponge was packed around them at the time the seedlings were transplanted. The Tygon tubes in the treatments that were to be aerated were attached to rubber tubes leading to a manifold through which CO_2-free air was pumped. The air was bubbled through two containers of saturated NaOH to remove CO_2 and was then washed by bubbling through two containers of distilled water before entering the manifold. The purpose of the aeration was to sweep as much CO_2 away from the root surfaces as possible.

In order to check whether the aeration of the plants in the Apatite treatment was having an effect other than to remove the CO_2, excreted by the plant roots, from the media, a control treatment using 1/2 Hoagland's No. 2 solution was run. It was assumed that the removal of CO_2 should have no effect on the absorption of phosphorus by the plants from a nutrient medium containing soluble phosphorus.

Experiment No. 5. The purpose of Experiment No. 5 was to determine quantitatively the contributions, to the over-all "feeding power" of squash, made by pH of nutrient medium, intimate contact between root and nutrient source, and calcium concentration of the nutrient medium. The treatments designed to measure CO_2 contribution to the total "feeding power" of squash were included in the experiment, but results of these treatments were so erratic as to be unintelligible.

One squash plant per test tube and six tubes per No. 12 can were used in all cases. The plants were grown in exactly the same fashion as in the previous experiments. Each treatment was tiplicated. The plants were allowed to grow for 16 days prior to harvesting and the nutrient solutions were replaced on the eighth day of growth. The treatments used were as follows:

Solution B - No Apatite	No Virginia Apatite used; Solution B used as nutrient.
Solution B - Apatite in bottom of can	20 grams of Virginia Apatite placed in bottom of can; Solution B used as nutrient.
Solution B - Apatite	10 grams Virginia Apatite (per test tube) mixed intimately with sponge; Solution B used as nutrient.
Solution B + 1/8 Hoag. Ca-Apatite . . .	10 grams Virginia Apatite (per test tube) mixed intimately with sponge; Solution B + 0.112 g. $CaCl_2$ per liter used as nutrient.
Solution B + 2 Hoag. Ca-Apatite	10 grams Virginia Apatite (per test tube) mixed intimately with sponge; Solution B + 1.78 g. of $CaCl_2$ per liter used as nutrient.

All treatments were run at pH 5.0, 6.0, and 7.5. The pH's were adjusted eyery other day using 0.01 molar HCl and NaOH solutions. The