THE EFFECT OF PHYSIOLOGICAL PRE-CONDITIONING ON ORTHOPHOSPHATE ABSORPTION BY WHEAT ROOTS

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I INTRODUCTION

The phenomenon of root absorption of ions has fascinated man for many years. In addition to absorption a root functions in several other ways. Anchorage, nutrient transport, and storage are functions that are relatively well defined. The function of the root as a storage organ is very important even for one that is not specifically adapted for storage. Even a young primary root serves as a storage organ and it is thought to be associated with storage capacity as manifest by potential energy expressed as carbohydrate.

The intake of water and salts in relation to root energy reserve has been studied by several investigators (38, 76, 97)[1]. These studies produced experimental evidence that the phenomena of absorption are dependent on metabolic activity associated with growth and differentiation and that the factors determining ion uptake are not necessarily the same as those responsible for the uptake of water. Therefore, ion absorption can be studied without regard to water absorption if a steady state system is maintained with respect to the aqueous phase and if the metabolic status of the root has also been controlled with respect to atmospheric and nutrient environment.

Ions in the root environment enter the roots. This absorption has been described as both an active and passive process (18). Active absorption is viewed as a dynamic process whereby ions are accumulated against

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[1]Figures in parentheses refer to Literature Cited, page 71.

a concentration gradient thus requiring an expenditure of energy. Therefore, a direct relationship is thought to exist between root carbohydrate storage, aerobic respiration, carbohydrate utilization, and ion uptake (56). The phenomenon has been studied with excised root systems and slices of storage tissue (37, 38, 87, 91, 97). With respect to ion uptake, the mode of entry of ions into cells is an important question.

Plant physiologists have postulated that the absorption capacity of roots can be changed experimentally (50). This absorption variation might represent a change in concentration of certain metabolic acceptors within a cell. Therefore, it was thought that the production of roots of varying energy levels and ion absorption rates might be helpful in understanding the phosphorus metabolism in plants.

It was the purpose of this study to: (a) produce root tissues ranging from low to high in carbohydrate energy reserve, (b) determine the rate of phosphorus absorption by roots of variable carbohydrate reserves, (c) estimate the quantity of acceptors present, and (d) attempt identification of some organic phosphorus compounds, the precursors of which might serve as metabolic acceptors of phosphate ions from the nutrient medium.

II LITERATURE REVIEW

Plant physiology and plant nutrition are disciplines that originated from the broad field of biology which dates from antiquity. The philosophy of the nutrition of plants has progressed through several evolutionary stages. The humus theory of nutrition was strongly advocated during the early part of the nineteenth century. In 1840 von Liebig introduced the theory of mineral nutrition (54). In 1851 von Mohl formulated a new concept of the absorption of water and dissolved salts by roots (76). He stated:

> "In all plants the fluid nutriment is taken up by absorption through cells. As the cell membrane has no orfices, only such matters as are actually dissolved, can be absorbed into the cell with the water which penetrates the cell membrane."

The next significant advance in the understanding of root absorption was made by Sachs (84) in his research with relations between root hairs and water films. He stated that the principal absorption region was a short distance back of the root tip and that root hairs absorb and deliver water to other cells of the root. Since the time of Sachs the subject of absorption has received a vast amount of study. Broyer (10) grew plants in culture solutions and concluded that the maximum rate of absorption is usually observed in cells a few centimeters from an apical meristem. In this area of maximum uptake, absorption is aided by lateral extension of epidermal cells into root hairs. The root hairs are considered to increase absorption capacity by increasing effective surface area. The most

active root hairs are coincident with the area of greatest absorption (25). Prevot and Steward (75) stated that the entire surface of a barley root was capable of absorbing bromine but the apical centimeter accumulated more than the older portion. Rosene (82) reported that onion root cells showed a maximum absorption rate at a distance of approximately 50 millimeters from the root tip. Hayward et al., (35) obtained similar results with corn. Handley and Overstreet (31) studied absorption associated with the corn root and suggested that it might be possible to separate respiratory stimulations resulting from the operation of the accumulation mechanism from those due to other causes, by dividing the roots into segments from the apical tip.

Energy Requirements

It is now accepted by most investigators that, by some special organization of the cell or subcellular particles, absorption requires an expenditure of metabolic energy. A great advance was made by Hoagland and Davis (39) when they showed that root absorption of mineral elements was light dependent. This indicated that light was an aid in the production of substances capable of furnishing energy for the absorption process. In another paper Hoagland and Martin (40) emphasized the importance of light and temperature relative to concentration of essential elements in plants.

The uptake of solutes from a dilute solution by plant roots attracted the attention of plant nutritionists at an early date. Steward (89) was perhaps the first to call attention to the relations between respiration and salt accumulation. Early in the 1920's it was demonstrated that

Nitella could absorb ions from the external solution against concentration and activity gradients (39). This was interpreted as being an attribute of living plant cells in a state of active growth and metabolism and that energy was expended in the process. Then Hoagland and Broyer (38) demonstrated that young barley roots, high in available carbohydrates and with suitable aeration, accumulated potassium and bromide with remarkable rapidity; provided the root tissue did not attain a condition the authors called a "high salt" condition. Van Andel et al. (97) investigated this concept further. They observed that ion absorption was continued in the dark as well as the light after the substrate had been fortified with sugar. Many other investigators (1, 21, 58, 81) also subscribe to the energy concept. However, in recent publications Handley et al. (31, 32) have studied the absorption characteristics of sections of the primary root of corn with respect to the uptake of sodium, calcium, and chlorine. They report that the uptake of the elements in the non-vacuolated meristematic portion of the root tip was a non-metabolic process. In the same study it was reported that the uptake of these ions into vacuolated portions of the root was largely metabolic.

Effect of Aeration on Absorption

The fact that an adequate oxygen supply is needed for ion accumulation by root tissue is well documented. Steward (88) proved that accumulation of potassium salts by discs of storage tissues occurs only when oxygen is supplied to the tissues. Rosenfels (83) has shown that accumulation of bromine by Elodea, in the dark, is likewise dependent upon aeration. In similar experiments, Hoagland and Broyer (38) and Petrie

(72) emphasized that when oxygen was withheld from excised barley roots in apparently healthy condition, they absorbed only small quantities of bromine and a very slight quantity of potassium. In marked contrast, aeration of the tissues resulted in the accumulation of large amounts of the two elements. Absorption was four to five times higher than for similar unaerated tissues. As a result of these studies all workers have recognized the value of aerating experimental tissue during absorption studies and all ion uptake research uses aeration as a standard procedure. Therefore, a combination of low salt (high sugar) roots and proper aeration combine to provide a basic technique for studying ion accumulation.

Salt Absorption

Salt absorption is generally considered from two view points (20). The literature has distinguished between the two processes by use of the terms "passive absorption and active absorption".

Passive absorption envisions a continuation of the nutrient solution into the "apparent-free-space" of the root. Cations and anions diffuse equally into this space. There are no energy requirements associated with the process. Ions do not compete for the space, there is no pH effect, and at equilibrium the concentration of ions in the "apparent-free-space" may equal the ionic concentration in the nutrient medium (22). Butler (14) estimated the apparent-free-space in wheat roots to be about 24 to 25 per cent of the tissues. Epstein (22) obtained a value of 25 per cent for excised barley roots. After invading the available space by diffusion, the ions are thought to move directly to the tops of intact plants or be bound by binding sites of metabolically produced carriers

or acceptors (20, 53).

Active absorption is thought to involve individual ions or ion groups and organic acceptors (3, 4, 8, 10, 18, 19, 30, 52). With the exception of Vervelde (100) there appears to be wide agreement that active ion absorption involves the operation of acceptors. Since anions and cations are absorbed separately it appears likely that different acceptors must be responsible for each. In recent years the idea has developed that both the role of metabolism and the specificity in the absorption process can best be accounted for by assuming that ion binding compounds are generated by metabolic processes. General reviews which make this point include those by Ussing (96), Robertson (80), Steinbach (91) and Jacobson et al. (45). These workers and others stress the need for assuming that the complex is labile and, after combining with the ion, breaks down again, releasing the ions. Several compounds and some sub-cellular constituents have been suggested as possible ion acceptors.

Ordin and Jacobson (69) propose that adenosine triphosphate (ATP) may react with Krebs Cycle intermediates to form acceptors. Tanada (94) supposes that the acceptors are ribonucleic acids, with the nucleic acid binding the cations and the protein moiety binding the anions. Stewart and Street (90) report that ribonuclease impairs ion accumulation in plant tissue, therefore, it is suggested that the ribonucleic acid (RNA) in cytoplasmic membranes may bind components essential to ion accumulation (51). Henson (34) and Steward and Street (90) suggest that the carriers are phosphorylated nitrogen intermediates in protein synthesis. The acceptor is supposed to release bound ions upon incorporation into protein, with release occurring at the site of protein synthesis. Robertson et al.

(81) implicate mitochondria in ion binding and transport and have found accumulation of both cations and anions in these protoplasmic bodies. Lundegradh's (56, 57, 58, 59) massive works have focused attention on the role of cytochrome-cytochrome oxidase in the active transport of ions. Certainly there is close correlation between cytochrome activity and ion uptake, but this is not an indication that anions are absorbed directly through the operation of the cytochrome system (3).

Other suggested mechanisms involve choline derivates. The "zwitter" ion of lecithin has been suggested as a possible ion acceptor (4). Supposedly ion-salt complexes are formed at the outside of the membrane, transferred across the membrane where lecithinase-D hydrolyzes the complex to choline and phosphatidic acid, liberating the salt. It is then postulated that choline-esterase transfers choline from the acetyl to the phosphatidyl radical. Consequently, lecithin is resynthesized at the outside of the membrane. A phosphatide cycle is thus postulated as an acceptor mechanism (4). In other work related to choline-bearing compounds Maizel et al. (60) identified phosphoryl choline as the major phosphate ester of plant saps and suggested that the "zwitter" ion nature of the compound might act as a phosphorus carrier capable of penetrating plant membranes.

Eddy et al. (16) related the binding of ions to carbohydrate metabolism. More specifically, Helder (36) found that the uptake of phosphorus by maize plants was intimately connected with respiration and phosphorylation and stated that the sugar content of the roots was of primary importance. In his conclusions Helder stated that the sugar status of roots had a strong influence upon phosphorus uptake from a

nutrient medium. Research with cotton indicates that ion uptake is dependent on carbohydrate supply (73). Studies with both excised roots and entire plants showed that the uptake of phosphate was enhanced by the administration of sugars (36, 44). The exact mechanism of sugar utilization for ion uptake is not understood. It certainly represents an energy supply. Surely metabolizable intermediates are responsible for energy release and perhaps some of the intermediates might be involved in a role of ion acceptor. The pathway involved is questionable. Glycolytic intermediates or Kreb's cycle acids might be involved. Also both pathways might contribute to the phenomena through phosphoenolpyruvic carboxylase whereby oxalacetic acid is converted to phosphoenolpyruvate, a high energy compound. In the glycolysis cycle it has been suggested that inorganic phosphate from external solution is incorporated at the glyceraldehyde-3-phosphate junction, forming 1, 3-diphosphoglycerate which reacts with adenosine diphosphate to produce 3-phosphoglycerate and adenosine triphosphate, a compound containing an additional high energy phosphate bond (74). The exact mechanism, relative to metabolic intermediates, seems to depend on the place of first binding in relation to cell anatomy.

The opinions of different authors are contradictory with respect to point of initiation of active absorption. Some investigators suggest a barrier to free diffusible ions and to exchange processes at the outer surface of the protoplasm. Becking (3) and Holm et al. (42) suggests that the protoplasmic membrane is the locale of active absorption. Other authors hold that the protoplasmic membrane is permeable to diffusible ions while active absorption in confined to the region of the

tonoplast membrane (9, 80, 92). Teleologically it can be argued that it is unlikely that the cytoplasm, which so closely regulates its own internal ionic composition, would be freely exposed to the ionic concentration of its own environment. Becking (3) also states that animal cells and meristematic plant cells without a vacuole show a highly selective ion-accumulation capacity. In a rather different approach Sandstrom (85) studied ion absorption of wheat roots after the epidermal cells had been stripped off with di-n-amylacetic acid as suggested by Burstrom (11). It was found that tissue free of epidermis exhibited a significant increase in ion absorption. Consequently, it was concluded that some part of the epidermis was concerned in the mechanism of ion absorption. A study of the absorbed ions and water in the cell indicated a ratio similar to that of the external solution. This is indicative of a wholly passive uptake of salts when the epidermis is missing. These variations in and interpretations of data amplify the uncertainty of ion absorption in relation to cell morphology. Regardless of the barrier location, the philosophy of energy requirement for absorption remains intact and centered around carbohydrates. Also the idea of acceptor mechanisms has stimulated considerable research and resulted in new concepts as attempts to unify experimental results continue.

Factors Essential to Development of Root Carbohydrates

Nutrition and environment can influence the carbohydrate content of roots (38). Therefore, the control and manipulation of some variables of nutrition and environment will alter root carbohydrate content. Hoagland and Broyer (38) have demonstrated that the ion absorption

capacity of young roots exhibits maximum potential only when the cells are high in sugar (low salt). When a reverse condition (low sugar-high salt) exists the root cells do not exhibit pronounced ion absorption.

Since the nutrition of root cells has a great influence on ion absorption, it is important to control the concentration, composition, and volume of the nutrient solution in which the plants are grown. When the nutrient solution is sufficiently reduced in supplying power, translocation from root to shoot exceeds absorption and a low salt, high sugar root is produced. If the supplying power of the nutrient solution is maintained a salt deficit does not occur, therefore, a high salt low sugar root is produced. In this manner root cells may be caused to vary in their sugar content and ion absorption rate. An initial low salt (high sugar) root condition has become a standard in studying ion absorption by root tissue (18, 23, 24, 29, 47, 52, 53, 68).

Hoagland and Broyer (38) showed that high sugar roots, suitable for ion absorption studies, were produced in 21 days when the nutrient solution was not changed during that time. Low sugar roots were produced when the solution was changed every day or every other day. From these data it seems logical to assume that root tissue having a sugar (energy) content ranging from low to high may be produced by a series of nutrient solution changes during a 21-day growth period.

The rate of chemical reactions increases with rising temperature. The same is true for plant respiration since the phenomenon is a consequence of temperature dependent chemical reactions. Therefore, the rate of respiration of a particular tissue may be controlled by controlling temperature. McAlister (61) has demonstrated this phenomenon in winter

wheat. Like many biological processes which depend on complex reactions, respiratory rate increases as temperature increases. The optimum temperature for growth of young wheat seedlings is about 20 to 22 degrees C. (71). Lowering the temperature below this optimum should decrease respiration and raising the temperature above this optimum should increase respiration. Therefore, lowering temperature in connection with Hoagland and Broyer's (38) nutrient solution depletion method should result in a substantial increase in root sugars.

In plants the reverse of respiration is photosynthesis. The limiting factors in photosynthesis are light intensity, carbon dioxide concentration in the atmosphere surrounding the plants, and temperature (98). Meyer and Anderson (64) and Hoover et al. (43) indicate that wheat plants become light saturated at approximately 1000 foot candles in a normal carbon dioxide atmosphere. Therefore, the photosynthetic rate no longer increases with increasing light.

When photosynthesis exceeds respiration the plant system should accumulate considerable amounts of photosynthetic products. Ultimately this phenomenon may result in an increase in the carbohydrate content of the roots. An increase in day length should aid in increasing total photosynthetic products. Long days in conjunction with low temperatures and nutrient solution depletion, should cause root sugar accumulation to approach a maximum. Conversely, short days, high temperatures, and high salt level nutrient solutions should cause root sugar accumulation to approach a minimum.

Techniques of Root Absorption Studies

It is generally accepted that ion absorption by root tissue can be studied most effectively by eliminating, during a brief experimental period, the complications of root and shoot relationships (8, 10, 11, 18, 29, 30, 31, 38, 52, 68, 81). Therefore, a common technique involves the use of young excised root systems. Six and 21-day-old roots have been used satisfactorily (38, 47).

Young excised roots must be handled carefully in order to preserve the ion accumulating system in an active form. Leggett (52) found that when excised barley roots remained in water at 24° C. for three hours, phosphorus absorption was reduced by 50 per cent. However, this absorption decline was finally prevented by protecting excised roots in a dilute calcium solution. The calcium solution is necessary for the maintenance of normal ion absorption integrity (12, 13, 48, 52, 70, 93, 99). Therefore, it is desirable that root tissue be preserved in this manner until the actual absorption experiment is conducted.

An absorption trial might provide several kinds of data. However, one of the initial considerations is the quantitative measurement of the total amount of ion or ion species transferred to the root from the external environment (29). The quantitative estimation of ion transfer is very difficult to determine when it is expressed as the difference between the initial and final amount of the ion in the nutrient solution (23, 29, 38, 97). The development of atomic physics and subsequent production of isotopes created a new research tool for biological studies. Consequently, isotopes were utilized in root absorption studies as a means of direct analysis of uptake. Various techniques were conceived and evaluated, and

at present quantitative ion absorption may be adequately evaluated by the use of radioisotopes and excised roots in the well defined environment of (a) a single salt solution, (b) constant hydrogen ion concentration, (c) small mass of roots per unit volume of solution, and (d) a short absorption period. This procedure permits a precise study of steady-state ion absorption (29, 52, 68).

Kinetics of Ion Absorption

The study of the kinetics of root absorption, as related to metabolic acceptors, is an intriguing line of research. The study of the phenomenon makes use of the concept as developed for and applied to enzyme systems.

According to Fruton and Simmonds (28) Wilhelmy showed that the rate of a chemical reaction in which one molecular species disappears is proportional at each instant to the amount of that species. This relation may be expressed mathematically as $-\frac{dC}{dt} = kC$, where C = the concentration of substance undergoing change, t = time, and k = a proportionality constant. This equation, usually designated as a first order reaction, also describes processes in which an amount of a component is changing with time, for example the decay of a radioactive element. The function of the differential equation ($-\frac{dC}{dt} = kC$) may be obtained by integrating between the limits of the concentration Co at time t = o and of the concentration of C remaining at time t. After integration the function of the equation is found to be $\ln \frac{Co}{C} = kt$ (28).

The theory of the kinetics (rate measurement) of enzyme action assumes that the enzyme D combines with substrate F to form a compound DF by a reversible reaction

$$D + F \underset{k_2}{\overset{k_1}{\rightleftharpoons}} DF \qquad 1$$

where k_1 is the rate constant for the formation of DF, and k_2 is the rate constant for the dissociation of DF to D and F. After combination with enzyme to form DF, F is converted into the products R of the over-all reaction, thus making D available for further combination with more F. The rate of the conversion of DF to the products of the reaction is indicated by the constant k_3 and the rate of the conversion of D and R back to DF is indicated by the constant k_4. It is usually assumed that the breakdown of DF is irreversible; therefore, k_4 is negligible (28). The complete process involves a stepwise series of reactions:

$$D + F \underset{k_2}{\overset{k_1}{\rightleftharpoons}} DF \underset{k_4}{\overset{k_3}{\rightleftharpoons}} D + R \qquad 2$$

DF is a unique material in that it is visualized as a labile enzyme-substrate complex.

The first satisfactory mathematical analysis of enzyme-catalyzed reactions was made by Michaelis and Menten (65). They considered enzyme and substrate to combine as shown in equation 2. They assumed that the rate of decomposition of substrate was proportional to the concentration of the intermediate enzyme-substrate complex. For a given substance S which can be considered to be equal to the total concentration of S if the amount of enzyme is small, as is usually the case, then using the following notations:

E = total enzyme concentration
S = total concentration of substrate
ES = concentration of enzyme-substrate complex
E-ES = concentration of free enzyme
P = product of the reaction

A reaction analogous to equation 2 can be written as:

$$(E\text{-}ES) + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} (E\text{-}ES) + P \qquad 3$$

and a steady-state velocity equation can be derived. Since k_4 is considered to be negligible the continued shift of the equation to the right depends upon the speed of dissociation of ES (a constant Km) for a given concentration of substrate. The constant then provides for free enzyme concentration (E-ES) at any time t. An expression for the constant is:

$$Km = \frac{(E\text{-}ES)\ (S)}{ES} \qquad 4$$

This equation is an expression for the dissociation constant of ES. Solving for ES,

$$ES = \frac{(E)\ (S)}{Km+S} \qquad 5$$

If the velocity constant for the decomposition of ES into (E-ES) and P is k_3, and the measured velocity is $v = \frac{-d(ES)}{dt}$, then

$$v = k_3(ES) \qquad 6$$

and $ES = \frac{v}{k_3}$. Substituting in the equation above $\frac{v}{k_3} = \frac{(E)\ (S)}{Km + S}$, and solving for v one arrives at a velocity equation

$$v = \frac{k_3\ (E)\ (S)}{Km + S} \qquad 7$$

Velocity v becomes maximum, V_{max}, when the concentration S is maximal, i.e., when all of the enzyme is bound by substrate, and ES = E. At this point velocity is independent of substrate concentration (zero-order kinetics) and $\frac{-d(ES)}{dt} = k_3E$ where E can be regarded as a part of the proportionality constant. Here the logarithmic equation on page 14 does not hold. Under these conditions:

$$V_{max} = k_3(ES) = k_3E \qquad 8$$

since we said $v = k_3(ES)$, equation 6. If V_{max} is substituted for $k_3(E)$ in equation 7 the Michaelis-Menten equation is obtained:

$$v = \frac{V_{max}\ (S)}{Km + (S)} \qquad 9$$

which holds for cases where all enzyme is bound by substrate. This equation has proved to be somewhat cumbersome and simpler procedures have been devised. Two variations of the equation are utilized for data plotting and subsequent kinetic analysis. The reciprocal of the Michaelis-Menten equation results in the following:

$$\frac{1}{v} = \frac{Km + (S)}{V_{max}\ (S)} \qquad 10$$

This is known as the Lineweaver-Burk (55) equation. If one plots $\frac{1}{v}$ against $\frac{1}{(S)}$ a straight line is obtained with its intercept on the ordinate at $\frac{1}{V_{max}}$. The slope of the line is $\frac{Km}{V_{max}}$, and, since V_{max} can be determined from the intercept, Km can be calculated.

A second variation of the Michaelis-Menten equation was proposed by Eadie (15). This equation has the form:

$$v = V_{max} - Km\ \frac{v}{(S)} \qquad 11$$

If one plots v against $\frac{v}{(S)}$ a straight line is obtained with its intercept on the ordinate at V_{max}. The slope of the line is -Km. For most enzyme studies, the two variations of the Michaelis-Menten equation are more satisfactory for estimating the values of V_{max} and Km.

The application of enzyme kinetics to ion absorption by roots involves acceptors as being analogous to enzymes. The kinetics of ion absorption by roots has been studied extensively by Epstein (21) and others (26, 29, 30, 52, 68). These studies have been conducted on the

assumption that acceptors possess reactive sites capable of joining with an ion and subsequently releasing it. Acceptor reactions are designated:

$$G + H \underset{k_2}{\overset{k_1}{\rightleftharpoons}} GH \qquad 12$$

$$GH \underset{k_4}{\overset{k_3}{\rightleftharpoons}} G^1 + H \qquad 13$$

where H = ion, GH = labile acceptor-ion complex, and G and G^1 represent different chemical states of the metabolically produced acceptor. The two processes differ in that the combination between enzyme and substrate produces a chemical change in the substrate molecules, whereas the combination of acceptor and the ion results in the transport of the ion from the substrate to the inside of the cell. Acceptor G undergoes a chemical change rather than the substrate. Even though this type of analysis was first applied to enzymes, it is valid whenever the mechanism of a reaction involves the general features embodied in equations 12 and 13. Also, it is noted that H denotes anions as well as cations (21, 23). Under these conditions it must be assumed that a membrane impermeable to free ions is traversed by GH. Thence, the data obtained from reactions involving the general features should correspond to a mathematical analysis similar to that for enzymes. Studies with ion absorption make use of equation 10, when an element (ion) is being absorbed by only one acceptor. Where an element is thought to be absorbed by a double acceptor system equation 11 is used.

Epstein and Hagen (23) used the Lineweaver-Burk equation to study the kinetic absorption of alkali metal ions by excised barley roots. The experiment was concerned with rubidium absorption and the effects of

potassium and cesium as reaction inhibitors. Potassium and cesium interferred competitively with rubidium absorption, and it was concluded that the three ions were bound by the same binding site or acceptor. A similar experiment was conducted with the alkali earth metals. Strontium was considered the substrate while calcium, magnesium, and barium were considered inhibitors. Again it was found that active absorption was consistent with the hypothesis of the involvement of complex intermediate formation between ions and acceptors. It was also concluded that calcium, strontium, and barium were competitors for the same acceptor site while magnesium appeared to be a non-competitor (24). In the light of these findings for cations the behavior of anions is also of interest.

Epstein (21) reports data concerning anion absorption. It was found that anion uptake was subject to the same kinetic analyses as were cations. In addition to the bromine anion studies by Epstein, considerable work has been done with the phosphate ion. Hagen and Hopkins (30) found that phosphate absorption by excised barley roots was somewhat more complex than had been found for other anions. Generally it was thought that phosphorus absorbed by higher plants was in the form $H_2PO_4^-$. However, this was based on simple correlation studies of phosphate absorption with $H_2PO_4^-$ solutions (62). The Hagen-Hopkins results indicate non-linearity for the absorption of phosphate in root systems when $\frac{1}{v}$ (reciprocal velocity) was plotted against $\frac{1}{S}$ (reciprocal substrate concentration). Since the plot of $\frac{1}{v}$ vs $\frac{1}{S}$ in previous ion absorption studies had resulted in linear plots, the result for phosphate absorption was unusual. The plot was curvilinear as shown in Figure 1.

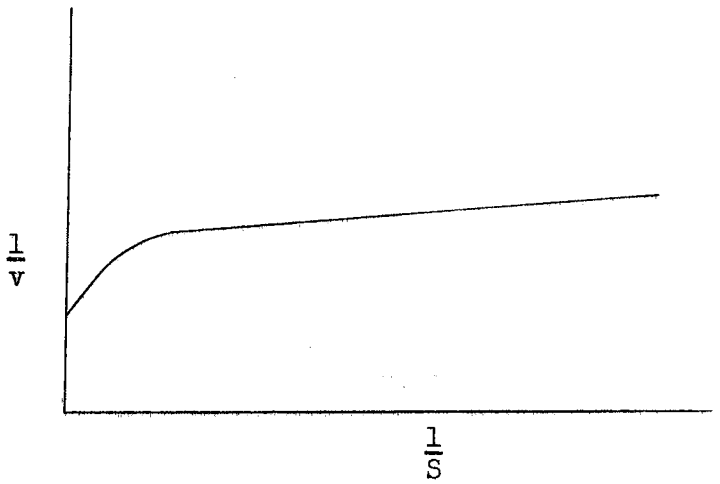

Figure 1. Double Reciprocal Plot of Phosphorus Absorption by Barley Roots. Hagen and Hopkins (30)

If the steady state absorption of phosphate involves a single first order reaction, described by a velocity equation, a double reciprocal plot should result in a straight line. The plot clearly indicates that the experimental results cannot be described by a single first order reaction. It was found by using a graphic representation as suggested by Eadie (15) and used by Hofstee (41) that the results yielded a plot as shown in Figure 2.

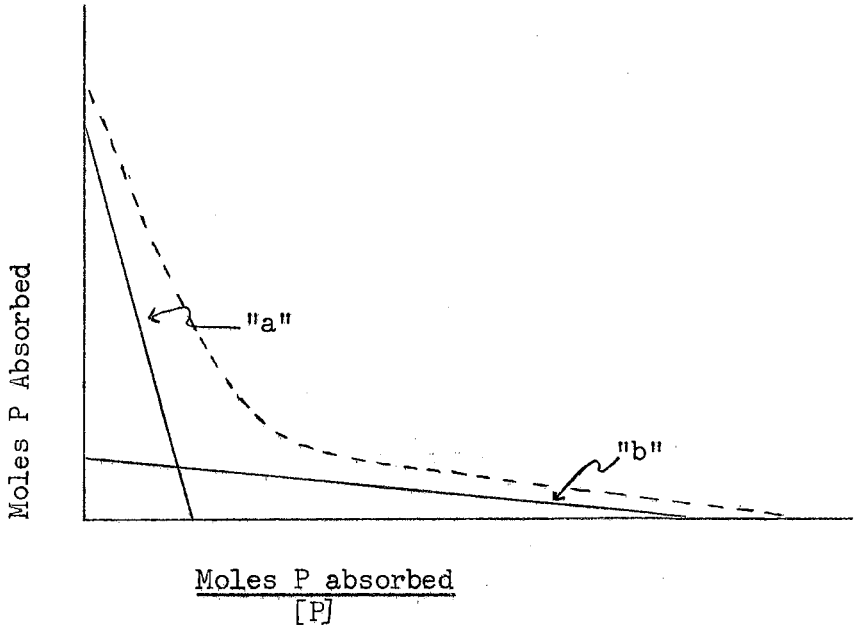

Figure 2. Hofstee Plot of Phosphate Ion Absorption by Barley Roots (30).

In Figure 2 the curved plot is obtained by plotting the moles of P absorbed against the moles of P absorbed divided by the concentration of the solution used for the study. The Hofstee plot reconciled the results obtained with the Epstein acceptor model in that two first order reactions were involved. It was suggested that one of the reactions involved $H_2PO_4^-$ while the other indicated that HPO_4^{--} was absorbed, with both reactions occurring simultaneously and independently. Therefore, it appears as though two different acceptors were involved. One acceptor was designated acceptor "a". The other was designated acceptor "b". In a more recent paper Noggel and Fried (68) compared the uptake of radioactive phosphorus by millet, barley, and alfalfa and interpreted their data with the kinetic analysis concept used by Hofstee. They showed that different plants take up phosphorus at different rates from equal concentrations of phosphorus in solution. The study indicated that the rate of phosphorus absorption is greater for millet than barley and greater for barley than alfalfa. The data also provided an estimate of the total amount of acceptor present in the roots of each plant, and it was concluded that the main difference in uptake between plants was the variation in concentration of acceptor. Fried and Shapiro (27) have compiled a table of kinetic constants of anion acceptor complexes in plant roots with appropriate references to specific authors. This table made only one reference to anion absorption by wheat roots in which the ion species studied was iodide. In studies of iodide ion uptake by wheat roots Boszormenyi (8) observed two active sites as has previously been observed for phosphate. In studying sulfate absorption Legett and Epstein (53) also noted a second concentration site for sulfate on barley roots.

Calculations of kinetic constants and estimations of quantities of acceptors present in roots have given indications of the presence of metabolic acceptors in root tissue.

The study by Noggle and Fried (68) assumed the acceptor-ion relationships as given in equations 12 and 13 to be the mechanism of ion accumulation inside a cell. Basically equations 12 and 13 are identical to the Michaelis-Menten enzyme equations 1 and 2. Therefore, using the following notations:

A = total acceptor concentration
P = total concentration of phosphorus
AP = concentration of phosphorus-acceptor complex
A-AP = concentration of free acceptor

A velocity equation applicable to root absorption may be derived which is analogous to equation 9 described by Michaelis and Menten (65). The velocity equation becomes:

$$v = \frac{V_{max}\ (P)}{Km + (P)} \qquad 14$$

which holds for cases where all acceptor is bound to phosphorus. The mechanism of phosphorus absorption is assumed to be:

$$(A\text{-}AP) + P \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AP \underset{k_4}{\overset{k_3}{\rightleftharpoons}} (A\text{-}AP)^1 + P \qquad 15$$

When the concentration of P is large all of A is in the AP form due to conditions for equilibrium whereby k_1 is much greater than k_2, and AP becomes a constant ($\underline{AP}$). Therefore, the only further reaction of $\underline{AP}$ is with respect to k_3 (moles of phosphorus per mole of AP per second) only since k_4 is considered to be negligible. Under these conditions $v = k_3(\underline{AP})$ where $v = \frac{-d(AP)}{dt}$ and here denotes the quantity of phosphorus released by the acceptor into root cells through the k_3 reaction. Consequently,

$$\frac{-d(AP)}{dt} = k_3\ (\underline{AP}) \qquad 16$$

The integral function of the differential equation may be obtained by evaluating between the limits of the concentration of $\underline{AP}$ at time t = o and limits of the quantity (q) of phosphorus released into root cells from $\underline{AP}$ at time t.

$$\int_{\underline{AP}}^{q} dAP = -\int_{o}^{t} k_3(\underline{AP})dt \qquad 17$$

Then $q = -k_3(\underline{AP})t + (\underline{AP})$ and since the system is assumed such that $\underline{AP}$ does not diminish and phosphorus release into cells by $\underline{AP}$ will be defined as positive it is correct to define k_3 such that:

$$q = k_3(\underline{AP})t + (\underline{AP}) \qquad 18$$

Equation 18 represents the quantity of phosphorus absorbed by the root and, at steady-state, the total phosphorus associated with the root includes both the phosphorus released inside the cell and that combined in the complex AP form.

Increased phosphorus in the external solution bathing the roots will cause an increase in the AP complex. At infinite external concentration, A will be completely saturated with P; hence all of A will be in the form of AP, i.e., AP = A, where A is the total amount of acceptor that will combine with phosphorus, and maximum absorption, Q_{max}, will occur. Equation 18 then becomes:

$$Q_{max} = k_3(A)t + (A) \qquad 19$$

By using an adaptation from the enzyme system to the root absorption system Noggle and Fried (68) studied phosphorus absorption by roots. The adaptation was accomplished by substituting the following notations into equation 11.

q = quantity of phosphorus absorbed
Q_{max} = Maximum phosphorus absorption at infinite concentration
P = Total concentration of phosphorus in absorption solution
Km = Dissociation constant of the acceptor-phosphorus complex

The equation then becomes:

$$q = Q_{max} - Km \frac{q}{[P]} \qquad 20$$

In this thesis the amount of phosphorus absorbed by the roots (q) was plotted against the amount absorbed divided by the total phosphorus concentration [P] in solution. The curved line resulting from the plot was similar to that obtained by Hagen and Hopkins (30). The curved line was resolved into two linear components "a" and "b" (Figure 2) by graphic methods. Line "a" was designated as a function of phosphorus absorption from high phosphorus concentrations, and line "b" was designated as a function of absorption at low phosphorus concentrations. This is comparable to the system described by Eadie (15) where two enzymes act simultaneously but independently on the same substrate. Q_{max} "a" and Q_{max} "b" are determined directly from the ordinate intercept of lines "a" and "b". Km for reaction "a", Km"a", and the Km for reaction "b", Km"b", are determined by calculating the slopes of lines "a" and "b".

In a second part of the kinetic analysis Noggle and Fried (68) studied the absorption of phosphorus from solutions of 1×10^{-6} molar and 5×10^{-4} molar phosphorus concentrations. Absorption was measured at 90 second intervals for a period of 10 minutes. The amount absorbed, plotted against time for each concentration, gave a straight line indicating steady-state uptake. The plot is shown in Figure 3. The plots for each phosphorus concentration were directly associated with the two linear components "a" and "b" of the curvilinear plot. The phosphorus associated

with the roots from the 1×10^{-6} molar phosphorus solution is a result of the reaction that is defined by line "b" of the curved plot which is analogous to acceptor "b" of the phosphorus-acceptor complex of the roots. Therefore, the rate constant, k_3 for acceptor "b", k_3"b", was obtained by dividing the slope of the line by the ordinate intercept. The ordinate intercept being $\underline{A"b"P}$ at $t = o$. The total amount of acceptor "b" per gram of roots (A"b") is obtained by substituting Q_{max}"b" and k_3"b" into equation 19.

The resulting steady-state straight line for the 5×10^{-4} molar concentration is a result of the reaction that is defined by both lines "a" and "b" in the curvilinear plot. Essentially total saturation of acceptor "b" is obtained at 5×10^{-4} molar concentration of phosphorus.

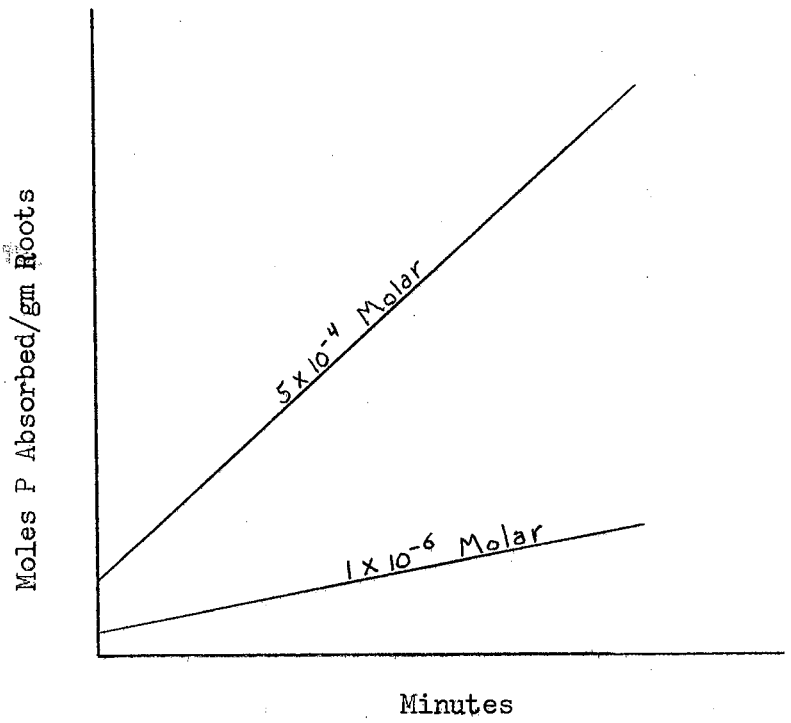

Figure 3. Plot of Phosphorus Absorption by Roots vs Time. (68)

Therefore, the value of $\underline{A"a"P}$, at $t = o$, is obtained by subtracting A"b" from the ordinate intercept, since the ordinate intercept of the time curve for concentrations of 5×10^{-4} phosphorus is a measure of the phosphorus in combination with both acceptors.

Where two acceptors are operating simultaneously the velocity equation representing total uptake must be a summation of the activity of both acceptors (28). Therefore, total uptake at 5×10^{-4} molar phosphorus concentration was determined by:

$$q = k_3"a"(\underline{A"a"P})t + k_3"b"(A"b")t + (\underline{A"a"P}) + (A"b") \qquad 21$$

where q is uptake from a 5×10^{-4} time plot in t seconds. One can solve for $k_3"a"$.

$$k_3"a" = \frac{q-(\underline{A"a"P})-(A"b")-(A"b")\ k_3"b"t}{(\underline{A"a"P})t} \qquad 22$$

where values of $\underline{A"a"P}$, A"b", and $k_3"b"$ are known and the value of q is obtained from the 5×10^{-4} plot at a time t. The substitution of this value into equation 19 gives an estimate for A"a". For determining the per cent saturation of A"b" at any substrate concentration:

$$\text{Per cent A"b" saturation} = \frac{q"b" \text{ at any substrate concentration}}{Q_{max}"b"} \times 100.$$

Chemical Characterization of Ion Acceptors

The chemical identification of ion acceptors in plant roots has been confined to those acceptors associated with phosphorus absorption. Jackson and Hagen (47) attempted to identify products of phosphate absorption by barley roots. After 10 minutes absorption time in a radioactive phosphorus solution, the ethanol soluble components were separated into five major

fractions. These components were separated by paper chromatography. Of the compounds separated, uridine-diphosphate-glucose, glucose-1-phosphate, and inorganic phosphate appear to be first in formation. P^{32} labeled adenosine phosphate was not found in the barley roots.

In this review, aspects of ion absorption in relation to stored potential energy has been documented. Various hypotheses concerning the mechanism of absorption have been reviewed. The kinetic analysis of ion absorption, as a tool for estimating ionic relationships and various biological constants, was mentioned as an empirical estimator of total acceptor concentration in root tissue. Only a few instances of attempts to characterize the acceptor mechanism chemically are recorded in the literature. The work of Hagen (29) and Jackson and Hagen (47) has demonstrated that the task of such a characterization is indeed a formidable one. The work also indicates that metabolic ion absorption is extremely complex.

III EXPERIMENTAL MATERIALS AND METHODS

Production of Roots with Graded Energy Levels

Plant Material: Experiments were made on the wheat plant (Triticum aestivum L.). Triumph, a variety of the commercial class Hard Red Winter Wheat, was used throughout the investiaation.

Wheat seeds were treated to control seed-born fungi spores. The seeds were immersed in 70 per cent ethanol for one minute to destroy surface tension. Immediately afterward they were placed in a 0.1 per cent mercuric chloride solution for one minute. To hasten germination the freshly decontaminated seeds (12 grams) were soaked in continuously aerated distilled water for 36 hours at 25°C. They were then distributed on cheesecloth supported by plastic coated stainless steel screens. The screens were placed on pyrex trays filled with tap water. The seeds rested on centimeter above the water level. Germinating equipment and germinating techniques are adequately described by Hoagland and Broyer (38).

Seedlings were grown to six and 29-days of age, each for a specific study. Roots six-days of age were studied to evaluate absorption characteristics when nutrition was limited to that furnished by the seed. Twenty one-day-old roots were studied to evaluate root absorption characteristics when produced under different environmental conditions. All six-day-old plants were grown in the dark at 25°C. At the end of the growth period, the roots were excised one-fourth inch below the seed,

rinsed three times in deionized water and placed in one liter of 0.00005 normal calcium hydroxide solution. When 21-day-old plants were desired etiolated seedlings were transferred to a low light intensity environment at five days of age. On the sixth day the seedlings were transferred to the direct light (2000 foot candles) of a plant growth chamber and placed in culture trays similar to those described by Hoagland and Broyer (38). The roots were allowed to dip into 3800 milliliters of nutrient solution and were grown in one of four environmental regimes for the purpose of obtaining roots of various energy (carbohydrate) levels.

Growth chamber experimental conditions are shown in Table 1. The roots were excised and samples obtained for carbohydrate analysis and ion absorption studies. Root tissue to be used for absorption studies was prepared and protected in a calcium solution as described for six-day-old roots.

Nutrient Solutions: The nutrient solutions used were those of Hoagland and Broyer (38) and van Andel et al. (97). Each solution was prepared in 18 liter batches in a constant temperature room (25° C). They were analyzed for actual concentration before use. The analytical methods are given in the Appendix. The composition of the solutions is given in Tables II and III.

Analytical Procedures for Carbohydrates

The root samples for carbohydrate analysis were preserved by the freeze-dry technique as described by Reed (77). The time required for drying was 60 hours. This technique insured the maintenance of the

TABLE I

GROWTH CHAMBER EXPERIMENTAL CONDITIONS

Condition	Temperature C.° Light	Temperature C.° Dark	Light Duration Hours	Nutrient Solution Changes *
I	10.0	3.3	15	0
II	15.5	5.6	13	1
III	21.1	11.1	11	3
IV	26.7	16.7	9	6

*per 14 days.

TABLE II

IONIC CONCENTRATION OF HOAGLAND'S SOLUTION (38)
(THEORETICAL VS ANALYTICAL VALUES)

Compound	Solution Concentration Molar	Solution Concentration gm/liter	Ion Concentration PPM (Theoretical Values) NH_3	K	NO_3	Ca	Mg	P
KNO_3	0.0025	0.2528	0.00	97.76	155.00			
$Ca(NO_3)_2 \cdot 4H_2O$	0.0025	0.4102	0.00		215.40	69.60		
$MgSO_4$	0.0010	0.1204	0.00				24.30	
KH_2PO_4	0.0005	0.0680	0.00	19.54				15.48
Theoretical Values			0.00	117.30	370.40	69.60	24.30	15.48
Analytical Values			0.93	118.00	373.40	63.00	21.00	15.60

Iron was supplied as monosodium-hydrogen-ferric diethylenetriamine pentaacetate (Geigy 330-10% Fe); 1.3% solution was added to the nutrient solution in the proportion of 1 cc per 3800 ml every other day.

TABLE III

IONIC CONCENTRATION OF VAN ANDEL'S SOLUTION (99)
(THEORETICAL VS ANALYTICAL VALUES)

Compound	Solution Concentration Molar	Solution Concentration g/liter	Ion Concentration PPM (Theoretical Values) NH_3	K	NO_3	Ca	Mg	P
KNO_3	0.0025	0.2528		97.76	155.00			
$Ca(NO_3)_2 \cdot 4H_2O$	0.0015	0.2461			129.20	41.80		
$MgSO_4$	0.0010	0.1204					24.30	
KH_2PO_4	0.0005	0.0680		19.54				15.48
$(NH_4)_2SO_4$	0.0005	0.0660	17.00					
Theoretical Values			17.00	117.30	284.20	41.80	24.30	15.48
Analytical Values			17.95	118.00	279.23	41.00	26.00	15.44

Iron was supplied as monosodium-hydrogen-ferric diethylenetriamine pentaacetate (Geigy 330-10% Fe); 1.3% solution, was added to the nutrient solution in the proporation of 1 cc per 3800 ml every other day.

chemical integrity of the various carbohydrate fractions, especially starch.

After drying, the root samples were ground in a "Micro Wiley Mill" to pass a 60-mesh screen. The samples were then placed in sealed brown manilla envelopes and stored in a vacuum dessicator containing "activated Alumina" until ready for analysis. Analyses were started on the day of grinding.

The analytical procedure for carbohydrates was essentially that of Nelson (67) for blood sugar as modified by Somogyi (86) and further modified by Reed (77) for plant tissue. A description of the modified procedure is given in the Appendix.

Phosphorus Absorption by Excised Roots and Estimate of Quantity of Acceptor Present

Absorption studies were made using single salt solutions of monocalcium phosphate. Solutions from 1×10^{-6} molar to 1×10^{-3} molar of phosphorus were made and labeled with P^{32}. The original activity of the solutions was approximately 15 millimicrocuries per milliliter. This activity was selected after preliminary work indicated that a series of 10 samples, representing absorption over the concentration range, could be counted within a three hour time period. This eliminated the necessity of calculations to compensate for radioactive decay.

The absorption procedure was a modification of that of Noggle and Fried (68). Two types of uptake studies were made. One study consisted of measuring phosphorus absorption from solutions with phosphorus concentrations of 1×10^{-6} molar and 5×10^{-4} molar at two-minute intervals for a period of ten minutes. The second study consisted of measuring

phosphorus absorption for a period of 10 minutes from 10 solutions with phosphorus concentrations that ranged from 1×10^{-6} molar to 1×10^{-3} molar.

Roots were removed from the dilute calcium hydroxide solution, into which they had been excised, blotted on washed, dry cheesecloth; and 0.5-gram portions were weighed out and again placed in a 50 milliliter beaker of dilute calcium hydroxide solution until actual absorption was begun.

For absorption, 150 milliliters of radioactive solution were measured into 250 milliliter polyethylene beakers. The pH of each solution was adjusted to 4.0 with 0.1 normal hydrochloric acid using a model N Beckman pH meter as suggested by Noggle and Fried (68), Tidmore (95) and Liggett (52). At the beginning of the absorption period the roots were suspended in the labeled solution and aerated constantly for the desired period of time. All uptake studies were made at 25° C. At the end of the absorption period the radioactive solution was decanted and the roots rinsed four times with deionized water to remove the phosphorus not associated with metabolic uptake (47, 52, 68). After rinsing, the roots were placed in a 30 milliliter beaker and digested in a nitric-perchloric acid mixture (66). The solution was evaporated to dryness, the residue dissolved in concentrated hydrochloric acid, transferred to a glass planchet, and the beaker rinsed with 1.5 milliliters of water containing one drop of a one per cent "Sterox"[2] solution. Digestion

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[2]Sterox, a special wetting agent for flame photometry. Obtained from E. H. Sargent and Co. Dallas, Texas.

beakers washed in this manner were free of radioactivity after one rinse. Samples prepared in this manner were placed on a sample spinner and evaporated to dryness under an infrared lamp prior to activity measurements. Aliquots of the original radioactive absorption solutions were pipetted into planchets, dried, activity measured and used as a standard for computing phosphorus absorption by roots.

All counting was performed with a counting system obtained from Nuclear-Chicago Corporation. The counting system consisted of a model 186 scaler and a model DS5-1P scintillation detector probe equipped with an XTB anthracene crystal. The detector was housed in a model 3053, aluminum veener, lead shield.

Kinetic analysis and calculation of the amount of acceptors present were made by the method of Noggle and Fried (68). The amount (q) of phosphorus absorbed from the various phosphorus concentrations (1 x 10^{-6} M to 1 x 10^{-3} M) was plotted against the amount absorbed divided by the concentration of phosphorus in solution [P]. The data were plotted in this manner for each of the experimental conditions studied. The curved plots were resolved into two linear components "a" and "b" by graphical methods (30). The various biological constants associated with the kinetic analysis of the plots were determined (68). Q_{max} "a" and Q_{max} "b" were determined directly from the ordinate intercept of lines "a" and "b". K_m "a" and K_m "b" were determined by calculating the slope of lines "a" and "b".

The amount of phosphorus absorbed from 1 x 10^{-6} molar and 5 x 10^{-4} molar phosphorus concentrations was plotted against time (68). The biological constants associated with these plots and directly applicable to the linear components "a" and "b" of the corresponding curvilinear

plots were determined (68)

Chromatographic and Electrophoretic Studies of Wheat Root Extracts

Plant Material: Experimental plant material consisted of excised roots from six-day-old and 21-day-old wheat seedlings. The six-day-old plants were grown in the manner previously described. The 21-day-old plants were grown in the plant growth chamber using experimental condition I as shown in Table 1. The excised roots were subjected to a 10-second absorption period in a 5×10^{-4} molar phosphorus solution labeled with P^{32}. After absorption the root tissue was washed four times with 250 milliliter portions of deionized water to remove root surface phosphorus.

Root Tissue Extraction: Extraction of P^{32} - labeled compounds from root tissue was essentially as described by Benson et al. (5) with modifications according to Runeckles (78). Water-rinsed roots were plunged into boiling ethanol (80 per cent) and extracted for 10 minutes using a reflux condenser to prevent loss of the azeotropic mixture. The supernatant liquid was decanted for immediate study or transferred with the extracted roots to a mortar and the entire mixture homogenized with sand. When homogenization was performed the removal of tissue residue was accomplished by centrifuging and decanting the supernatant liquid. Before chromatrographing the supernatant solution was concentrated approximately 10 times in vacuo at 20° C. (5, 79).

Chromatographic Separations: Sugar phosphate esters were separated by the two-dimensional method of Bandurski and Axelrod (2). Single batch sheets of Whatman No. 1 filter paper (28 cm. x 28 cm) were used. It was

found necessary to wash the paper prior to use. This was carried out in large enamel developer pans by allowing batches of 24 sheets to soak overnight in one-normal formic acid solution and then in 0.5 per cent ethylenediamine tetracetic acid brought to pH 8.5 with NaOH (17). The papers were then transferred to a large polyethylene pipette washer and washed with large amounts of distilled water. A final wash in 95% ethanol prevented the paper from wrinkling on drying (79). The machine direction of the paper was marked on each sheet and chromatograms developed in a sequence relative to machine direction.

Electrophoresis Separations: Electrophoresis was by the hanging strip principle suggested by Block et al. (6). A "Spinco", model R, Durrum type electrophoresis cell was used. Sheets of Whatman No. 1 filter paper (28 cm. x 30 cm.) were used. The paper was prepared in the same manner as described for chromatography. Sugar phosphates were separated by a modification of the Runeckles and Kortkov procedure (79).

Concentrated root extract was applied to electrophoresis paper at a point five centimeters in from each edge of the lower right hand corner of the paper. The paper was dipped into an electrolyte solution (five per cent pyridine and 0.5 per cent glacial acetic acid, pH 6), wetting the sheet to a distance of two centimeters on either side of the origin, and forming a dry band extending across the sheet. Prepared in this manner the sheet was mounted in the electrophoresis cell with edges making contact with heavy filter paper wicks extending into an electrolyte reservoir below. The wetting fronts were allowed to unite across the four centimeter dry band by capillarity before electrical potential was applied. Electrophoresis was carried out for seven hours at nine volts per centimeter of paper.

For two-dimensional examination chromatography was performed at right angles to the direction of electrophoresis using the solvent of Runeckles and Kortkov (79). The mechanics of chromatographing followed the method used by Bandurski and Axelrod (2).

Phosphoryl choline was separated by electrophoresis using the method of Maizel and Benson (60). The process was continued for 14 hours at 50 volts per sheet of paper.

Detection of Compounds: The chromatogram and/or electrophoresis papers were air dried and all phosphorus containing compounds detected by the method of Hanes and Isherwood (33) as modified by Bandurski and Axelrod (2). P^{32}- labeled fractions were detected by radioautographs (7). The dried chromatogram was placed on a 14 inch by 17 inch Kodak "no-screen" X-ray film with the origin at the lower right hand corner. The film was then exposed to the chromatogram for four to six days in a light proof "Kodak X-ray Exposure Holder", which was weighted with plate glass and lead "bricks" to equalize pressure distribution (5).

IV RESULTS AND DISCUSSION

The results are reported in four sections: (a) comparison of nutrient media, (b) root production with graded energy levels, (c) absorption kinetics, and (d) chromatographic and electrophoresis studies of root extracts following short term absorption from radioactive solutions.

Comparison of Nutrient Media

In the process of comparing the two nutrient media, the van Andel solution consistently proved inferior to the Hoagland solution. Appearances of the foliage of the wheat seedlings growing in the two solutions were not appreciably different. However, a very noticeable difference was evident in growth of the roots. The van Andel nutrient solution was inferior insofar as general appearance and quantity of root tissue produced per plant. In comparison the Hoagland nutrient solution indicated a much more favorable root environment from the standpoint of general appearance and quantity of root tissue produced per plant. The difference became more pronounced when the roots were examined quantitatively.

Root tissue grown in the two solutions was excised from the shoot and the yield and sugars determined. These data gave an immediate indication of the quantity of roots available and the carbohydrate levels. Differences obtained by experiments with the two solutions, as well as tap water, are summarized in Table IV. The striking differences obtained

between the two solutions cannot be attributed to a great variation in original elemental concentration. Neither can the poor results obtained with the van Andel solution be attributed to an overall nutrient deficiency. This conclusion is evident from the results obtained with tap water as the nutrient solution. The analysis of tap water is given in Table V. Results of these analyses are indicative of the fact that large ionic concentrations are not necessary for good root production, since those roots grown in tap water were produced in greater quantity and contained a much greater concentration of reducing sugar than those grown in the van Andel solution. The cause associated with the growth of inferior roots in this solution may be attributed to an impairment of root tissue. This impairment appears to be caused by root absorption activity which results in a major chemical change in the nutrient medium (97). The chemical change will finally establish a new pH environment that is not conducive to maximum root production and carbohydrate storage.

The pH of the two nutrient solutions is almost identical. Hoagland's solution has a pH of 5.7. Van Andel's solution has a pH of 5.65. Both solutions are poorly buffered. During absorption experiments the pH of Hoagland's solution changed considerably, rising to values as high as 8.0 as shown in Figure 4. The pH of van Andel's solution changed in the opposite direction to values as low as 3.7. The pH reported was that of the bulk solution. The pH of the root surface was at least one unit and perhaps as much as two units less (63). In the case of the solution having a pH of 3.7, the pH in the vicinity of the root surface could constitute an environment conducive to root cell disintegration. Visual evidence of root disintegration was very noticeable in roots grown in this nutrient

TABLE IV

REDUCING SUGAR AND YIELD DATA OF WHEAT ROOTS PRODUCED IN THREE NUTRIENT MEDIA AT VARIOUS TEMPERATURES*

Nutrient Solution	Percent Reducing Sugar (as glucose)			Mass per 168 plants (gms)		
	10°C.	15.5°C.	22.2°C.	10°C.	15.5°C.	22.2°C.
Hoagland	5.31	4.86	3.41	2.46	4.54	1.48
van Andel	1.17	0.96	--	1.05	1.76	--
Tap Water	4.03	--	--	2.05	--	--

*Average of 4 Trials

TABLE V

CHEMICAL ANALYSIS OF TAP WATER (OKLAHOMA STATE UNIVERSITY, JULY 1961)

Constituent	Concentration ppm
K	4.50
Ca	33.00
Mg	12.50
NH_3	0.75
NO_3	4.66

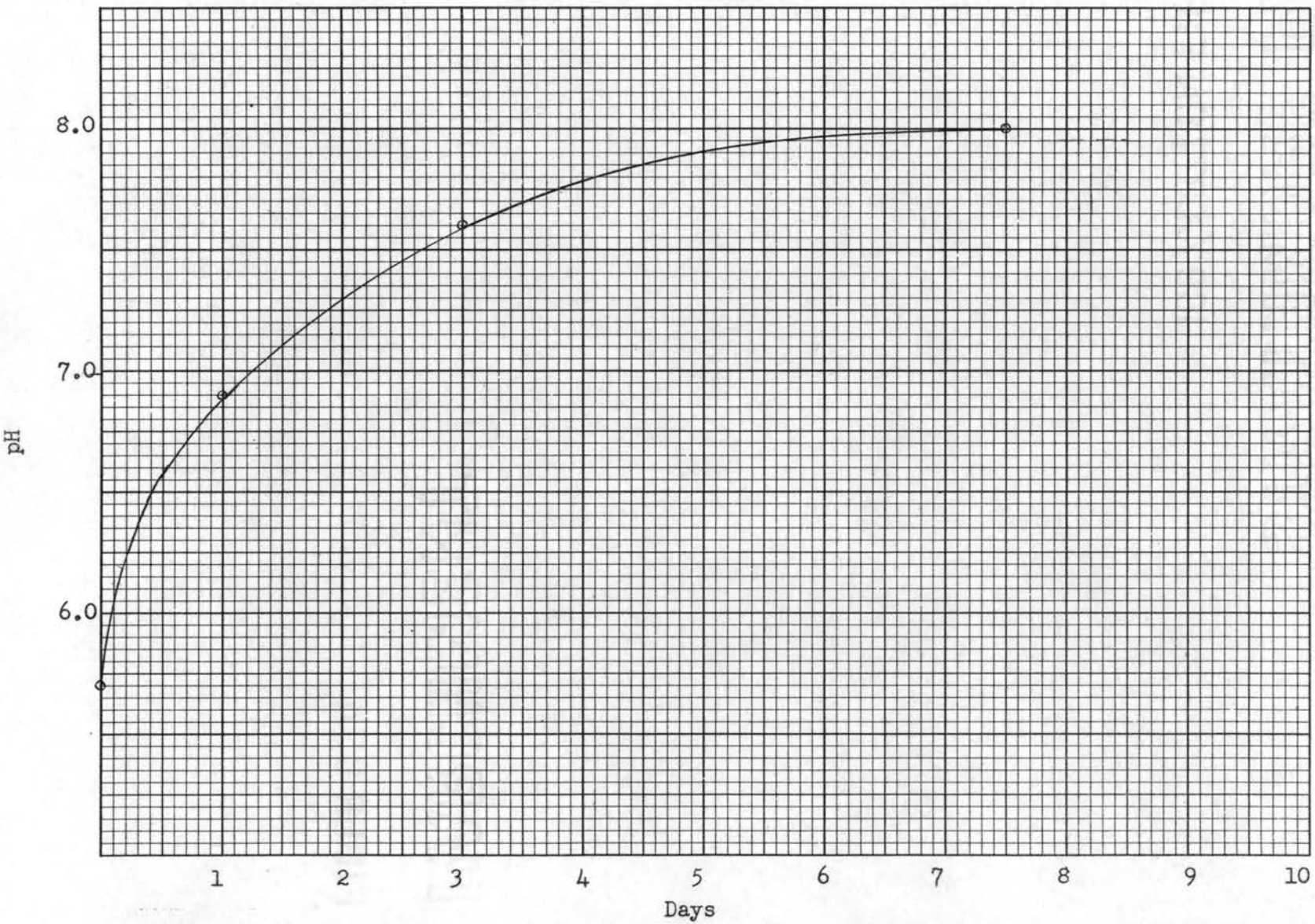

Figure 4. pH Change of Hoagland's Nutrient Solution During Time of Growth of Wheat Seedlings

solution. The solution contained a quantity of gelatinous material similar in appearance to that obtained after root tissue has been immersed in deionized water for several hours. This suggests a decline in the calcium activity of the solution. Burstrom (12, 13) has found calcium necessary for the growth of wheat roots.

Based on the results obtained, it was clearly established that the van Andel solution was inferior for the production of wheat roots having characteristics suitable for the continuance of this study. The Hoagland solution proved to be well adapted and was selected for use throughout the duration of the study.

Growth of Roots With Graded Energy Levels

A major purpose of this study was to learn more concerning the alteration of the carbohydrate content of roots. This was to be accomplished by changing certain environmental conditions.

Results obtained by holding light intensity and carbon dioxide constant while varying day length, temperature, and root environment are given in Table VI. The data in this table illustrate that the selected experimental conditions did result in a variation of carbohydrate content of root tissues. Although the reducing sugars present in this phase of the study are somewhat below those values reported earlier (Table IV), the trend is identical. No explanation can be given as to the wide variation noted here. However, Hoagland and Broyer (38) have reported a similar effect. It was their experience that root tissue produced during different seasons of the year varied considerably in root characteristics. The reducing sugar values reported in Table IV were from roots grown during the fall season of 1960. Reducing sugar values reported in Table VI were

TABLE VI

EFFECT OF TEMPERATURE, DAY LENGTH, AND ROOT ENVIRONMENT
ON CARBOHYDRATE STATUS OF ROOT TISSUE

Exp. Cond.	Day Temperature degrees C.	Light Duration Hrs.	Nutrient Solution Changes*	Per cent Reducing Sugars	Per cent Non-Reducing Sugars	Per cent Starch	Per cent Hemi-cellulose
I	10	15	0	4.74	2.50	0.02	10.72
II	15.5	13	1	1.55	1.58	0.15	12.79
III	21.1	11	3	0.52	0.48	0.04	8.49
IV	26.7	9	6	1.40	0.64	0.04	10.00

*Per 14 days

data from roots grown during the spring season of 1961. Reducing sugars and non-reducing sugars followed a similar pattern for the four experimental environments studied. These patterns are shown in Figure 5. The patterns for the polysaccharides are given in Figure 6.

Examination of the data indicates that, under the conditions of this study, increasing temperature, shorter days, and increasing salt content of the nutrient solution decreased the sugar content of roots for the first three regimes studied. There was a reversal of the trend for the fourth regime. Here there is a trend toward an intermediate carbohydrate content.

Results for starch and hemicellulose follow a similar pattern for the four environments studied. Results of the effect of environment on the total of the four carbohydrates studied are presented in Figure 7. The plotted data show a decrease in the total carbohydrate for the first three environments with a reversal for the fourth environment similar to that for sugars.

The reversal of the results of the fourth experimental condition cannot be explained at this time. At first one might suspect that the temperature may have gone beyond the respiration optimum, a complex temperature-respiration interaction whereby respiration decreases beyond a given temperature (64). However, McAlister (61) and Todd[2] have shown that the rate of respiration for wheat, does not decline at temperatures above 90 degrees Fahrenheit. Therefore, it seems unlikely that decreased respiration was responsible for the results obtained.

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[2]Todd, G. W. Private Communication. Department of Botany and Plant Pathology, Oklahoma State University, 1961.

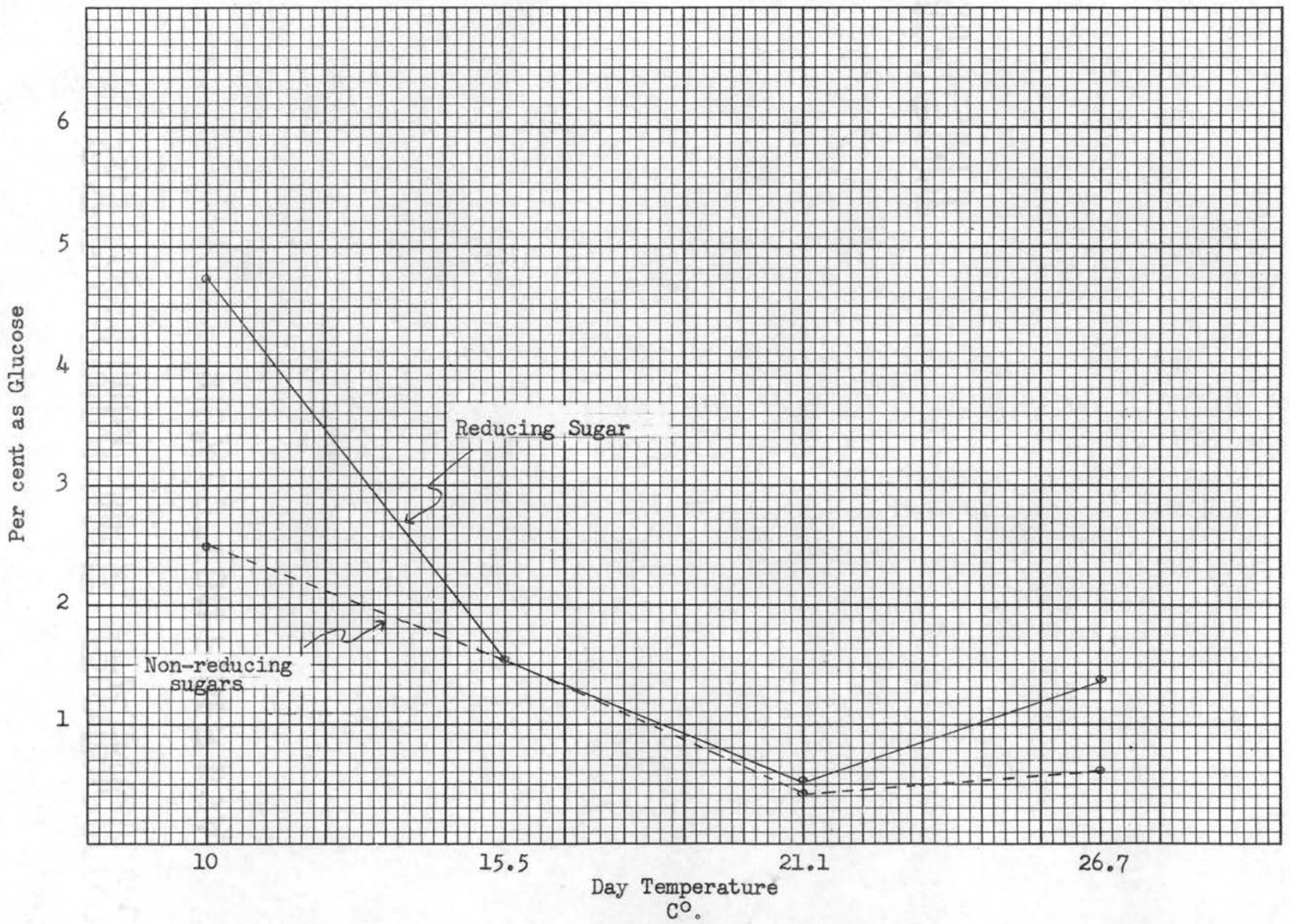

Figure 5. Effect of Experimental Environments on Monosaccharides and Oligosaccharides in Wheat Roots.

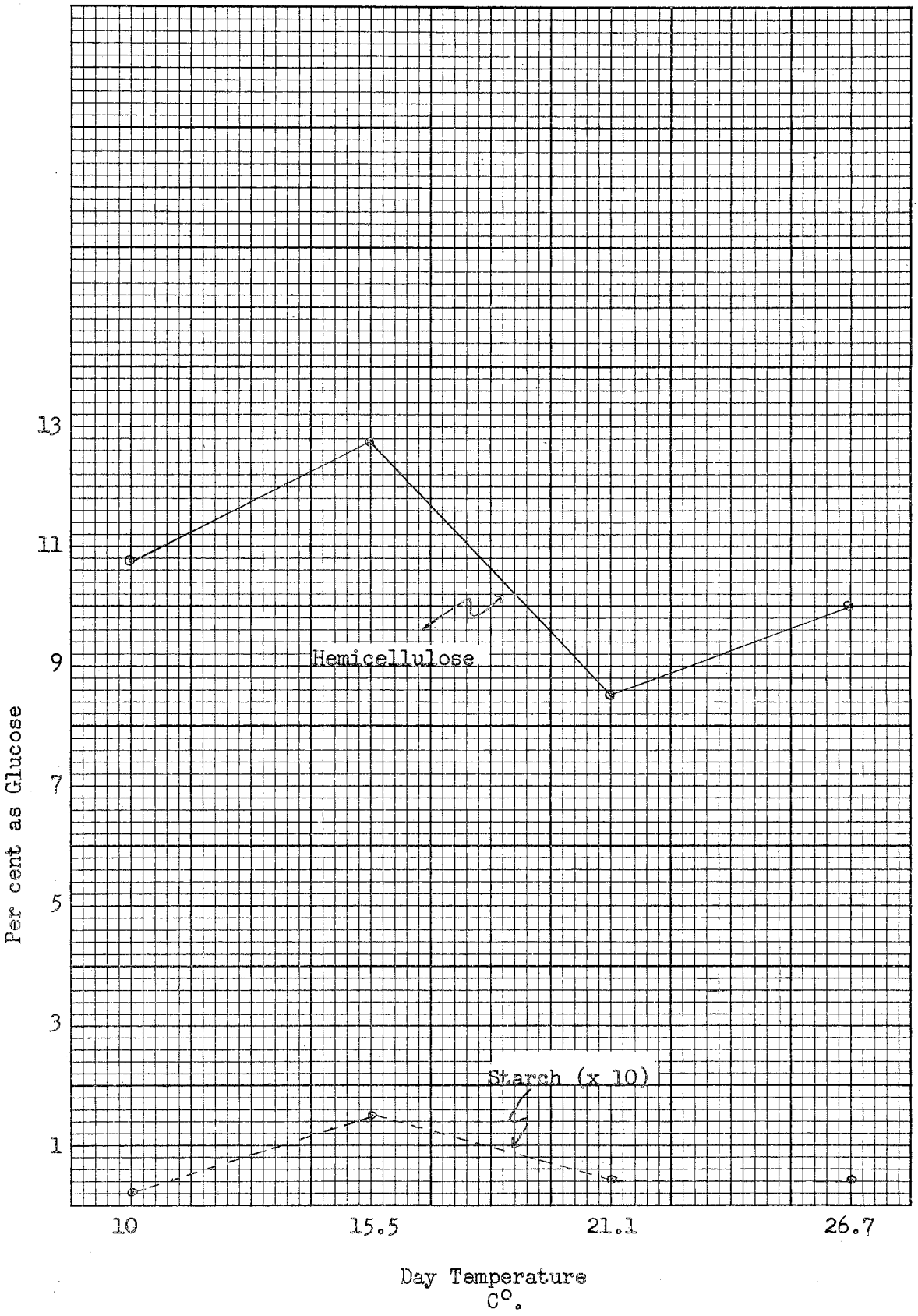

Figure 6. Effect of Experimental Environments on Polysaccharides in Wheat Roots.

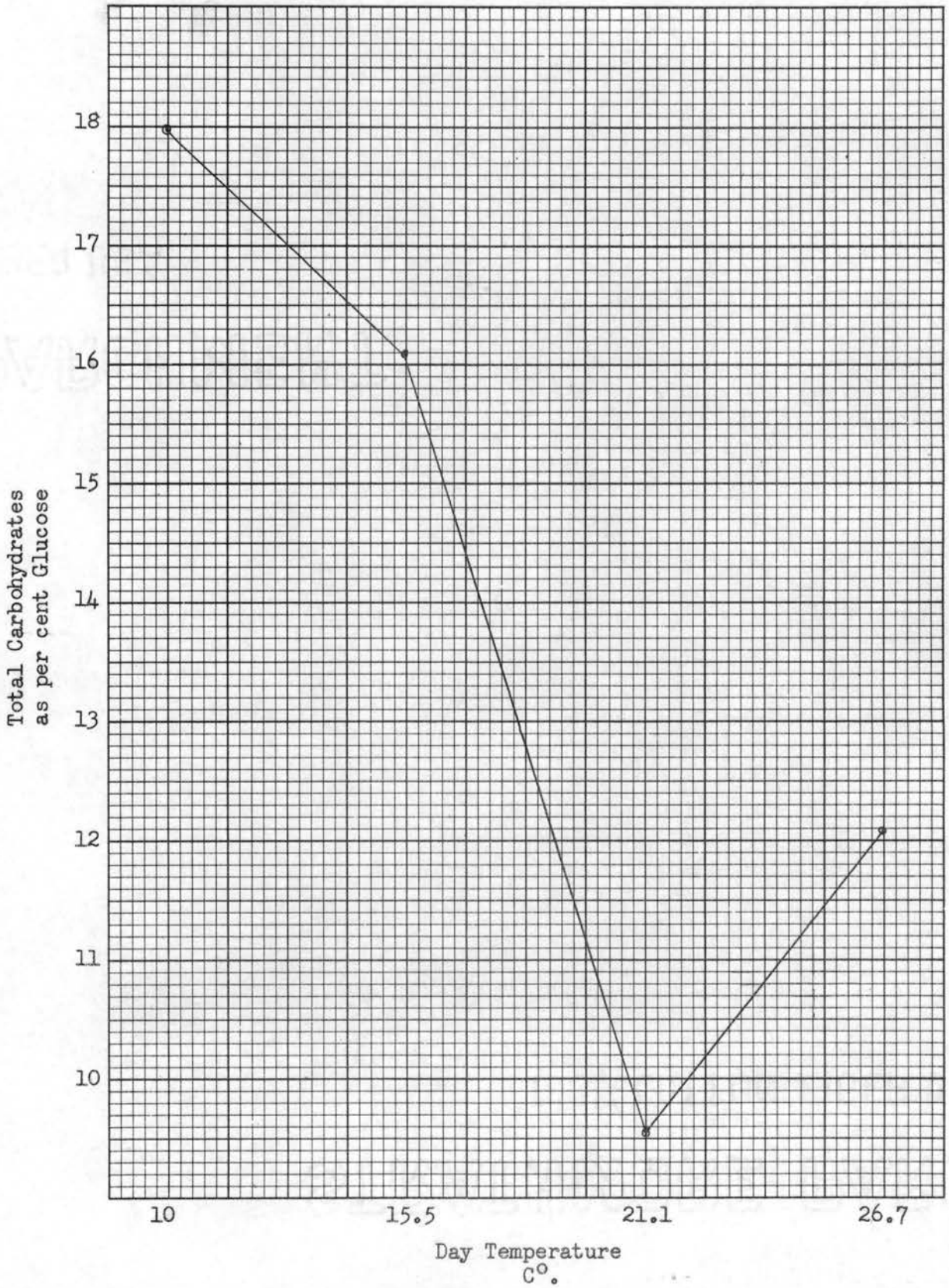

Figure 7. Effect of Experimental Environments on Total Carbohydrate in Wheat Roots

Kinetics of Phosphorus Absorption

The absorption of phosphorus from solutions of various concentrations (1×10^{-6} molar to 1×10^{-3} molar) was measured for each environmental regime. The amount of phosphorus absorbed was plotted against the amount absorbed divided by the solution concentration as shown in Figures 8, 9, 10, and 11. These data show that the uptake of phosphorus when plotted in this manner is curvilinear. This curvilinear relationship suggests two acceptor systems which operate simultaneously and independently on the same substrate (phosphorus). Apparently wheat absorbs phosphorus by two independent absorption reactions. This is consistent with the results of Hagen and Hopkins (30) for phosphorus uptake by excised barley roots. The curvilinear plots were each resolved into two linear components according to graphic methods (30). The linear plots were designated as "a" and "b". Each of these lines provided maximum absorption values (Q_{max}) for each acceptor ("a" and "b") as well as apparent dissociation constants (Km) for each acceptor complex. The values for Q_{max} and K_m for the four experimental conditions are shown in Table VII.

The absorption of phosphorus from solutions of 1×10^{-6} molar and 5×10^{-4} molar phosphorus concentrations was measured at two-minute intervals for a period of 10 minutes for each experimental condition. The amount of phosphorus absorbed was plotted against time as shown in Figures 12a and 12b. The plots gave straight lines indicating that steady-state uptake occurs during this period. Since most of the total phosphorus absorbed by the roots from 1×10^{-6} molar solution is contributed by the reaction that is defined by line "b" in the curvilinear

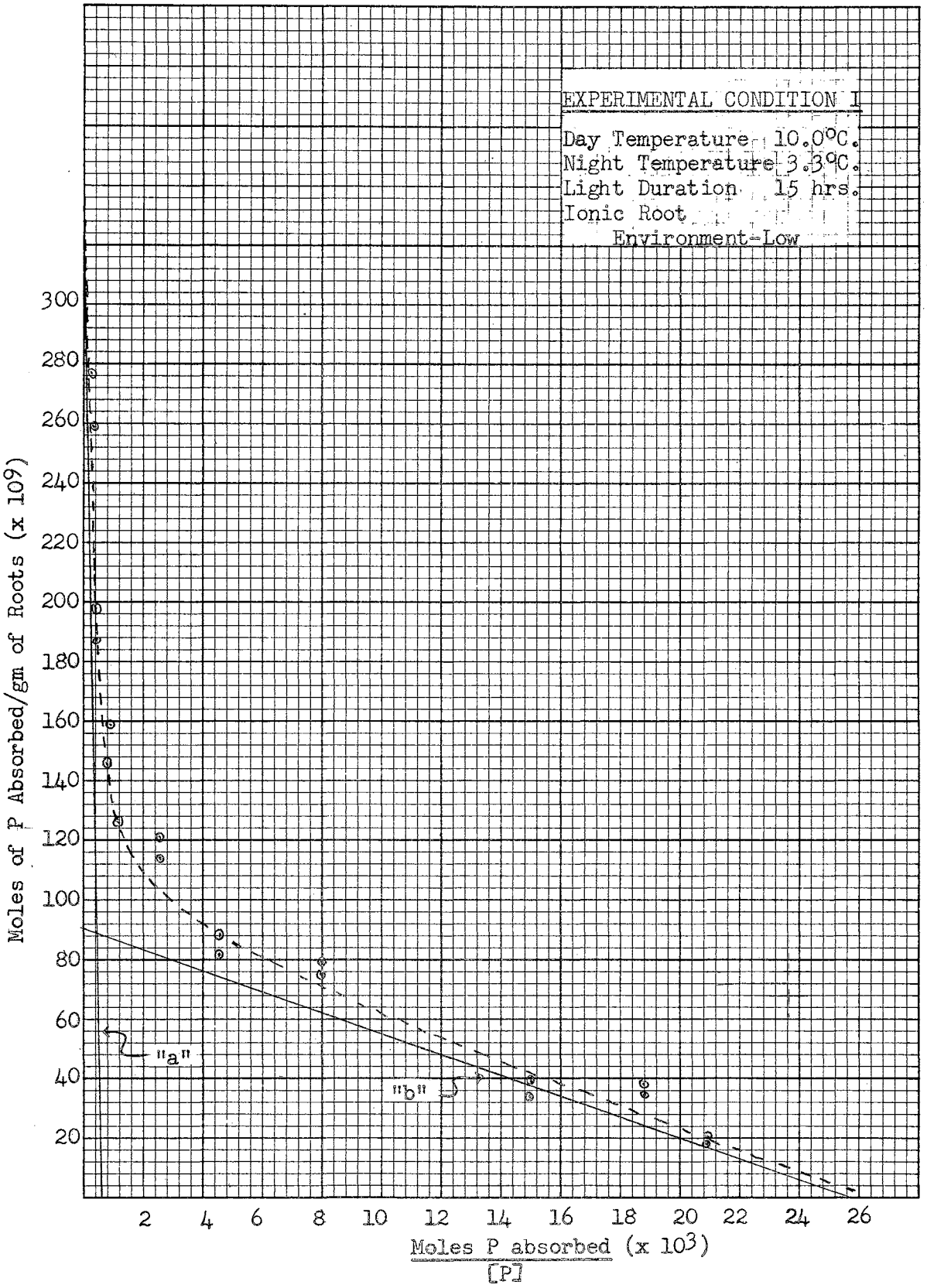

Figure 8. The Effect of Phosphorus Concentration upon the Uptake of Phosphorus by One Gram of Excised Wheat Roots in 10 Minutes. Experimental Condition I.

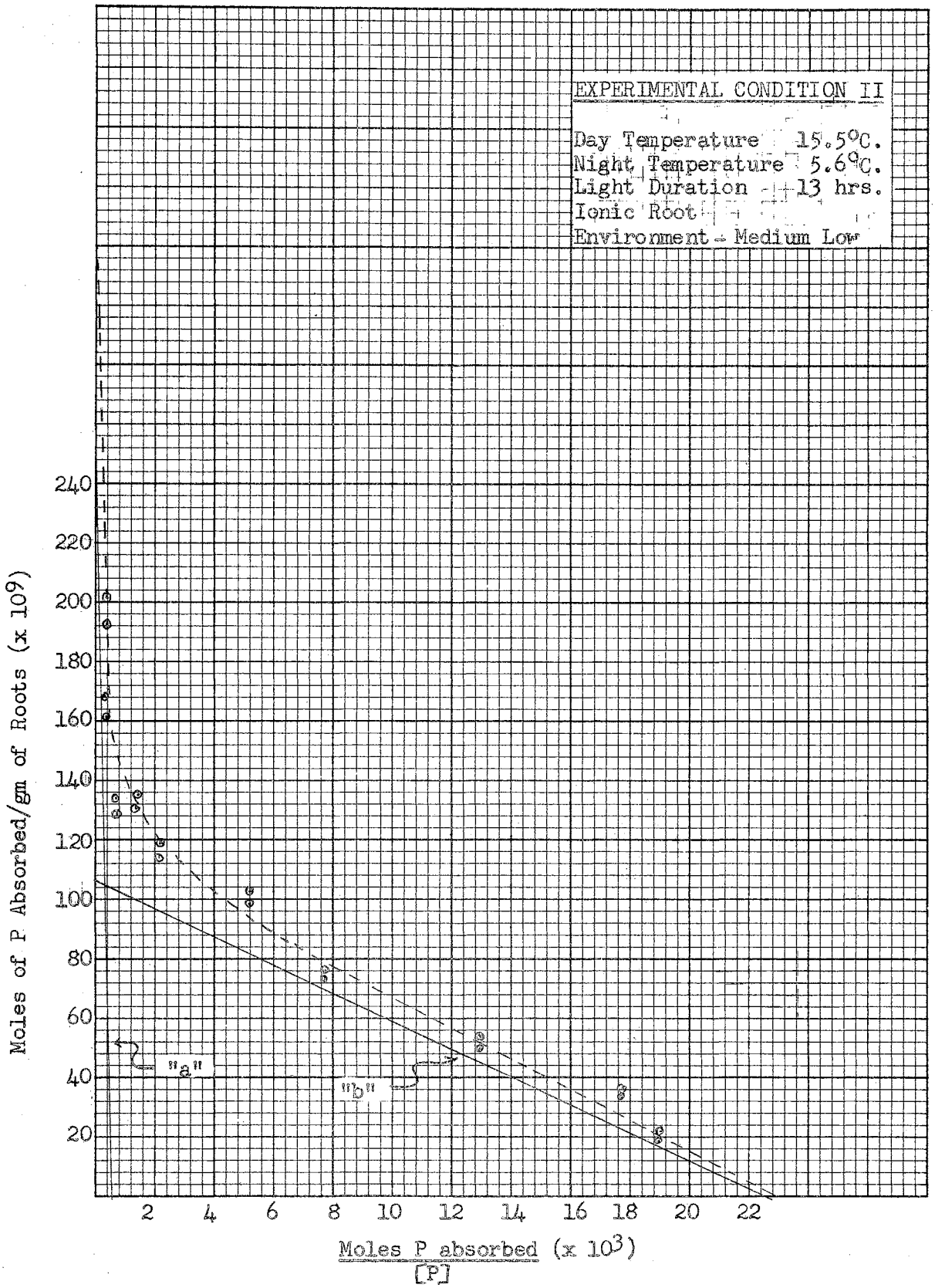

Figure 9. The Effect of Phosphorus Concentration upon the Uptake of Phosphorus by One Gram of Excised Wheat Roots in 10 Minutes. Experimental Condition II.

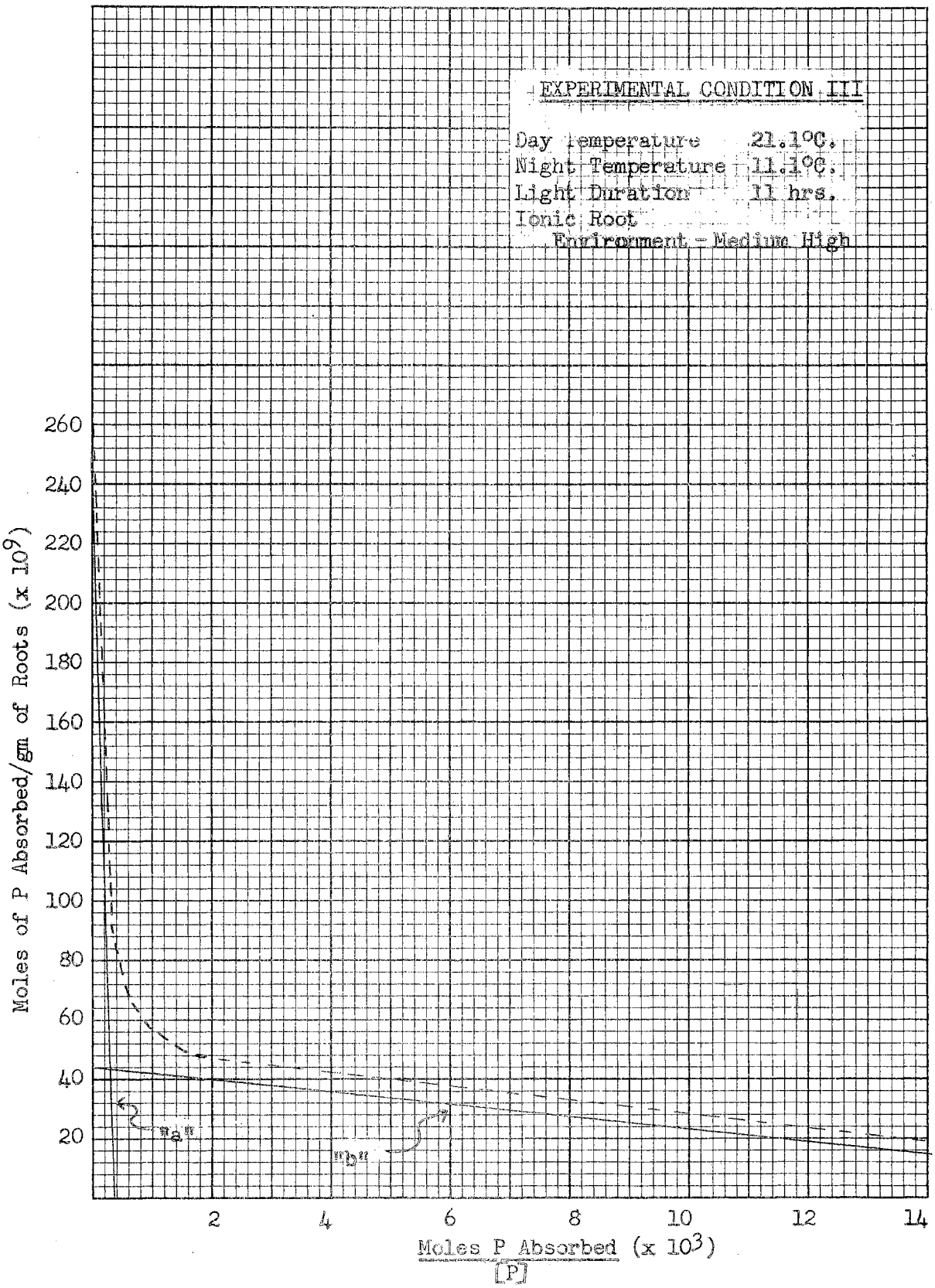

Figure 10. The Effect of Phosphorus Concentration upon the Uptake of Phosphorus by One Gram of Excised Wheat Roots in 10 Minutes. Experimental Condition III.

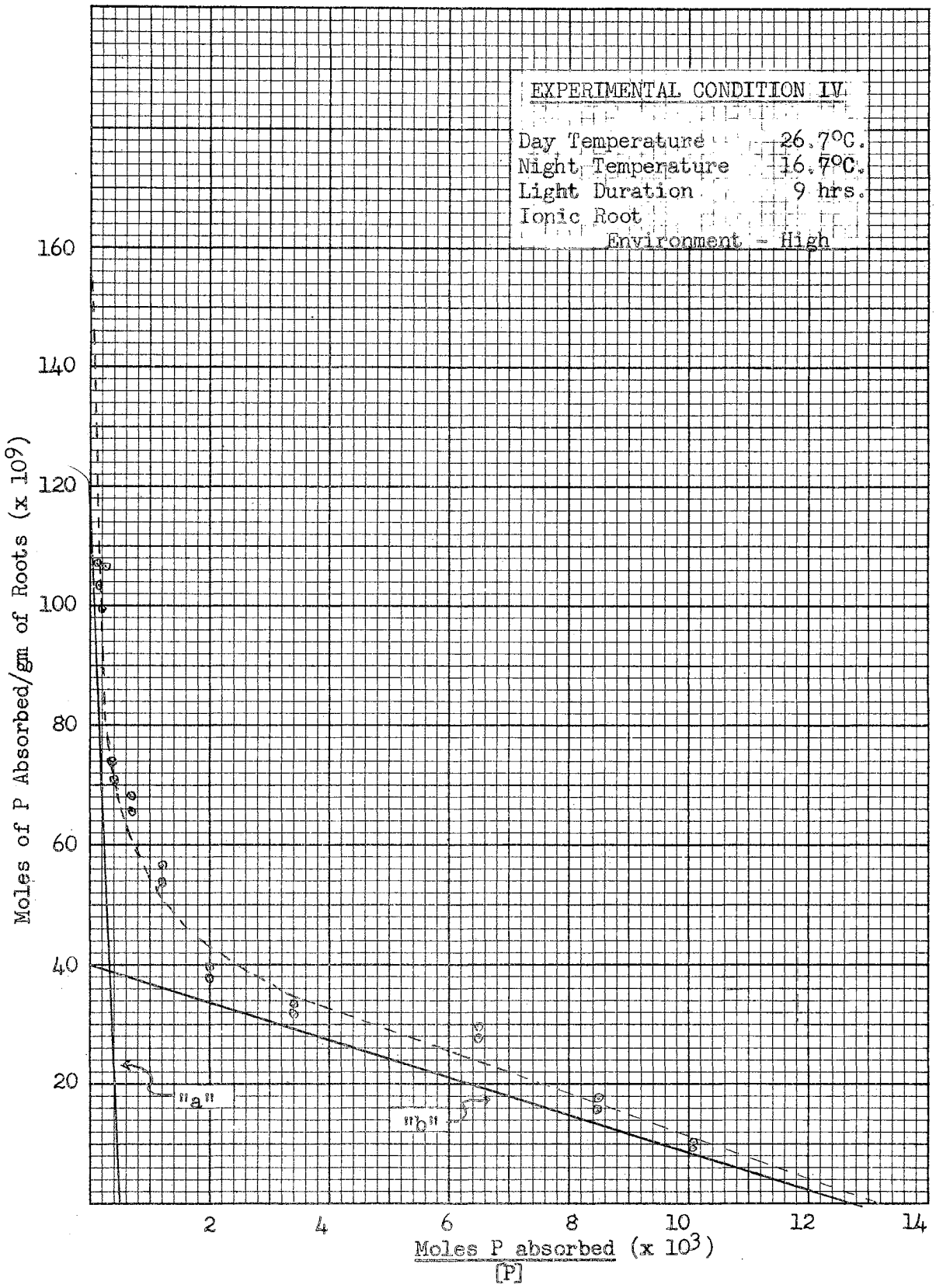

Figure 11. The Effect of Phosphorus Concentration upon the Uptake of Phosphorus by One Gram of Excised Wheat Roots in 10 Minutes. Experimental Condition IV.

TABLE VII

V_{max}, DISSOCIATION CONSTANTS, RATE CONSTANTS AND CONCENTRATION OF THE TWO ACCEPTORS INVOLVED IN PHOSPHORUS UPTAKE BY WHEAT ROOTS

Experimental Condition	Q_{max} Moles P x 10^9/gm roots "a"	Q_{max} Moles P x 10^9/gm roots "b"	Dissociation Constants K_m"a" x 10^{-4}	Dissociation Constants K_m"b" x 10^{-6}	Rate Constants Moles P x 10^3/Mole AP/sec. k_3"a"	Rate Constants Moles P x 10^3/Mole AP/sec. k_3"b"	Acceptor Concen. Moles x 10^8/gm roots A"a"	Acceptor Concen. Moles x 10^8/gm roots A"b"
I	296	91	4.6	3.6	2.9	6.3	10.8	1.9
II	252	106	4.2	4.7	2.5	8.4	10.2	1.8
III	216	45	6.6	2.4	0.8	21.3	14.3	0.3
IV	127	41	2.6	3.2	5.1	4.9	3.1	1.1

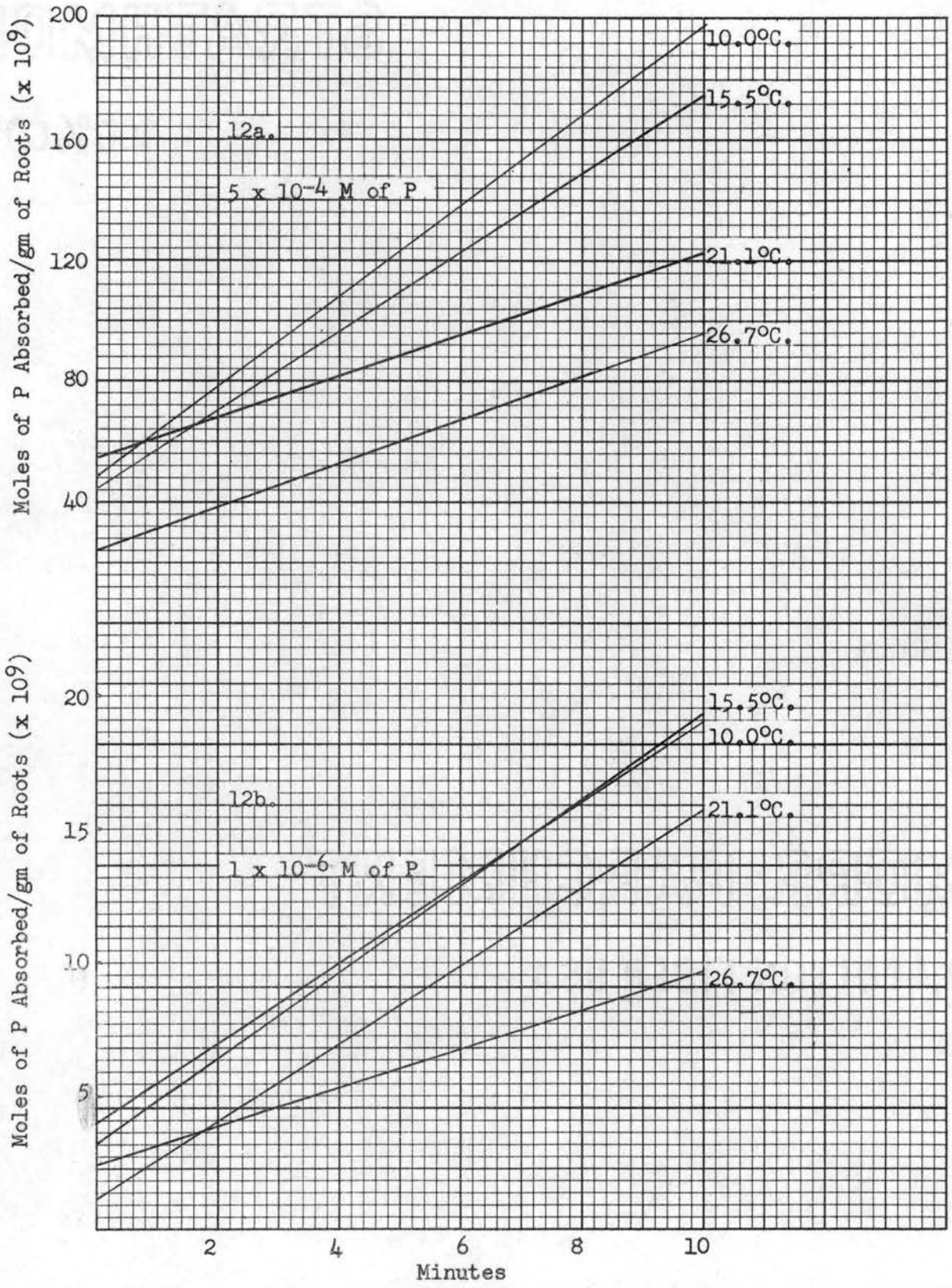

Figure 12. Rate of Phosphorus Uptake From Two Concentrations For Wheat Roots Produced by Four Different Environments.

plots, a determination of k_3"b" was made for each experimental condition from Figure 12b. Also a value for A"b" per gram of roots for each condition was obtained by substituting k_3"b" and Q_{max}"b" into equation 19. Values of k_3"a", associated with line "a" of the curvilinear plots, were obtained for each experimental condition by subtracting A"b" from the ordinate intercept of the 5 x 10^{-4} molar time plot (Figure 12a) and substituting the difference (A"a"P) into equation 22. A value for A"a" per gram of roots was obtained by substituting k_3"a" and Q_{max}"a" into equation 19. The k_3"b", A"b", k_3"a", and A"a" values for the four experimental conditions are shown in Table VII.

The data from the plots of the amount of phosphorus absorbed versus time show that the rate of phosphorus uptake from equal concentrations of phosphorus is generally greater for roots having a higher energy reserve. More specifically those roots grown in the long day, cool temperature environment had a greater phosphorus absorbing capacity than those grown in a short day, higher temperature environment. Phosphorus absorbing capacity decreased with each increase in temperature and each decrease in day length. Only one exception was noted in this respect. The rate of phosphorus absorption from the 1 x 10^{-6} molar solution is almost identical for experimental conditions I and II. Therefore, it appears that absorption rates from very dilute phosphorus solutions is not materially changed by conditions I and II. The absorption rates from the more concentrated phosphorus solution (5 x 10^{-4}M) are noticeably changed by the same regimes. Examination of the various biological constants in relation to rate of absorption are of interest. In experimental conditions I and II there is some difference in the

acceptor concentrations associated with reactions "a" and "b" of the curvilinear plots. There is, however, a greater difference in Q_{max} values for the two reactions. Both k_3 and acceptor concentration affect the Q_{max} of their respective acceptors. Also at low external phosphorus concentrations K_m affects absorption. The increase of Q_{max}"b" in experimental condition II over experimental condition I is due primarily to the increase in k_3"b". At the same time K_m"b" is contributing to an increase in Q_{max} from the standpoint of per cent acceptor saturation. Growth environment III presents a situation where the acceptor is 33 per cent saturated at 1×10^{-6} molar concentration. Although the rate constant is large, phosphorus absorption is restricted because of the low concentration of acceptor. At high external phosphorus concentrations both acceptors approach 100 per cent saturation with phosphorus and maximum uptake occurs for both acceptors. K_m"a" is not a principle factor here; Q_{max} is governed only by total acceptor concentration in connection with K_3"a". In all four environments Q_{max}"a" continues to decrease as temperature increases and day length decreases. Under experimental condition IV, the concentration of A"a" is decreased by a factor of three to four when compared to the other systems studied. This large decrease in acceptor concentration is somewhat offset by an increase in the rate constant (k_3"a"). It appears that a decrease in ion acceptor concentration in the wheat root is partially accompanied by an increase in the acceptor rate constant. This increased rate factor might be a compensator for the loss in phosphorus absorbing capacity associated with decreased acceptor concentration.

In this study it was found that the uptake of phosphorus by excised wheat roots could be controlled by environmental pre-conditioning. Envi-

ronmental conditions favorable to the accumulation of photosynthetic products in root systems enhanced the phosphorus uptake capacity of roots. In general phosphate absorption appears to be associated with the sugar reserve contained in roots, however, acceptor concentration is not proportional to root sugar reserve. Ion acceptor concentration values per gram of roots indicate that the total concentration of both acceptors per plant is dependent upon the amount of roots produced per plant. It is also possible, however, that under a given environment, any practice conducive to the development of an extensive root system might result in a dilution effect of total acceptor concentration instead of a proportional increase. Although there are differences in the total acceptor concentrations between the various environmental conditions, the greatest differences governing phosphorus absorption are in the rate constants governing the release of phosphorus from the acceptor complex into the cell and vascular system of the plant.

In another kinetic study the experimental plant material consisted of excised roots from six-day-old etiolated wheat seedlings. Results of this study are presented in Figures 13, 14, and 15. The purpose of this experiment was to obtain phosphorus absorption information for comparison with some results obtained by Noggle and Fried (68) for barley, millet, and alfalfa.

Kinetic data from this study were calculated and the several biological constants evaluated as in the previous study. These biological constants are presented in Table VIII along with constants for millet, barley, and alfalfa as determined by Noggle and Fried (68). The data show the relative phosphorus absorbing power of wheat roots from equal

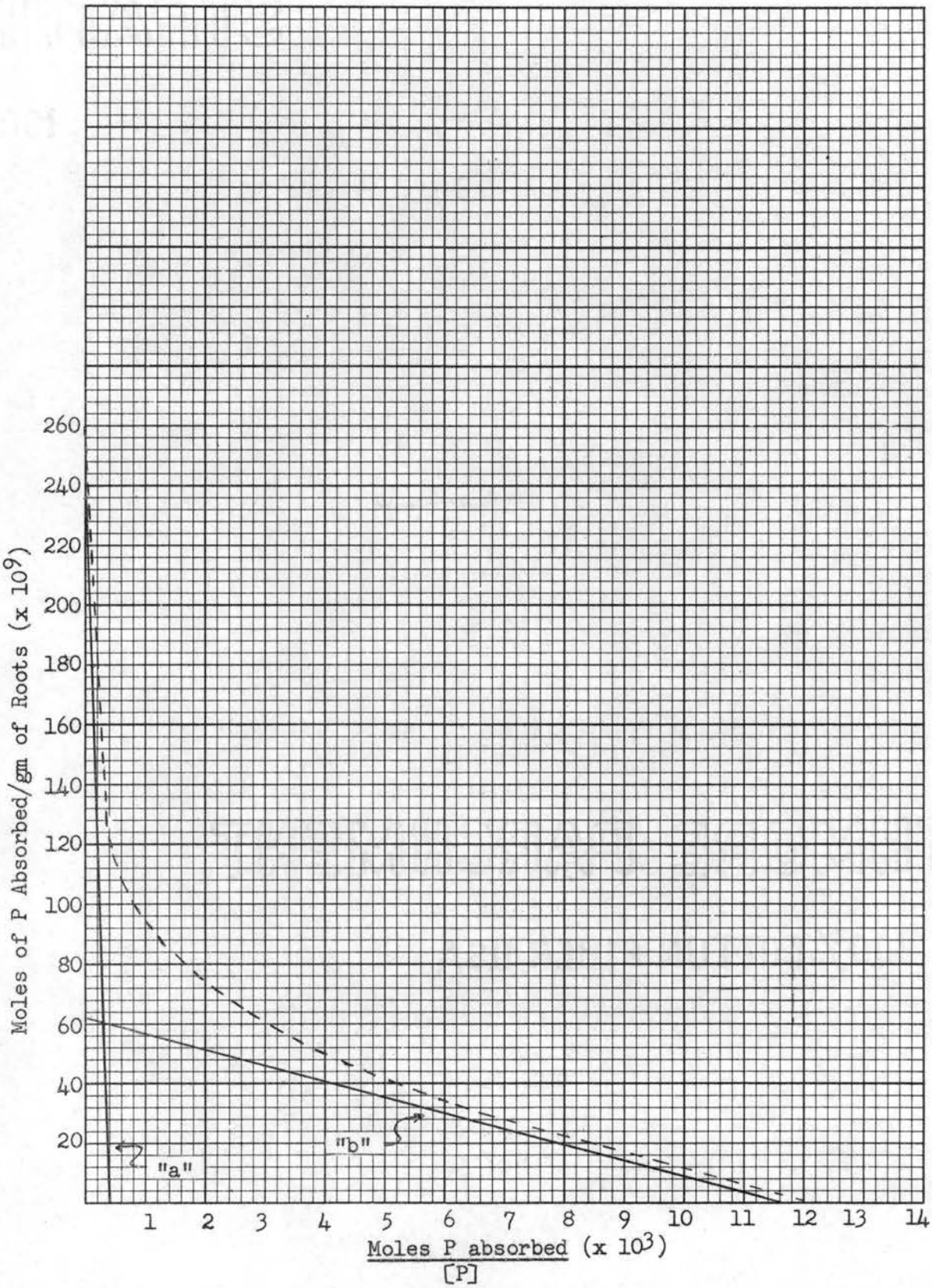

Figure 13. The Effect of Phosphorus Concentration upon the Uptake of Phosphorus by One Gram of Excised Six-Day-Old Wheat Roots.

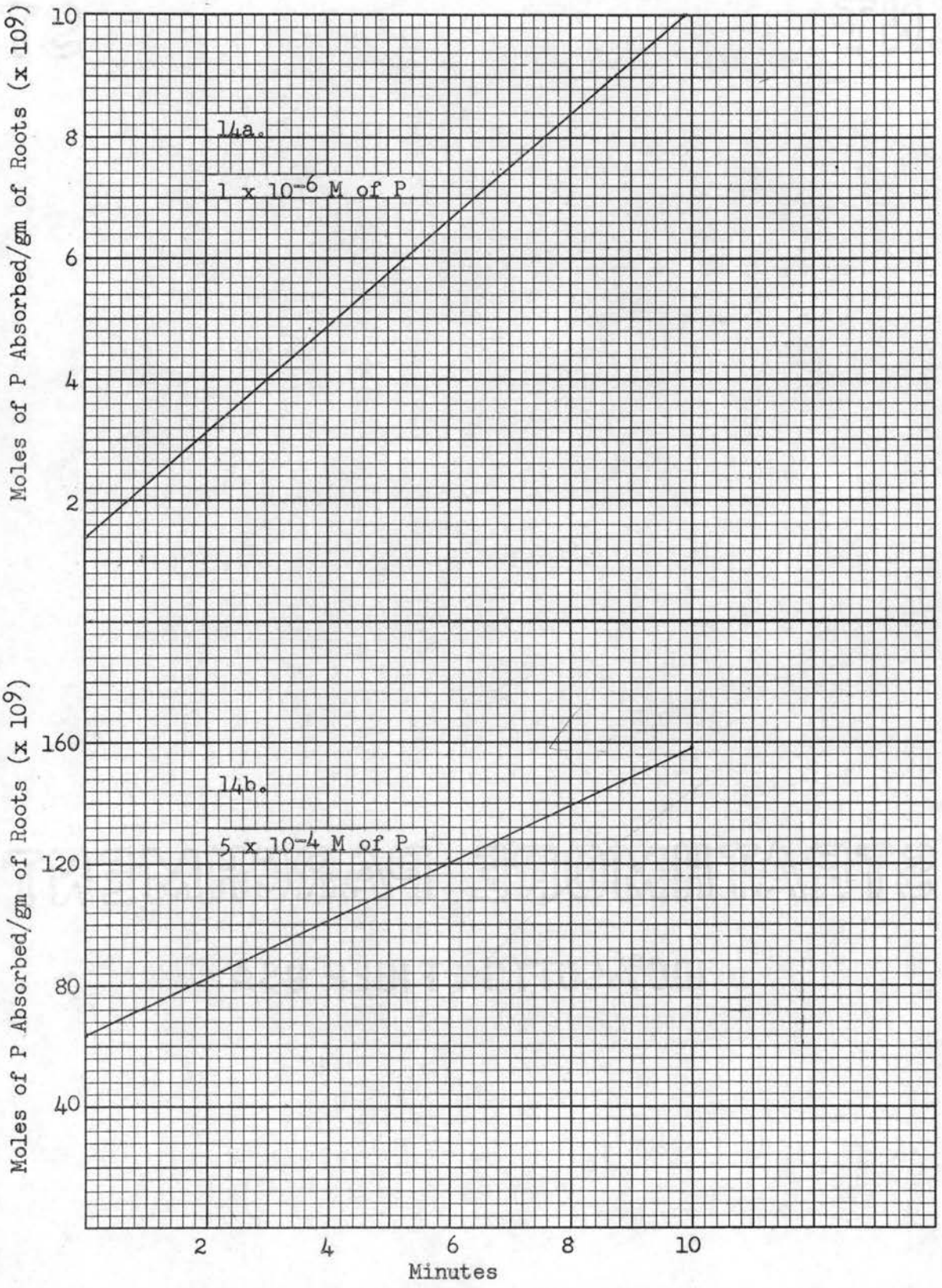

Figure 14. Rate of Phosphorus Uptake From Two Concentrations For Six-Day-Old Wheat Roots.

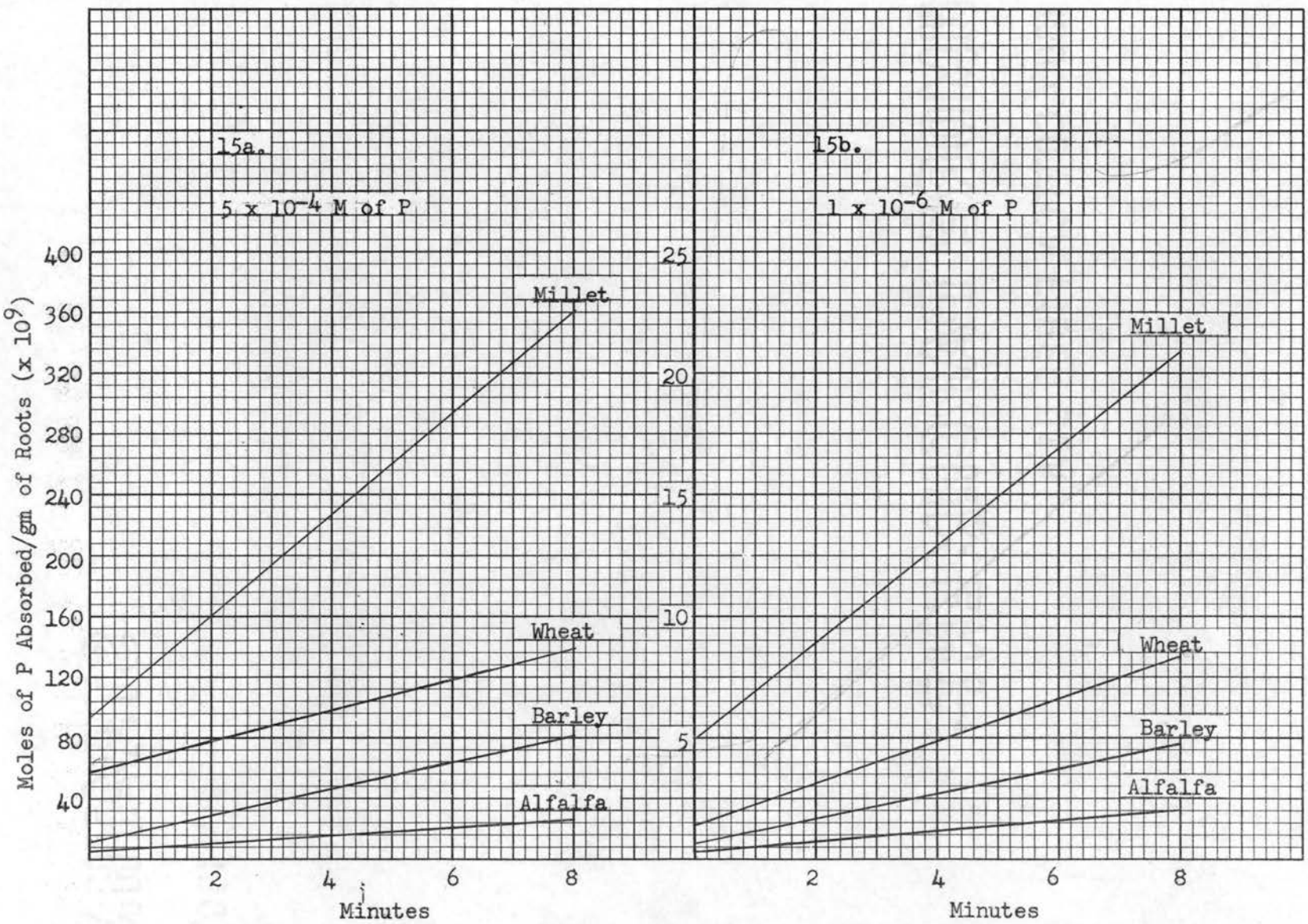

Figure 15. Phosphorus Uptake by One Gram of Excised Roots of Millet (68), Wheat, Barley (68), and Alfalfa (68) at Two Phosphorus Concentrations.

TABLE VIII

THE DISSOCIATION CONSTANTS, RATE CONSTANTS, AND CONCENTRATION OF THE TWO ACCEPTORS INVOLVED IN PHOSPHORUS UPTAKE BY MILLET (68), WHEAT, BARLEY (68), AND ALFALFA (68).

Plant	Dissociation Constants		Rate Constants Moles P x 10^3/Mole AP/Sec.		Acceptor Concentration Moles x 10^8/gm roots	
	K_m"a" x 10^4	K_m"b" x 10^6	k_3"a"	k_3"b"	A"a"	A"b"
Millet	9.6	3.5	5.4	7.0	18.9	2.2
Wheat	5.2	5.4	2.0	10.7	10.7	0.8
Barley	9.0	8.0	12.6	16.6	2.0	0.7
Alfalfa	9.4	2.7	8.1	15.0	0.7	0.1

phosphorus concentrations when compared with other plants as given in Figure 15. Evaluation of the rate constants indicates that the difference in phosphorus absorption between genera, wheat included, is due primarily to a difference in the amount of acceptor present in the roots. This was also the conclusion of Noggle and Fried (68) with regard to millet, barley and alfalfa.

The pooled data for the four genera rank them in decreasing rate of phosphorus uptake as millet, wheat, barley and alfalfa respectively. At high phosphate concentrations wheat absorbs phosphorus at a more rapid rate than barley because of the presence of five times as much acceptor in the root tissue. At very dilute phosphate concentrations wheat absorbs phosphorus at a more rapid rate than barley because of the difference in per cent saturation of acceptor "b". Noggle and Fried (68) reported that acceptor "b" for barley is only 11 per cent saturated at 1×10^{-6} molar concentration of phosphorus while the data for wheat indicate that acceptor "b" is 14.4 per cent saturated at the same phosphorus concentration. These kinetic data indicate that the phosphorus absorbing power between genera is largely due to a difference in the total amount of acceptor present for a given mass of roots.

Chromatographic and Electrophoretic Studies of Wheat Root Extracts

The purpose of this portion of the study was to learn more about the metabolic products labeled by radioactive phosphorus in the studies with wheat roots. Theoretically any knowledge gained in this study should have application to other plant tissues. Also experience gained from the work might be helpful in future research of a similar nature. With this

in mind the isolation of some products of phosphate absorption by roots was attempted. In general no positive results were obtained, insofar as identification of a labeled acceptor was concerned. In two instances it was thought that positive identification had been obtained; however, the results could not be duplicated.

When 21-day-old wheat roots were used for absorption experiments, no measurable amounts of P^{32}-labeled or unlabeled adenosine triphosphate (ATP) were found. This was true when the two-dimensional chromatographic procedure of Bandurski and Axelrod (2) was used as well as the electrophoresis procedure of Runeckles (78). Both of these methods are well adapted for the detection of ATP. Since the 21-day-old roots had been grown in a phosphorus containing nutrient medium, it was thought that the phosphorus turn-over rate might be retarded for short term absorption due to an adequate supply of cellular phosphate. Subsequently, a set of six-day-old wheat roots was grown, using tap water as substrate, thereby limiting phosphorus metabolism to that originally contained in the wheat seed. After a 10-second absorption period in a radioactive phosphorus solution, the chromatographed root extract migrated to a location identical to ATP. Autoradiography confirmed that the location was radioactive. All attempts to duplicate this result were failures. Therefore, it was not possible to obtain a chromatographed sample for co-chromatography with an authentic sample of ATP. A second occasion involved a tentative identification of fructose 1,6-diphosphate but it was not possible to duplicate the result.

Another phase of the study was the examination of wheat root extracts for phosphoryl choline. The identification method is very

specific for the compound. All electrophoresis studies of root extract gave negative results for phosphoryl choline. When the root extract was electrophoresed alone, inorganic phosphate was the only compound detected. When root extract was electrophoresed with reagent phosphoryl choline, both inorganic phosphate and the phospholipid were readily detected. Therefore, phosphoryl choline was not found in wheat roots by the extraction procedure that was used. Attempts to identify lecithin were carried out in a similar manner. In no case was lecithin present in wheat root extracts in measurable amounts.

Considerable effort was devoted to the chromatographic and electrophoretic studies of some of the sugar phosphates, of which the sugar moiety might function as a short term metabolic intermediate of phosphorus absorption. One phosphate location was obtained when wheat root extracts were examined. The spot was always identified as inorganic phosphate. Autoradiography and a survey meter confirmed the presence of radioactive phosphorus in the location. Assuming that all non-metabolically absorbed phosphorus was removed from the roots by four washings with distilled water (47, 52, 60), it was evident that radiophosphorus had been incorporated metabolically into root cells. Based on the assumption that the inorganic radioactive phosphorus had entered via the acceptor route, it seemed logical that the acceptor compound should have been adequately labeled. However, it was thought that perhaps 10 seconds was not a sufficient time period to allow adequate P^{32} to combine with the acceptor for detection by autoradiographic methods. Subsequently, several 60-second absorption studies were performed and, after electrophoresis, the chromatograms were examined both colorimetrically and autoradiographically. The results were identical with those

obtained with a 10-second absorption period. Only inorganic phosphate was present. This phenomenon was also encountered by Kamen and Spiegelman (49), who found that animal cells with normal active metabolism tend to dispose of large quantities of unstable bound phosphate.

Jackson and Hagen (47) found at least five phosphate compounds in extracts from barley roots. Three of the compounds were identified after a 10-minute absorption period. Ten minutes seem to be a rather long time period when one is attempting to determine the identity of a "first-tagged" acceptor. It is highly probable that those compounds identified by Jackson and Hagen (47) were the products of cellular metabolism beyond the acceptor mechanism. A 10-second absorption period is probably not too short. The phosphorus content of roots, following a 10-second absorption period from a radioactive solution, showed that phosphorus uptake, due to metabolic absorption, was approximately 5000 counts per minute. It seems more reasonable to assume that the acceptor concentration is present in such minute quantities that it is not capable of being detected by the procedures used. Actually the extraction procedure did not remove all of the metabolically absorbed phosphorus. After extraction, the root residue was assayed for radioactivity. It was found that large amounts of metabolically bound P^{32} remained. Therefore, it was apparent that considerable amounts of the incorporated phosphorus were not removed by the alcohol extraction method. Extraction of the roots and measurements on the various fractions showed that only 41 per cent of the total phosphorus absorbed in 10 seconds by six-day-old wheat roots is soluble in ethanol. Further

fractionation of tissue by alcohol extraction plus homogenization of the root tissue with sand, in a mortar, shows that only 38 per cent of the total phosphorus absorbed in 10 seconds is soluble. It was somewhat surprising to notice that grinding root tissue with sand, after alcohol extraction, resulted in a decrease in the amount of phosphorus removed. Repeated investigations gave similar results. Therefore, it was concluded that fractionation by grinding with sand was not a desirable procedrue, and under the conditions of this study did not contribute to phosphorus removal from root tissue. Extraction of phosphorus from 21-day-old wheat roots showed that 64 per cent of the total phosphorus absorbed in 10 seconds was soluble in alcohol. Thus the activity associated with the alcohol insoluble fraction contained up to 59 per cent and 36 per cent of the total absorbed P^{32} after a 10-second absorption period, for six-day-old and 21-day-old roots, respectively. The difference exhibited by the two root systems is not readily explained at this time. There is a possibility that further study of the non-extracted P^{32} in both root systems might reveal information concerning the absorption mechanism. At least it should lend itself to investigation.

V SUMMARY

Wheat seedlings were propagated in a plant growth chamber and in a constant temperature room, each providing a degree of controlled environment. In the beginning two nutrient solutions were compared to determine the one best suited for the experiment. Results indicated that a nutrient solution devised by Hoagland was far superior to a nutrient solution formulated by van Andel. For this study Hoagland's solution produced extensive and healthy-appearing root systems capable of storing considerable quantities of energy in the form of carbohydrates. The growth of wheat roots in the van Andel solution was meager, and the roots were considerably lower in carbohydrate than those grown in the Hoagland solution.

Wheat plants were grown in the Hoagland nutrient solution. Four different experimental conditions were used for the growth of wheat in the plant growth chamber. The roots produced under each condition were studied from the standpoint of the effect of each environment on the carbohydrate content of roots and on the absorption rate of phosphorus. The results indicated that roots grown in a low temperature and long day environment accumulated more carbohydrate and exhibited a more rapid rate of phosphorus absorption than those roots produced in an environment of high temperature and short days. Kinetic analysis indicated that metabolic acceptor concentration did not vary proportionately

with carbohydrates in the roots.

Phosphorus absorption appeared to be a function of acceptor rate constant and percent acceptor saturation, and quantity of acceptor per gram of roots. The results also indicated a trend in rate constants as the amount of acceptors varied. In general, as acceptor concentrations increased, rate constants decreased.

If the results obtained in this study are compared with the results of Noggle and Fried (68) for millet, barley, and alfalfa, it may be considered that wheat absorbs phosphorus at a rate slower than millet but faster than barley or alfalfa. The variation in rate between genera was confirmed as being primarily due to a difference in root acceptor concentration.

The final phase of the investigation was concerned with the extraction and chemical identification of some possible metabolic intermediates that might function as phosphorus acceptors in transporting phosphorus from the solution external to the cell into the cell. After a short time absorption from a radioactive phosphorus solution, the root tissue was extracted. Boiling ethanol (80 per cent) was used as the extracting solution. The concentrated root extract was studied by chromatographic and electrophoretic methods in an attempt to identify compounds containing phosphorus that had been labeled with P^{32}. Several compounds were investigated, including phosphoryl choline, lecithin, and several phosphorylated sugar intermediates. Results were negative insofar as organic phosphate compounds were concerned. However, in two instances, organic phosphates, ATP and fructose 1,6-diphosphate, were tentatively identified. Attempts to duplicate these findings were

unsuccessful. The results indicated that inorganic phosphorus was the only material that was identifiable after metabolic incorporation.

Studies of the root residue after ethanol extraction indicate some problems for further studies of this metabolic system. Specifically, the ethanol extraction procedure does not remove all metabolically absorbed phosphorus from root tissue. Only 41 per cent of the total phosphorus absorbed in 10 seconds by six-day-old wheat roots was extracted by alcohol. In contrast, 64 per cent of the total phosphorus absorbed in 10 seconds by 21-day-old wheat roots was extracted by alcohol.

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APPENDIX

APPENDIX

Analytical Procedures for Nutrient Solutions and Tap Water

Cations: Fifty milliliters of each nutrient solution (250 ml. of tap water) were mixed with 2 grams of Dowex 2-X8 anion exchange resin (Cl^- form) and agitated for thirty minutes on a wrist action shaker. The solutions were filtered to remove the resin, and analyzed for calcium, potassium, and magnesium by use of a Beckman flamespectrophotometer with photomultiplier attachment in an oxy-hydrogen flame. Ammonia nitrogen was determined separately by a Kjeldahl technique (46). The residue from the ammonia determination was saved for nitrate analysis.

Anions: The solution samples used for phosphorus analysis were mixed with a cation exchange resin (H^+ form) to convert all anions to the hydrogen form. Fifty milliliters of each nutrient solution (250 ml. of tap water) were mixed with 2.5 grams of Rohm and Haas Amberlite cation exchange resin (IR-120) and agitated for thirty minutes on a wrist action shaker. The solutions were filtrated, to remove resin, and analyzed for phosphorus by the Jackson No. 1 method (46).

Nitrate nitrogen analyses were made on the residue remaining from the ammonia analysis by the method of Jackson (46) for total nitrate nitrogen in waters.

Modified Analytical Procedures for Carbohydrates (77).

Root tissue that had been previously prepared by freeze-drying and grinding (60 mesh) was used. A 0.2 gram sample was weighed into a 15 milliliter pyrex, long tapered, centrifuge tube and 10 milliliters of boiling hot water were injected into the sample with a syringe. The sample was allowed to set for 15 minutes and then centrifuged for 15 minutes. The tubes were then rotated 180 degrees and centrifuged again for 15 minutes.

The resulting amber colored liquid was carefully decanted into 50 milliliter centrifuge tubes and the root tissue extracted a second time in the same manner. Root residue was saved for starch and hemicellulose analysis. The two extracts were combined and the water soluble proteinaceous material precipitated by adding one milliliter of a saturated solution of lead acetate. After mixing, the tubes were allowed to stand until a coagulus had formed. The solution was then filtered through a 12.5 centimeter, #30 Whatman filter paper, the paper finally being washed with warm distilled water.

Afterward two milliliters of a saturated solution of potassium oxalate were added to the filtrate to precipitate the lead. The lead oxalate was filtered off and the filtrate volume adjusted to 100 milliliters.

Reducing Sugars: A five milliliter sample of root extract was pipetted into a 50 milliliter volumetric flask and five milliliters of deionized water added, followed by two milliliters of freshly mixed alkaline-copper solution. The flasks were placed in boiling water and boiled for exactly 20 minutes, cooled under running tap water and

two milliliters of an arsenomolybdate solution added. The solution was thoroughly mixed and allowed to stand five minutes to insure that all metallic copper had been dissolved and that the color reaction had been completed. The sample was then diluted to 50 milliliters, mixed, and its optical density determined on a Bausch and Lomb colorimeter using a 500 millimicron wave length setting. The reading obtained was referred to a standard curve, which had previously been prepared by using "Primary Standard Grade" glucose. Results were calculated and reported as per cent reducing sugar or glucose.

Total Sugars: The solution remaining from the original extract was used for total sugars. A twenty milliliter aliquot was pipetted into a 100 milliliter volumetric flask containing 50 milliliters of a sodium acetate-acetic acid buffer mixture (pH 4.77) and one milliliter of assayed 0.1 per cent invertase solution in five milliliters of water. The mixture was allowed to react for 20 minutes with occassional shaking. Since invertase cannot be removed from solution by lead acetate or suitable protein coagulants, it was necessary to run a control blank on the invertase.

After 20 minutes the volume was adjusted to 100 milliliters with deionized water and a 10 milliliter aliquot removed for analysis by the method described for reducing sugars. Results were calculated and reported as per cent non-reducing sugars expressed as glucose.

Starch: The root residue remaining in the centrifuge tubes after sugar extraction was treated with five milliliters of water containing one milliliter of a one per cent solution of analytical grade alpha-amylase. The reaction was allowed to continue for 12 hours. After 12

hours the residue was extracted with boiling water as described for sugars. The extract was treated with lead acetate and potassium oxalate as previously described, the volume adjusted to 50 milliliters with deionized water, and a 10 milliliter aliquot removed for analysis. Results were calculated and reported as per cent starch, expressed as reducing sugars.

Hemi-celluose: The residue remaining in the centrifuge tube from the starch analysis was treated with 10 milliliters of a N-solution of hydrochloric acid and digested in a boiling water bath for two hours. The tubes were removed, cooled, and the pH adjusted to 7.0 with 6N sodium hydroxide. One milliliter of lead acetate was added directly to the sample in the centrifuge tube; cellulose and protein coagulum were filtered off and the lead removed with potassium oxalate. The filtrate was made to a 100 milliliter volume, five milliliters removed, placed in a 50 milliliter volumetric flask and reducing sugars determined. The results were calculated and reported as per cent hemi-cellulose, expressed as reducing sugars.

VITA

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