INVESTIGATIONS CONCERNING PROPOSED CATION UPTAKE MECHANISMS IN PLANTS

By

ARTHUR B. ONKEN

Bachelor of Science
Texas College of Arts and Industries
Kingsville, Texas
1959

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1963

Submitted to the Faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
DOCTOR OF PHILOSOPHY
August, 1964

INVESTIGATIONS CONCERNING PROPOSED CATION

UPTAKE MECHANISMS IN PLANTS

Thesis Approved:

Lester W. Reed
Thesis Adviser

John F. Stone

Eddie Basler

Dale E. Weibel

Billy B. Tucker

Dean of the Graduate School

ACKNOWLEDGMENTS

The author wishes to express his appreciation to the staff of the Agronomy Department of Oklahoma State University for their helpful advice, constructive criticisms and cooperation. The author is grateful for the use of the laboratory facilities, chemicals and equipment that made this study possible.

Special appreciation is expressed to Dr. Lester W. Reed, thesis adviser, for his patience, advice, encouragement and helpful criticisms during the course of this research and in the preparation of this thesis. Special thanks go to Dr. Billy B. Tucker, Dr. John F. Stone, Dr. Dale E. Weibel and Dr. Eddie Basler for their guidance and helpful criticisms.

The author wishes to give special recognition to his wife, Cara, not only for typing this manuscript, but also for her patience, encouragement, and understanding which contributed much to this work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Prediction is one of the greatest contributions man can obtain from science. Science and common-sense both gather and classify knowledge, but science also attaches an explanation. This is how the discipline of science has helped man to progress beyond any point he could have reached by strictly empirical means (common-sense). Science does not discard common-sense knowledge, but expands it. From the gathered and classified knowledge, theories are worked out from which predictions can be made.

When a phenomenon is observed in reference to a given set of conditions, unless some method of prediction is available when one or more of the conditions are changed, the phenomenon must be observed again in reference to the changes made. However, if the relationship between the phenomenon and the conditions has been described, then the changes could be predicted and the additional observations would not be necessary. Theories can be built upon. If a theory is found to be adequate with respect to certain variables, other variables can be introduced until a total system is described. Changes in that system can then be predicted in reference to changes in any of the factors that influence it. Thus, logical empiricism is not enough for the rapid advancement of man's knowledge.

In effect, there is a hierarchy of statements in science. The first of these are the individual statements, e.g. measurements and magnitudes. The second level contains the general statements. These are statements of functional relations between magnitudes, e.g. theories. The third level consists of principles. These give the relation between functional relations, e.g. the conservation of energy links together chemistry, physics, mechanics, and biology.

Early in the nineteenth century, Justus von Liebig, Sir John Lawes and Von Mohl instigated the first scientific research into plant nutrition. Since that time, scientists interested in plant nutrition have been working at the first two levels of scientific statements. Much progress has been made, particularly in the last three decades. The use of high analysis fertilizers has shown that it will eventually be necessary to describe more fully the plant-soil-nutrient source system.

The doubling of plant material yields by fertilizer addition to soil never fertilized before can be easier than increasing the doubled yield by adding more fertilizer to the soil. In other words, each additional increment increase in yield is harder to obtain. The same principle holds for quality of plant material produced. Introduction of new varieties helps, but cannot alone solve the problem. Geneticists are faced with much the same type of problem. It is not unreasonable to suspect that being able to describe the plant-soil-nutrient source system and being able to predict from that description would be of benefit in helping to gain that next increment in plant material yield and/or quality.

The total system in which agronomists are interested is too complex to be worked with as a whole and must be broken down. Each fragment must be worked on individually and later incorporated into a description of the total system. The research reported in this thesis was concerned with cation uptake. The objectives of this research were: 1) to investigate the mechanism of cation uptake using a system more nearly approaching the natural conditions of the plant and environment than excised roots and forced aeration. 2) to evaluate the proposed mechanisms of cation uptake with measured quantities in addition to kinetics.

CHAPTER II

LITERATURE REVIEW

Cation uptake by plants has been considered to be passive, active and a combination of both. Passive uptake is thought to be characterized by diffusion along a concentration gradient, it is nonlinear with time, ions taken up are readily exchangeable with other ions in the external solution, Q_{10} is around one, it is not highly selective and it does not require oxygen or metabolic energy. In contrast to this, active uptake is thought to be characterized by absorption against a concentration gradient, chemically similar materials are often mutually antagonistic, chemically similar materials are selectively absorbed, a supply of oxygen is required, a source of energy is required, the uptake process may be reversibly reduced by narcosis enzyme inhibitors. In the following review the most prominant theories of passive and/or active cation uptake will be discussed. By the foregoing definition, any time uptake is considered to take place against a concentration gradient and if energy is expended it is active, regardless of the mechanism involved, e.g. physical or enzymatic.

Plants have been found to take up cations against a concentration gradient (59)[1]. The second law of thermodynamics forbids this movement

[1] Figures in parenthesis refer to Literature Cited.

without energy expenditure. Therefore, diffusion, as thought of in the usual sense, would not have contributed to the movement of the cations into the root cells.

At present there are three general theories about active cation transport. Each of these general theories may be modified slightly by various investigators, but the fundamental concepts remain the same. These theories are: 1. The carrier mechanism (15, 16). 2. the sodium-potassium pump theory (11). 3. the association-induction theory (34).

The carrier mechanism is visualized as follows: a semi-permeable membrane surrounding the cytoplasm of a cell limits the free movement of a cation from the external solution into the cell cytoplasm. In this semi-permeable membrane (plasmalemma) are carrier molecules. When a carrier molecule is at the outer boundary of the plasmalemma it can accept or combine with a cation in solution. The carrier-ion complex then traverses the plasmalemma and the ion is released into the cytoplasm. The carrier molecule then returns to the plasmalemma outer boundary. In this process energy is thought to be needed for carrier ion synthesis, formation of carrier-ion complex, movement of the carrier-ion complex across the plasmalemma, release of the ion and return of the carrier to the plasmalemma outer boundary. This energy is thought to come from metabolic processes.

Various carriers have been hypothesized. Among these are peptides (49), lecithin (8), protein (50), and ribonucleic acid (51). That these carriers might act in a manner analogous to enzymes was first put forth by Epstein and colleagues (15, 16) and later by Fried and colleagues

(19, 20), Wallace and colleagues (26, 57) and Bange (4). The methods of characterization have been by the use of kinetics. Epstein and co-workers (15, 16) have used the Lineweaver and Burk plot of the Michaelis-Menten equation.

$$\frac{1}{v} = \frac{1}{V}\left[Km \neq \frac{Km\ (I)}{Ka}\right]\frac{1}{(S)} \neq \frac{1}{V}$$

where: v = observed velocity of the reaction

V = maximum velocity at which the enzyme is saturated with substrate

Km = Briggs-Haldane constant of substrate

(I) = concentration of inhibitor

(S) = concentration of substrate

Ka = dissociation constant of inhibitor

Using excised barley roots, Epstein and Hagen (15) found that potassium and cesium interfered competitively with rubidium absorption. Consequently, the conclusion was reached that these three ions were bound to the same binding sites on the metabolic acceptor. Sodium did not interfere competitively with rubidium uptake except at high concentrations. Lithium was found to be competitive with rubidium, however, at low lithium and rubidium concentrations, lithium increased the rubidium uptake rate. They considered the evidence sufficient to conclude that cations are taken up by metabolic acceptors and that there are several distinct binding sites of which one group binds potassium, rubidium and cesium in preference to sodium and lithium.

In contrast to the experiment by Epstein and Hagen, Huffaker and Wallace (26) found for corn, soybeans and radishes that potassium had

a competitive inhibition on sodium absorption.

The conflicting results of the two previous investigations have been made somewhat more compatible by subsequent investigations. Epstein (14) found that calcium was essential for the integrity of selective ion transport sites in excised barley roots ($1x10^{-3}$ N Ca^{++}). He concluded that potassium and rubidium were transported through two carrier sites, one inhibited by sodium, the other largely indifferent to this ion. Sodium was also found to have two carrier sites, one of which was indifferent to potassium and rubidium. Bange (4) described two carriers for potassium and sodium in intact maize seedlings; one specific for potassium and the other for both sodium and potassium.

Tromp (53) concluded that a carrier mechanism was responsible for the uptake of ammonia, potassium and sodium by wheat roots. He further concluded that the absorption rate of the inhibiting ion and not the concentration of the inhibitor in the experimental solution determined the inhibitions found.

Fried and Noggle (19) reported that for excised barley roots two carrier sites exist for rubidium, potassium, sodium and strontium absorption. One site dominated at low ion concentrations. Apparent dissociation constants were determined and the magnitude for sodium was somewhat higher than the rest, which were all similar. Hydrogen ions were found to compete directly for each of the sites and to dominate at lower ionic concentrations. Rubidium and potassium were found to compete for the same site. Bange and Overstreet (5) concluded that a dual carrier mechanism was in operation for cesium absorption by excised barley roots. Boszormenyi and Cesh (9, 10) found a complex

relationship between the halide ions with respect to their absorption. They concluded that a multiple carrier system operated.

Moore et al. (41) established the metabolic nature of magnesium absorption by excised barley roots. Magnesium absorption in single salt solutions was found to be more nearly like the alkali cations than calcium. They further found that a large amount of magnesium absorption was blocked by small amounts of calcium being present in the solution.

Handley, Dios and Overstreet (23) and Handley and Overstreet (24) found that the non-vacuolated cells (apical 2mm.) of corn roots took up calcium, sodium and chlorine non-metabolically. The vacuolated cells were found to take these ions up metabolically. The region of cell elongation was found to be the most active in ion uptake.

Passive uptake of ions is generally considered to be a consequence of root cation exchange capacity. However, the role of root cation exchange capacity in cation uptake has not been completely described. Many of the investigations designed to evaluate this factor have been conducted with the postulates of Jenny and Lundegardh in mind.

Lundegardh (36) states that the first step in nutrient uptake is ion exchange controlled by the charge on the root surface. He further states that cations enter the root passively, while anions move into the root as a function of a metabolic process as indicated by a linear relationship with respiration. Becking (7) maintains that Lundegardh's own results show that cation uptake, if the cations are separated by species, also show a linear relationship to respiration. He presents further evidence that ammonium ion uptake was independent of the uptake

of other ion species and that an increase in respiration was due only to active ammonium uptake.

In 1939 Jenny and Overstreet (30) proposed a contact exchange theory as a possible mechanism by which plant roots accumulate ions. However, they tested this hypothesis by using the reverse process, contact depletion. Loss of potassium from low salt and potassium deficient barley roots to distilled water, dilute salt solutions and bentonite clay suspensions was measured.

Loss of potassium to distilled water and dilute salt solutions was negligible. Potassium loss to dilute clay suspensions ranged from 15 to 90% depending on the clay concentration, particle size and species of ion adsorbed. If the roots were suspended in a semi-permeable membrane such that the roots were separated from the clay, but ion movement was not restricted, the depletion was not observed. On the basis of these data the authors concluded that the phenomenon of contact exchange did occur. Overstreet and Jenny (44) carried this experiment one step further. They compared sodium uptake by excised barley roots from a sodium clay suspension and the inter-micellar liquid of the clay suspension. Low salt roots were used. From the data the authors concluded that it would be expected that contact phenomena would be a predominant process in absorption in those cases where the level of exchangeable nutrient ions or the degree of saturation of the soil was low and the availability of the root surface limiting.

Bartlett and McLean (6) found a diversity of results in measuring the effect of potassium and calcium activities in clay suspensions

and solutions on plant uptake. Some evidence was found for contact exchange for both potassium and calcium.

Olsen and Peech (43) and Lagerwerff (31), using clay and resin systems and their equilibrium dialyzates and a resin plus glass bead system respectively, came to the conclusion that their results were at variance with Jenny's theory. They further concluded that under conditions of equilibrium the composition of the solution phase of the growth media fully characterized the environment of the plant root; the adsorbed cations having no direct effect.

Fried et al. (20) have used a further modification of the Michaelis-Menten equation to determine the relationship between adsorption and absorption of cations and the concentration of the acceptor. They concluded that ions are adsorbed and absorbed by specific ion bonding sites and that only negligible quantities of the cations used were adsorbed nonspecifically. They found that the root cation exchange capacity was ten times as great as the concentration of carrier. Thus, it was concluded that measurements of root cation exchange capacities include not only the site for the cation used, but also a large amount of other titratable groups. Therefore, although correlations between cation uptake and root cation exchange capacity would exist, it would appear that the capacity of the plant to adsorb the ion of interest would be a more specific observation than root cation exchange capacity.

By comparison of the calculated percentage saturation of barley root exchange sites with rubidium and calcium with the amounts of these cations absorbed by the roots, Lagerwerff and Peech (32) concluded that the rate of accumulation of the two cations was not determined primarily

by the relative amounts of these two cations adsorbed on the exchange sites. They further concluded that exchange adsorption and cation accumulation are two independent co-existing processes.

Evidence conflicting with the above conclusions has been presented by Epstein and Leggett (16). They presented evidence that metabolic absorption removed strontium from exchange sites. Further, they showed that absorption of the alkaline earth cations would fit the criteria of the carrier mechanism as described by the Michaelis-Menten equation.

Wallace (57) found that both the action of some enzyme systems and ion uptake varied with substrate concentrations according to C^{ln2} where C is the concentration of the substrate. However, a saturation point was reached where this relationship no longer held. Wallace stated that this equation in no way violated the Michaelis-Menten equation. Wallace's statement is probably true and the relationship that he observed might be expected. His equation is of the Freundlich type while the Lineweaver and Burk equation is a Langmuir type equation. The Freundlich equation and Langmuir equation both describe a Type I adsorption isotherm, but the Freundlich equation breaks down at high concentrations and the Langmuir equation does not.

Etherton and Higinbotham (17) measured potential differences in oats sufficient to increase the potassium content in the plant 100 times that in solution. They also concluded that the plasmalemma did indeed exist and that the cytoplasm was not a part of free space.

Dainty (11) maintains that ions move under the influence of electrochemical potential gradients, not chemical potential gradients. In view of this he has proposed that ion pumps exist in higher plants.

Briefly they are supposed to work as follows: Inwardly directed anion pumps are located in the tonoplast and are the most important in higher plants. These are supposed to produce the high internal pressure in the vacuole and, thus, the turgor pressure of the cells. A sodium extrusion pump is located in the plasmalemma. Essentially the sodium extrusion pump would work on a carrier system. On the inside boundary of the plasmalemma the carrier would pick up a sodium ion, traverse the membrane, release the ion to the outside, pick up a potassium ion, again traverse the membrane, release the potassium ion to the inside of the cell and start the whole process again. The pumps, therefore, produce differences in ion concentrations across the membrane and this produces membrane potentials. These membrane potentials are diffusion potentials and arise in the following manner.

The outwardly directed sodium pump produces a lower inside electrochemical potential and the inwardly directed potassium pump a higher inside electrochemical potential. Thus, the sodium would tend to diffuse inward and the potassium outward. However, most membranes are more permeable to potassium, so a higher electrochemical potential is created for sodium than potassium. This system would have energy requirements similar to those of the enzyme carrier system.

Ling (34) has shown rather conclusively that the sodium pump theory is inadequate for animal tissue. He has shown that the energy available for the operation of such a mechanism is insufficient. His conclusion is of course based on what is currently known about cellular energy sources.

In view of this Ling (34) has proposed an association-induction

theory of ion uptake. Essentially it is as follows: Cells contain a fixed-charge system. Cytoplasmic inclusions such as nucleii and mitochondria as well as the cytoplasm itself are three dimensional lattice works of protein. This protein contains the charged groups of amino acids such as glutamic acid, aspartic acid, arginine and lysine. Water and ions are associated closely with the protein molecules.

Further, the primary function of the highly polarizable resonating protein chain is to provide a way for the ready transmission of an inductive effect from one functional group to another. Just as the substitution of three chlorine ions for three hydrogen ions radically changes the properties of acetic acid, so does the association of an ion with one fixed-charge group change another fixed-charge group in close proximity. Thus, a chain of events such as falling dominos can be instigated. Ling considers it to be an all or nothing effect. This would also provide for key positions that could regulate the entire process. Since ATP is found in close association with protein, Ling considers it to be used in this regulartory function rather than as an energy source. He has presented evidence that ATP has a role in selective ion uptake by animal tissues.

The association-induction theory makes ion uptake an exchange process, which in turn makes it a diffusion process. Ling considers two sources of ion exchange limitation, surface-limited and bulk-phase-limited. Surface-limited exchange would be where the rate limiting step was at the surface of the fixed-charge system, e.g. the plasmalemma. Bulk-phase-limited ion exchange would be where the rate limiting step was in diffusion through the cytoplasm.

The ions would move in the fixed charge system by adsorption-desorption migration. According to Ling the most likely type of migration would be triplet migration. In order for an ion to move it would have to gain sufficient kinetic energy to jump out of its adsorbed position. An anion-cation pair in the fixed-charge system would be polar. A second counter cation would be repulsed or attracted depending on its direction of approach. As the second counter cation approached the ion pair, it would cause the first cation to be held less tightly. Finally, an equilibrium state would be reached. Thus, a triplet would be formed and the kinetic energy necessary for the first cation to be released would be reduced.

By use of the theory given above, Ling has derived an equation for competitive entry of ions. This derivation is given in the Appendix. Ling's equation is similar to the Lineweaver and Burk plot of the Michaelis-Menten equation.

$$\frac{1}{v_i} = \frac{1}{V_i}\left[K_i + \frac{K_i \lceil P_j \rfloor_{ex}}{K_j}\right]\frac{1}{\lceil P_i \rfloor_{ex}} + \frac{1}{V_i}$$

where: v_i = the initial rate of entry of the ith - ion

V_i = the maximum rate of entry of the ith - ion

$\lceil P_i \rfloor$ = the ith - ion concentration

$\lceil P_j \rfloor$ = the jth - interfering ion concentration

K_i = the dissociation constant for ith - ion adsorption

K_j = the dissociation constant for jth - interfering ion adsorption

The difference between this equation and the Michaelis-Menten equation

is that K_i in Ling's equation is a true dissociation constant while it is not in the Michaelis-Menten equation. Ling's derivation, although analogous to the Michaelis-Menten derivation, does not involve the chemical changes involved in enzymatic type reactions.

Cations can accumulate passively against a concentration gradient in two ways. These are: 1. Donnan equilibrium and 2. diffusion through an ion exchange membrane. Ions have been considered to accumulate in the Donnan-free space of plants at a concentration higher than in the external medium. The mechanism involved is thought to be the Donnan equilibrium. Satisfactory limits have not been set on the extent of Donnan-free space.

Ionic diffusion through an exchange membrane is governed by a number of conditions. The conditions are membrane thickness, water film thickness, exchange capacity of the membrane, ionic species, ionic concentration, number of ion species, temperature, pH, physical structure of the membrane, ion pair formation and association, electrostatic attraction, London interactions, complex formation in solution, formation of precipitates, ionic solvation and swelling pressure. One of the most outstanding characteristics of exchange membranes is their selective permeability. This selective permeability is governed by diffusion and, therefore, by the ion, solution and membrane characteristics given above. Ions of similar characteristics could compete for movement through the same membrane, but one would be preferred. Equations describing ionic diffusion across an exchange membrane are much more complex than Ficks law of diffusion (25). The use of the foregoing theory for ion uptake depends on the presence of

an ion exchange membrane. Two possibilities are immediately apparent for plants. These are the pectin layer found by Jenny (29) and the plasmalemma.

At this point it might be of value to discuss the limitations of mathematical characterizations of experimental data. Substitution of experimental data into an equation that describes a particular theory and determining if the proper relationships exist between terms is one of the most useful methods for evaluation of a particular theory. One of the techniques most often used is graphical analysis. Two components of the descriptive equation are plotted against one another to determine if the expected relationship exists.

If the components do not have the expected relationship, then the theory can be rejected. However, a rejection can be made only if the investigator can be certain that the experiment as designed and executed actually gave a measure of the components used. If the components do have the relationship expected, the theory is not necessarily proved. The fact that one descriptive equation will satisfy the experimental data does not preclude another descriptive equation of a totally different theory from satisfying the same experimental data.

With this in mind it becomes apparent that the cation uptake theories discussed above must not only describe uptake in relation to time and ionic concentration, but they must also be compatible with such things as free energy changes, enthalpy changes, activation energies and ion selectivity.

Even these quantities can be of dubious value for predicting

mechanisms. For enzymatic reactions, free energy changes to form the enzyme-substrate complex can be less than 10 kcal. per mole. Total enthalpy changes for enzymatic reactions varies with temperature and has been known to range from -5 kcal. per mole to 20 kcal. per mole for the same reaction. Activation energies for enzymatic reactions also vary with temperature and have been known to range from 9 kcal. per mole to 15 kcal. per mole for the same reaction (12).

Q_{10} is related to activation energy "E" by the equation

$$E = \frac{R\ T_1\ T_2\ \ln Q_{10}}{10}$$

where: R = gas constant

T = absolute temperature

Q_{10} values for enzymatic reactions generally range between one and two (12). Adsorption generally increases 4% to 8% per degree centigrade (25). This places it in the same Q_{10} range as enzymatic reactions. Q_{10} values for ion uptake have been shown to vary not only with temperature, but also solute concentration (58).

Adsorption energies are generally less than 10 kcal. per mole. Enthalpy changes for adsorption are generally less than 2 kcal. per mole, but have been known to go as high as 10 kcal. per mole (25, 37). Thus, it is obvious that thermodynamic quantities of physical and enzymatic reactions overlap and caution must be used when predicting mechanisms based on measurements of these quantities.

Other than Q_{10} measurements and cation adsorption energies to the plant root exchange complex, energy measurements for cation uptake are lacking. Adsorption energies for several cations on the plant root exchange complex for several plant species have been measured (30).

The range of energies was 55 to 1629 calories per mole. The adsorption energy was found to depend on the plant and cation species. Since Woodruff (60) has shown that Marshall's bonding energy concepts can readily be used in studying exchange phenomena of roots, ionic concentrations would also be expected to affect adsorption energies. Ratio of ions and total ion concentration would both be expected to contribute. Since differences in adsorption energies represent a selective mechanism, these same factors would be expected to affect selective adsorption.

Some work has been done on selective ion uptake. The criteria used has been tissue composition. From the previous discussion on the theories of the cation uptake mechanism, it is evident that the manifestation of uptake phenomena in plant composition might produce a variety of results. In view of this, Specht (48) has proposed a variation-control mechanism in plants which controls degrees of variability of element concentration in tissues. To test this hypothesis Specht grew rose clover, soybeans and rice in solutions varying in magnesium, phosphorus and manganese respectively. Roots and tops were analyzed. The evidence led the author to conclude that a control mechanism does exist in plants. The author further stated that each plant or even each cell in a tissue has a definite minimum and maximum tolerance for each element and that this tolerance is relative to specific growth functions. Further, the action of the variation-control mechanism is low or absent so long as the supply of an element is more than adequate, but not excessive. Therefore, in this range, the competitive action of ions in nutrient absorption results in relatively wide variations within limits permitted by cation-anion balance. The

author also states that when the plant is subjected to a progressive change from an adequate nutrient supply to a deficient or toxic concentration that activation of cellular processes tends to counteract the direction of change in concentration. This was said to be done in two ways: 1. By causing a more uniform distribution throughout the plant tissues. 2. By either decreasing or increasing the feeding power of the roots. Also, under extreme deficiency or toxic conditions the element variation-control processes are impaired, allowing a relatively wide variation to occur. As described here, this mechanism would be physiological in nature, but it would be hard to explain the observations of Epstein (14), Viets (56) and Tanada (52) by its use. Various other investigations have been conducted with the theory that physical processes might account for selective ion uptake.

In an experiment by Huffaker and Wallace (26) it was found that potassium, magnesium and calcium contents of different plant species grown under similar conditions could be related by the equation

$$\frac{CEC_1}{CEC_2} = \frac{K_2}{K_1} = \left[\frac{Ca_1 \neq Mg_1}{Ca_2 \neq Mg_2} \right] 1/2$$

where: CEC is the root cation exchange capacity

K, Ca and Mg are concentrations of the ions represented

Subscripts 1 and 2 represent different plant species

This equation was derived using the Donnan concept. The idea that root cation exchange capacity is related to ion uptake indicates that root cation exchange capacity as a physical process is the preliminary step in cation uptake and does not imply a subsequent metabolic process.

The theory is that root cation exchange capacity due to the density of negative charge regulates the relative proportion of divalent and monovalent ions absorbed by regulating the relative proportion of these ions entering the free space.

This equation was investigated by growing several species of plants under varying conditions. The authors concluded that the evidence presented was circumstantial, but gave an indication that such a relationship did exist. Some of their data indicated that either the apparent root exchange capacity was too high or not all of it was functional in adsorption. This substantiates the ideas of Fried et al. (20) discussed earlier.

Gray et al. (22) also investigated the possible relationships between root cation exchange capacities and plant composition, but from a more practical standpoint. They investigated the possibility that root cation exchange capacities as related to potassium composition might be used to explain the disappearance of legumes from grass-legume pasture. Ladino clover, smooth bromegrass, Kentucky bluegrass and bentgrass with root cation exchange capacities of 43, 24, 21 and 16 meg. per 100 grams respectively were used.

It was found that potassium uptake by individual plant species was correlated with root cation exchange capacities. However, a concentration factor was found in that differences between species was reduced by higher additions of potassium. Ladino clover grew best with smooth bromegrass and poorest with bentgrass. In fact, 120 pounds of potassium per acre added to the clover-bentgrass mixture was not enough to meet the demands of the clover for potassium. This was said to be

in agreement with the law of differential mono-divalent cation adsorption by colloids. The higher the cation exchange capacity, the more divalent ions that are attracted to the exchange complex. Thus, the effect would be a compound one in that not only would plants with low root cation exchange capacities have a greater attraction for potassium, but the divalent ions would have a greater affinity for the high cation exchange capacity roots.

In conjunction with the effects of plant properties on cation uptake and plant composition, the effect of the plant root environment must also be considered.

It seems apparent that ions have various effects on each other in relation to their uptake as manifested in plant composition. In some cases ions have been found to compete with one another and in other cases one will stimulate the uptake of another (1, 4, 15, 16, 18, 19, 26, 41, 45, 47, 52, 56). In at least one case calcium was thought to be necessary for the selective ion uptake mechanism to function (14). Concentration effects of ions have also been observed. Ions have been found to be non-competitive at one concentration and then to be competitive at another (15). Others have found, even with widely varied ratios of ions, that some plants took up a relatively constant number of milliequivalents of cations per unit weight (2, 3, 28, 35, 39, 55). It has been reported that this cation equivalent constancy can be disrupted by high concentrations of one ion (13).

From the review presented, it is apparent that cation uptake, as indicated by plant composition, is regulated by a complex array of factors. Plant effects discussed were passive uptake, active uptake

and root cation exchange capacity. Evidence discussed in this review supports more strongly a connection between passive and active uptake than the proposition that they are separate and unrelated processes. There is also an indication of a relationship between selectivity of cation uptake and root cation exchange capacity.

Plant root environmental factors considered include exchange complexes, cation ratios and cation concentrations present. All these factors seem to interact with the plant factors in different ways and under a variety of conditions to produce changes in plant composition, e.g. the indicated relationship between selective cation uptake and root cation exchange capacities, cation ratios and cation concentrations. Thus, there appears to be little doubt in the value of obtaining relationships from which predictions could be made.

CHAPTER III

MATERIALS AND METHODS

Plant Production

Sugar Drip forage sorghum, Sorghum vulgare Pers., was the indicator plant used throughout this entire series of experiments. Approximately twenty grams of seed were washed for two minutes in 80% ethyl alcohol to eliminate contaminating organisms. The seeds were then washed with deionized water to remove the alcohol and placed in 800 ml. of continuously aerated deionized water. Aeration of the water was continued for three days. The water was changed each day.

The germinated seeds were then planted on a single layer of cheesecloth over three liters of a solution containing $1x10^{-4}$ molar calcium sulfate and $1x10^{-4}$ molar magnesium sulfate. The cheesecloth was spread over glass rods held in a wooden frame. The corners and two edges of the cheesecloth dipped into the solution. The seeds were approximately 2.5 cm. above the liquid. A glass pan 4 cm. deep, 22 cm. wide and 35 cm. long contained the calcium-magnesium solution. Seeds of as uniform germination as possible were planted. The plants were grown in the dark in a 24°C constant temperature room for nine or twelve days before use. During this interval, the calcium-magnesium solution was added as needed to maintain the liquid level.

Absorption

At the end of the growth period the plants were removed from the cheesecloth one at a time in a random manner, but in groups of sixteen. They were suspended in approximately 200 ml. of the calcium-magnesium solution. The plants were removed one at a time from the solution, blotted between two pieces of tissue paper and the roots inserted through holes in a piece of lucite plastic 0.5 cm. thick and 4 cm. square. The seeds were allowed to rest on top of the plastic. A piece of string was used to tie the plant tops together loosely in order to keep them erect. During the process of placing the plants in the plastic holders, the roots were not allowed to dry out. Immediately after each plant was placed in the plastic holder the roots were dipped into deionized water. Less than a minute lapsed between the time a root was blotted and it was dipped into the water. After a holder was filled, the roots were washed for two minutes in 500 ml. of deionized water.

Upon removal from the water, the roots were put into their respective radioactive absorbing solution. The roots of the plants extended 2.5 cm. above the surface of the liquid. Approximately 81 ml. of the absorbing solution were held in 90 ml. plastic centrifuge tubes. The ions used were cesium, sodium, potassium and calcium. Tracer ions used were Cs^{137}, Na^{22} and Ca^{45}. The cations were all chloride salts.

Preparation of Plants for Analyses

After the appropriate absorption period, the roots were removed from the absorbing solution and washed twice for one minute in 500 ml.

of distilled water. The roots and tops were then separated and weighed. Roots and tops were placed in a 30 ml. beaker, digested together in 3 ml. of a 3:1 mixture of 15.7 normal nitric and 11.6 normal perchloric acids and taken to dryness. Digestion and drying were conducted on a carefully controlled hot plate. The digested material was taken up in approximately 3 ml. of 12.1 normal hydrochloric acid and the volume reduced to approximately 0.5 ml. by heating. The contents of the beaker were then washed into a glass planchet using six to nine approximately 0.5 ml. portions of a solution containing 1 ml. of 1% Sterox[2] per 30 ml. of water and/or 2N HCl. Each time the planchet was filled, the liquid was evaporated under an infrared lamp. For the final evaporation the planchet was placed on a sample spinner. The samples were then ready for radioactive assay.

Assay of Radioactivity

When only one of the ionic species in solution was radioactive, beta particles were counted. The beta particles were counted using a Nuclear-Chicago model 186 scaler and a model DS5 scintillation detector probe equipped with an XTB anthracene crystal. The detector was housed in a model 3053, aluminum veneer, lead shield.

When two ion species in the solution were radioactive, a Nuclear-Chicago model 1820A recording spectrometer and a Harshaw

---

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

808F4 NaI (Tl) crystal were used in conjunction with the equipment described above. The scaler and probe were attached to the radiation analyzer.

It was found that the Cs^{137} gamma spectrum did not coincide with the Na^{22} gamma spectrum of the 1.28 Mev. Na^{22} gamma energy peak. Thus, when Cs^{137} and Na^{22} were mixed, the gamma count from Na^{22} could be determined without interference from Cs^{137}. An integral gamma count was made by setting the base level of the radiation analyzer at the appropriate value with a window width of 10 volts and using the scaler to record the time necessary to obtain a given number of counts.

The ratio of beta counts to gamma counts for Cs^{137} and Na^{22} was worked out using a set of concentration standards. By using the beta-gamma ratio for Na^{22} the amount of beta count from Na^{22} could be determined and subtracted from the total beta count of a Cs^{137} and Na^{22} mixture and, thus, the amount of Cs^{137} determined. Since Ca^{45} emitts only a beta particle, the same technique could be applied for a mixture of Ca^{45} with Na^{22} and Cs^{137}. The beta-gamma ratio of the standards was determined each time a series of experimental determinations were to be made. This eliminated the effects of any changes in geometry that might have occurred due to changing crystals or repositioning the probe. When betas were counted, 100,000 counts were made. When gammas were counted, three counts of 10,000 counts each were made.

Absorption Studies

A series of experiments consisting of ion uptake for various lengths of time were conducted in single salt solutions. Another

series of experiments involving varying concentrations of single and double salt solutions were conducted. One series of double salt solution experiments involved the use of two radioactive tracers. In this series of experiments one set contained the salts in varying ionic ratios, but constant solution concentration. The other set contained the ions in constant ratio, but varying total solution concentration. All concentrations are indicated where the results are reported. All data were reported on the basis of fresh plant weights. Absorption studies were conducted in a constant temperature room at 24°C unless otherwise stated.

Temperature and ionic concentration of the absorbing solution were variables in one experiment. Five concentrations and two temperatures were used. The lowest temperature of the absorbing solution was 19°C and maintained by use of a constant temperature room. The higher temperature was 29°C and was maintained constant by use of a constant temperature bath. No deviations in temperature were found to occur during this experiment. Activation energies, Q_{10}'s and overall uptake enthalpy were calculated from these data.

It was found that the plants would take up enough tracer ion to give a reasonably short counting time when the solutions were labeled to give approximately 10,000 counts per minute per milliliter. All solutions used were made by dilution of stock solutions. When two absorption studies were compared, the plants were from the same planting.

A series of studies were conducted using $1x10^{-3}$N ICH_2CO_2H and $1x10^{-3}$N NaCN in a $1x10^{-4}$M $CaSO_4$ and $1x10^{-4}$M $MgSO_4$ solution as

metabolic inhibitors. The plants were positioned as discussed previously and 99.995% N_2 was bubbled into the solution to exclude oxygen from the solution and from around the root portion above the liquid. Iodoacetic acid inhibits the glycolysis cycle and proteinases (21, 34). Nitrogen and cyanide inhibit oxidative phosphorylation. The first study conducted was for determination of the length of time in the inhibiting solution necessary to inhibit ion uptake. The second study was to determine the length of time necessary for increasing absorption by inhibited plants to cease.

After these determinations were made, a study was conducted to determine the maximum amount of ATP available for ion uptake. This study was conducted twice. In the first study the roots of six sets of sixteen plants each were placed for one hour in the inhibiting solution. The roots of two sets were washed for two minutes in 500 milliliters of distilled water and placed in a $6x10^{-3}N$ CsCl solution containing Cs^{137} as a tracer. After a three hour absorption period, the plants were removed and treated as discussed previously.

Another two sets were placed in a non-radioactive $6x10^{-3}N$ CsCl solution for three hours. Nitrogen was bubbled into the absorbing solution to exclude oxygen from around the root portion projecting above the liquid surface. After removal from the absorbing solution the roots were washed, separated from the tops, fresh weights obtained and ATP plus ADP determined by the method outlined by Umbreit, Burris and Stauffer (54). The procedure was modified in that two extractions of 0.1 N perchloric acid were used.

The roots of the other two sets were removed from the inhibiting

solution, separated from the tops, fresh weights obtained and ATP plus ADP determined. The first study yielded such small quantities of ATP plus ADP that the experiment was repeated using thirty-two plants instead of the usual sixteen.

The decision not to use aeration of the roots while in the absorbing solution was based on experimental evidence. Two studies were conducted to determine the necessity for aeration if 2.5 cm. of the roots were exposed to the air. One experiment involved absorption with varying time from solutions of constant concentration and the other involved absorption from solutions of varying concentrations with time in the solution constant. The roots of one group of plants were aerated and those of the other group were not while they were in the absorbing solution. The results are given in Figures 1 and 2.

Microequivalents of cesium taken up per gram of fresh roots were plotted versus time in the absorbing solution in Figure 1. Three plots are given in Figure 2. The microequivalents of cesium taken up per gram of fresh roots per two hours were plotted versus cesium concentrations in the absorbing solution directly, $\log_e$-$\log_e$ and reciprocally. The reciprocal plots were made according to the method of Lineweaver and Burk (21) and Ling (34).

The results show, in both cases, that aeration had no effect on the relationships shown. Differences in the positions of the lines were due to variation when plants from two different plantings were used. Therefore, in all other experiments reported, aeration was not used.

A study to compare methods of growing the experimental plants was

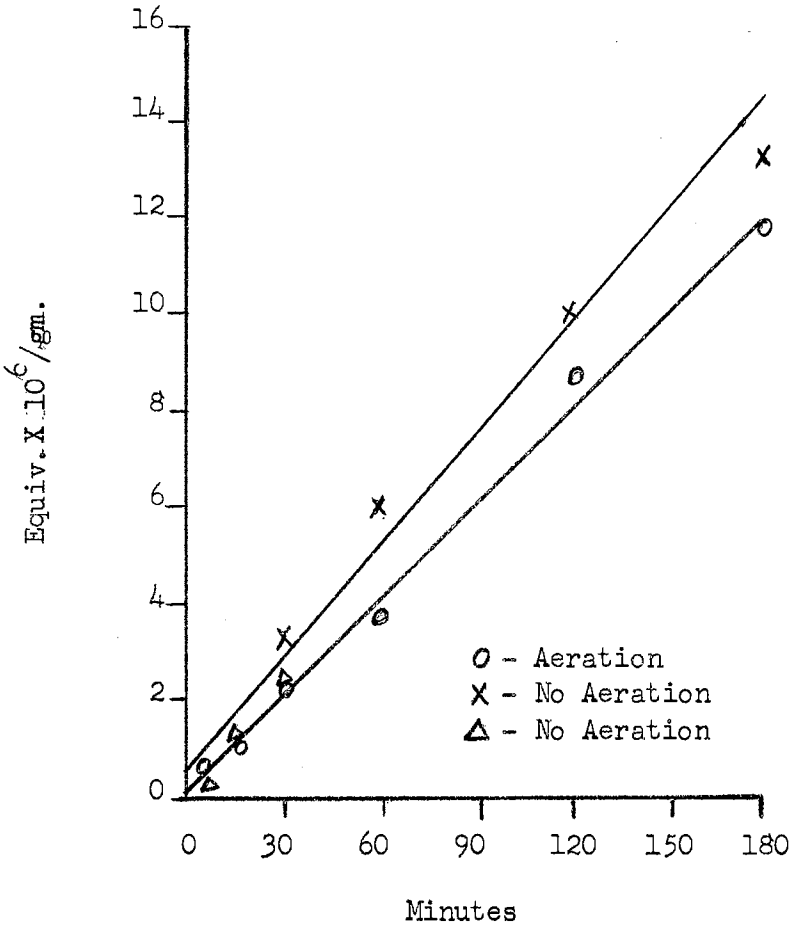

Figure 1. Effect of Aeration on Cesium Uptake as a Function of Time. Solution Concentration- 5×10^{-3}N CsCl.

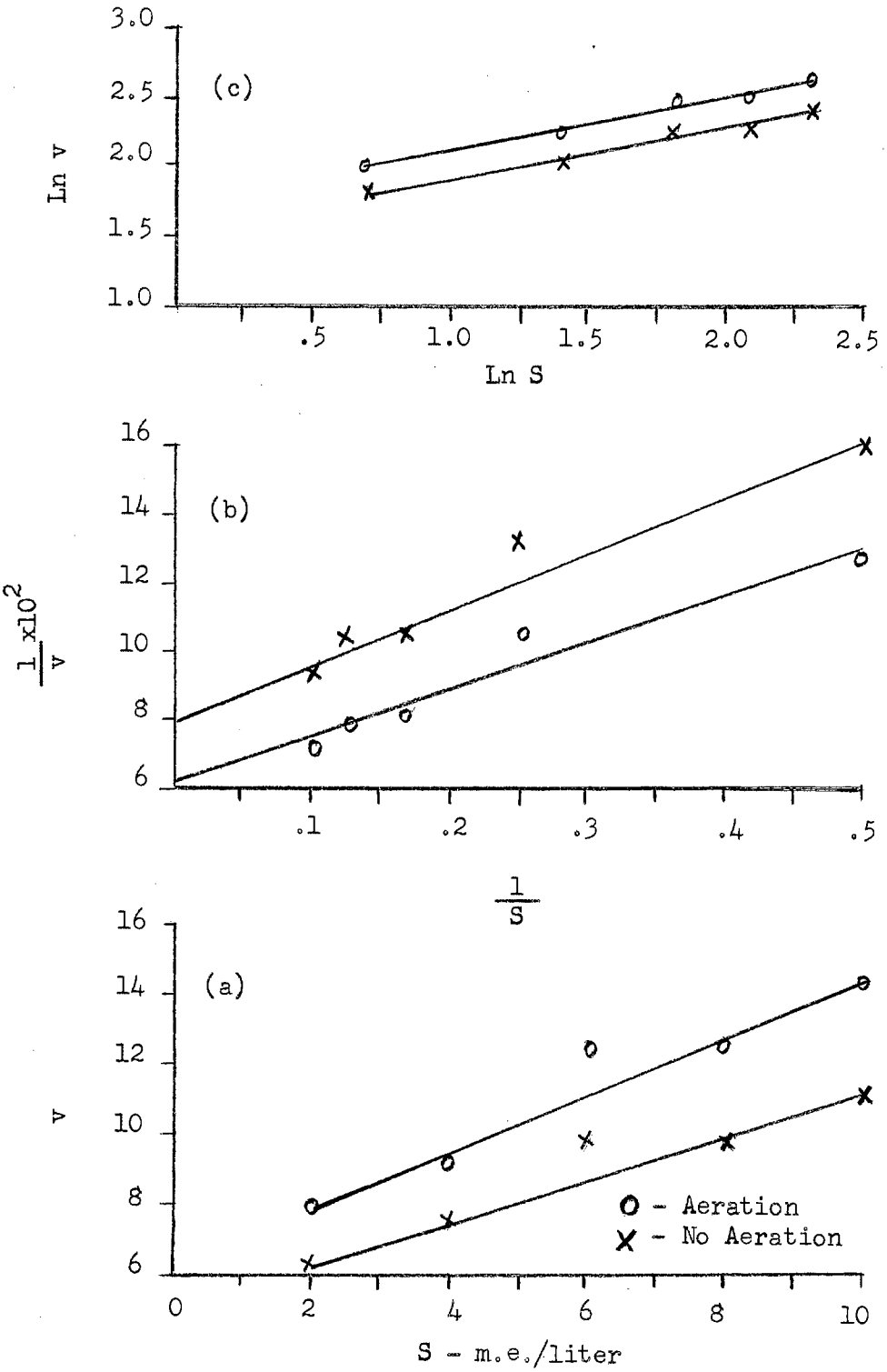

Figure 2. Effect of Aeration on Cesium Uptake as a Function of Cesium Concentration. v = microequivalents per gram of Fresh Roots per Two Hours.

also conducted. Three groups of seed were treated as discussed previously up to time of planting. One group of seed were planted over a calcium-magnesium solution as discussed previously. Another group was planted over $1x10^{-4}$M $CaSO_4$. The third group was left in the aerated deionized water for a total of nine days. The water was changed each day. The planted seeds were used six days after planting.

The results of this experiment are given in Figure 3 and Figure 4. Microequivalents of cesium taken up per gram of fresh roots were plotted versus time in the absorbing solution. The results show no differences in uptake versus time. The decision to use the roots grown in the calcium-magnesium solution was based on ease of handling the roots. The roots grown in the deionized water were turgid and stiff. They also had a tendency to curl. The combination of stiffness and curling made it difficult to put the roots through the holes in the holder without breaking them. Roots grown in the calcium-magnesium solution were used, because they appeared to be the straightest, toughest and most limber. These qualities facilitated their use with the plastic holders.

The pH of the solutions used was approximately 7.0. Changes in pH of the absorbing solutions were not detected. A Beckman model N pH meter was used to determine pH.

Statistical Analyses

All straight lines were plotted by the method of least squares. The methods for least squares and "t" tests were taken from LeClerg, Leonard and Clark (33).

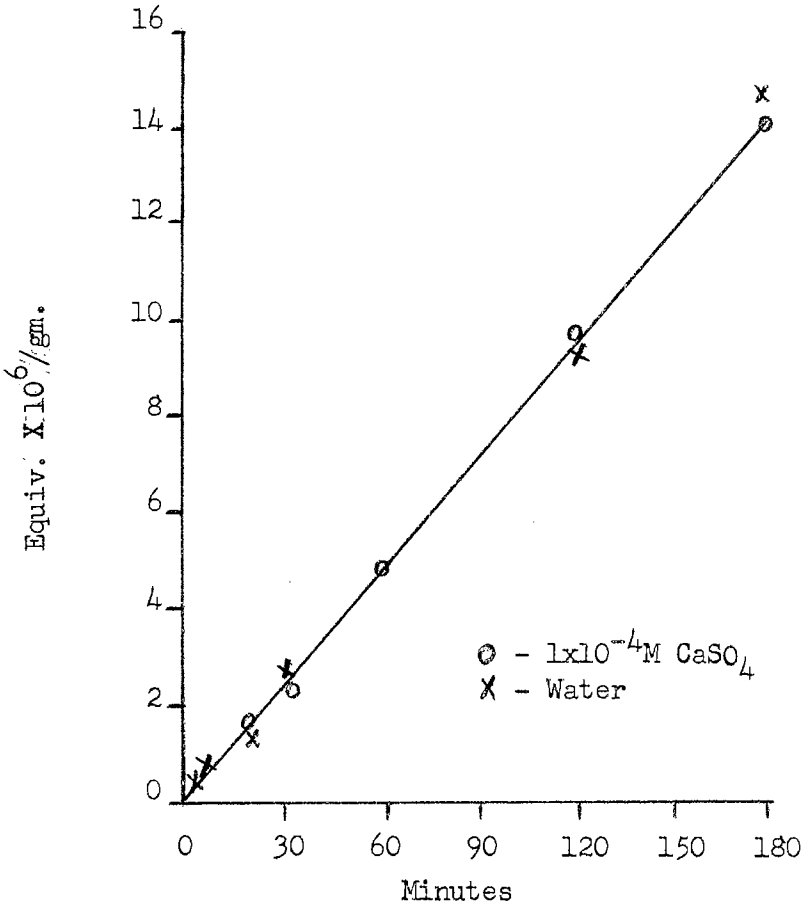

Figure 3. Effect of Growth Solutions on Cesium Uptake as a Function of Time.

CHAPTER IV

RESULTS AND DISCUSSION

For presenting results and discussion of results, the various experiments conducted will be grouped according to the primary effect being evaluated. Thus, this section will be divided into time and concentration studies, energy magnitude studies, selectivity studies and energy source studies.

Time and Concentration Studies

Cesium, sodium and calcium uptake as a function of time is illustrated in Figure 4. Regression equations and coefficients of determination are given in Table I.

TABLE I

REGRESSION EQUATIONS AND r^2 VALUES OF LINES FITTED TO DATA IN FIGURE 4

Ion	Regression Equation	r^2
Na	$y = 0.14(x) \neq 1.17$	0.98
Cs	$y = 0.062(x) \neq 1.95$	0.98
Ca	$y = 0.019(x) \neq 1.47$	0.98

The lines representing cesium and sodium exhibit a slight tendency for an adsorption shoulder as compared to the line for calcium.

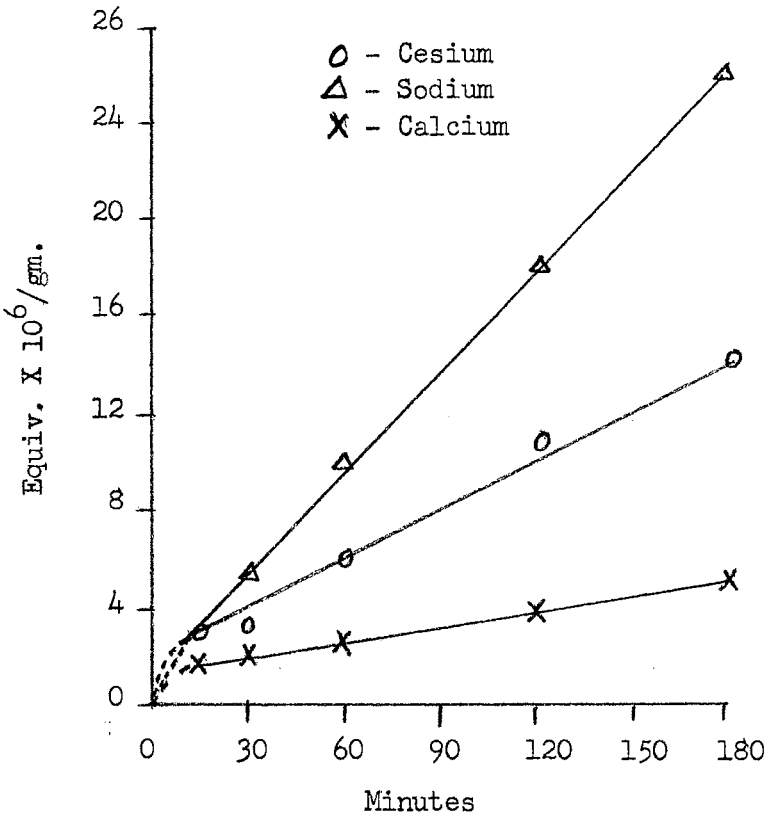

Figure 4. Uptake of Cesium, Sodium and Calcium as a Function of Time.

From previous studies, this would be expected (15). The plants were grown in a calcium-magnesium solution, consequently, the root cation exchange complex should have been saturated with calcium and magnesium. Monovalent ions, in the concentrations used, would not be expected to displace an appreciable amount of divalent ions as compared to the amounts taken up. This would be due to the fact that divalent ions would be held more tightly to the exchange complex than monovalent ions. The calcium curve exhibits a more pronounced adsorption shoulder when compared to the amounts taken up. Again this is similar to previous results (16). It would be expected that calcium in the absorbing solution would replace more calcium and magnesium on the root cation exchange complex in proportion to the amounts absorbed than would cesium or sodium.

With all three ions, a steady state condition of uptake was well established at 120 minutes. Therefore, in subsequent experiments involving ion concentrations as a variable, the absorption time was two hours.

Results of a replicated experiment of cesium uptake as a function of cesium concentration in the absorbing solution are given in Figure 5. This experiment was conducted not only to determine the pattern of cesium uptake, but also to determine the validity of comparing sets of plants selected at random from the same planting. Ten sets of sixteen plants each were selected at random from the same planting and subjected to the same solution concentrations in groups of two.

The data obtained were plotted three ways. A plot of velocity versus concentration was made and illustrates a steady state condition.

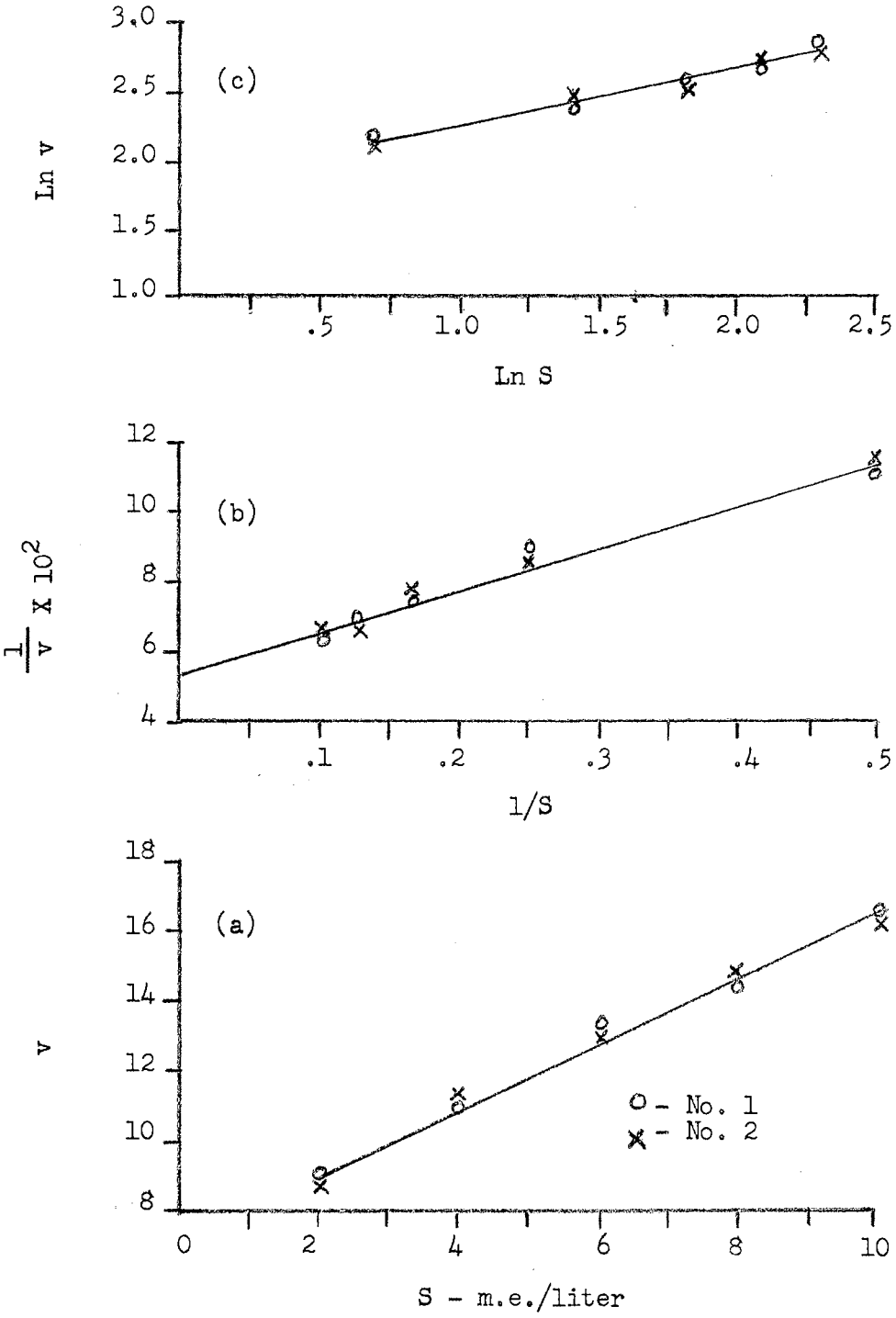

Figure 5. Replicate Experiments of Cesium Uptake as a Function of Cesium Concentration. v = Microequivalents of Cesium per Gram of Fresh Roots per Two Hours.

A Lineweaver-Burk or Ling plot was also made. It will be remembered that the reciprocal plot would give the same results for both the carrier mechanism (Lineweaver-Burk) or for the adsorption mechanism (Ling). The reciprocal plot is a Langmuir type plot. The ln-ln plot is a Freundlich type plot. Both Langmuir and Freundlich type equations represent a type I adsorption isotherm. The Freundlich equation breaks down at high concentrations, whereas, the Langmuir equation does not. Wallace (57) found that ion uptake and enzymatic reactions followed a Freundlich type equation up to certain concentrations.

The data presented in Figure 5 fit all three types of plots. There was no significant difference between the two replications. Only one line was plotted, but equations for both lines and coefficients of determination are given in Table II.

The velocity versus concentration plot shows that a steady state condition existed over the concentration range used. The reciprocal and ln-ln plots show that the data fit both the carrier and association-induction theories.

Absorption data for sodium and calcium as a function of concentration are illustrated in Figures 6 and 7. Regression equations and coefficients of determination are given in Table III. These data are subject to the same interpretation given previously for cesium.

The next series of experiments was conducted to determine the effects of sodium, potassium and calcium as interfering ions on cesium uptake. These experiments were replicated. Each replication represents a single growth of plants. Sodium interference with cesium uptake is illustrated in Figures 8 and 9. Regression equations and

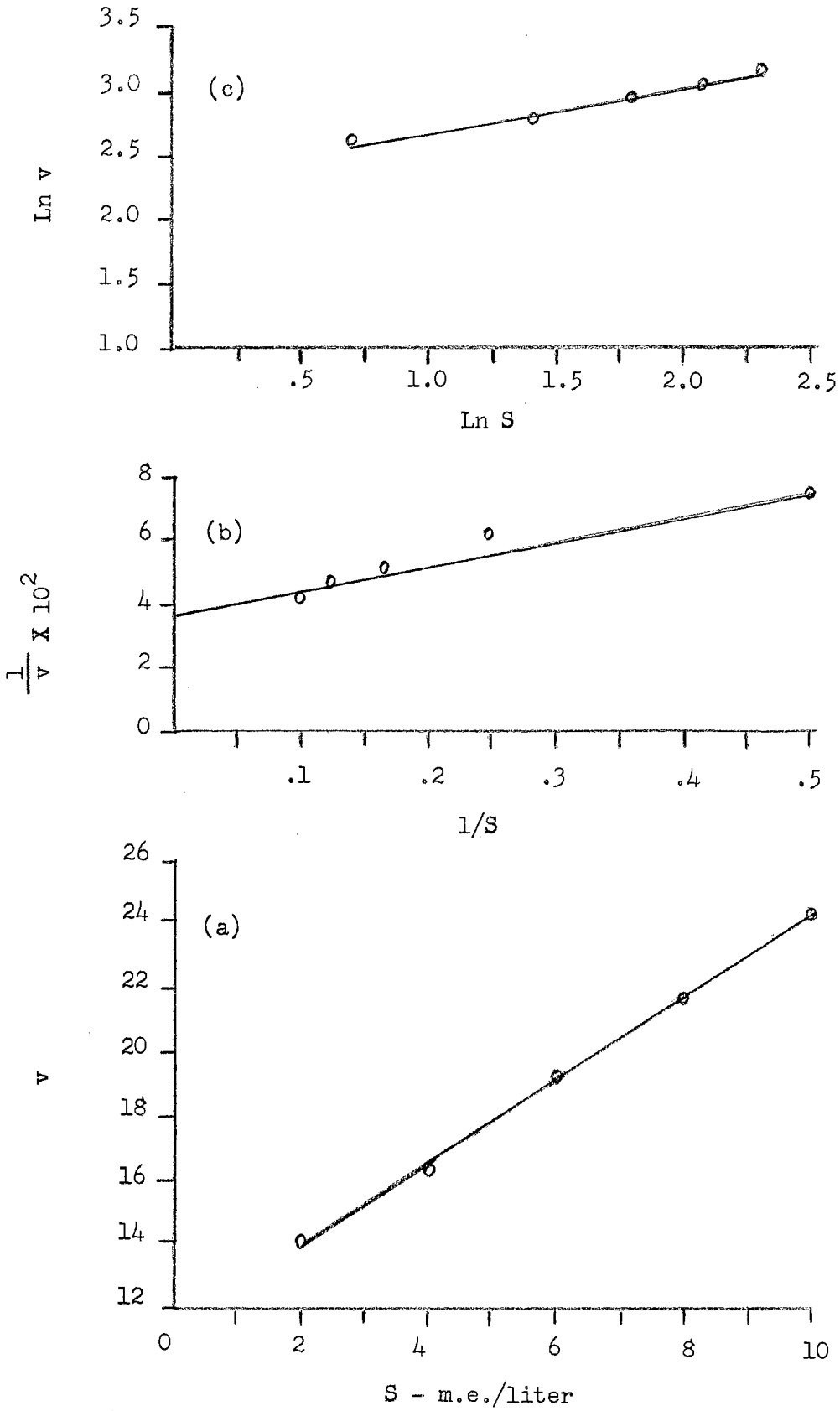

Figure 6. Sodium Uptake as a Function of Sodium concentration. v = Microequivalents of Sodium per Gram of Fresh Roots per Two Hours.

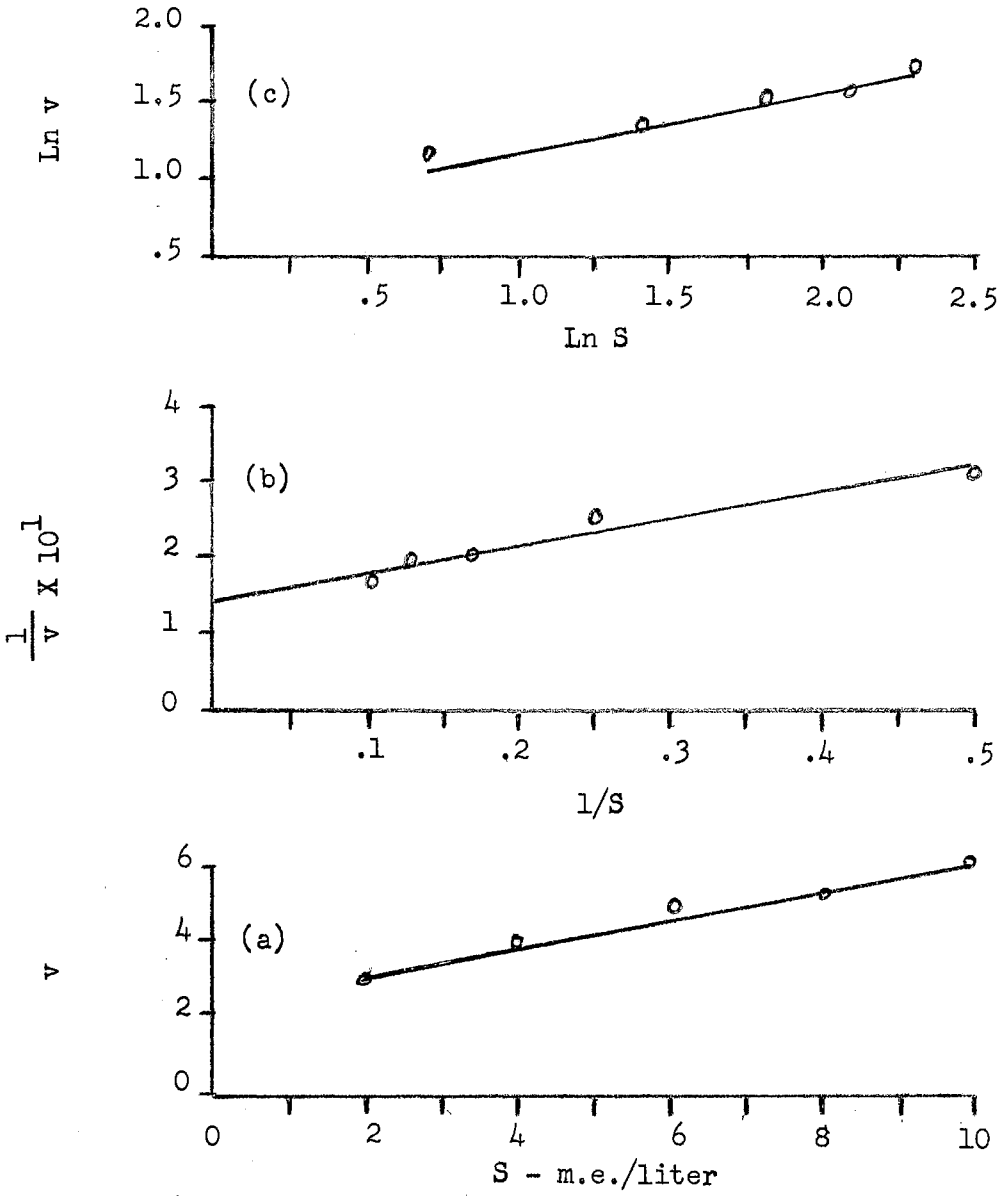

Figure 7. Calcium Uptake as a Function of Calcium concentration. v = Microequivalents of Calcium per Gram of Fresh Roots per Two Hours.

TABLE II

REGRESSION EQUATIONS AND r^2 VALUES OF LINES FITTED TO DATA IN FIGURE 5

Rep.	Plot	Regression Equation	r^2
I	(a)	$y = 0.93(x) \neq 7.35$	0.98
II	(a)	$y = 0.94(x) \neq 7.28$	0.98
I	(b)	$y = 11.78 \times 10^{-2}(x) \neq 5.41 \times 10^{-2}$	0.94
II	(b)	$y = 12.59 \times 10^{-2}(x) \neq 5.24 \times 10^{-2}$	0.97
I	(c)	$y = 0.37(x) \neq 1.92$	0.98
II	(c)	$y = 0.39(x) \neq 1.90$	0.94

TABLE III

REGRESSION EQUATIONS AND r^2 VALUES OF LINES FITTED TO DATA IN FIGURES 6 AND 7

Ion	Plot	Regression Equation	r^2
Na	(a)	$y = 1.34(x) \neq 10.94$	0.98
Na	(b)	$y = 7.58 \times 10^{-2}(x) \neq 3.76 \times 10^{-2}$	0.90
Na	(c)	$y = 0.35(x) \neq 2.34$	0.86
Ca	(a)	$y = 0.33(x) \neq 2.63$	0.96
Ca	(b)	$y = 3.31 \times 10^{-1}(x) \neq 1.52 \times 10^{-1}$	0.92
Ca	(c)	$y = 0.34(x) \neq 0.91$	0.88

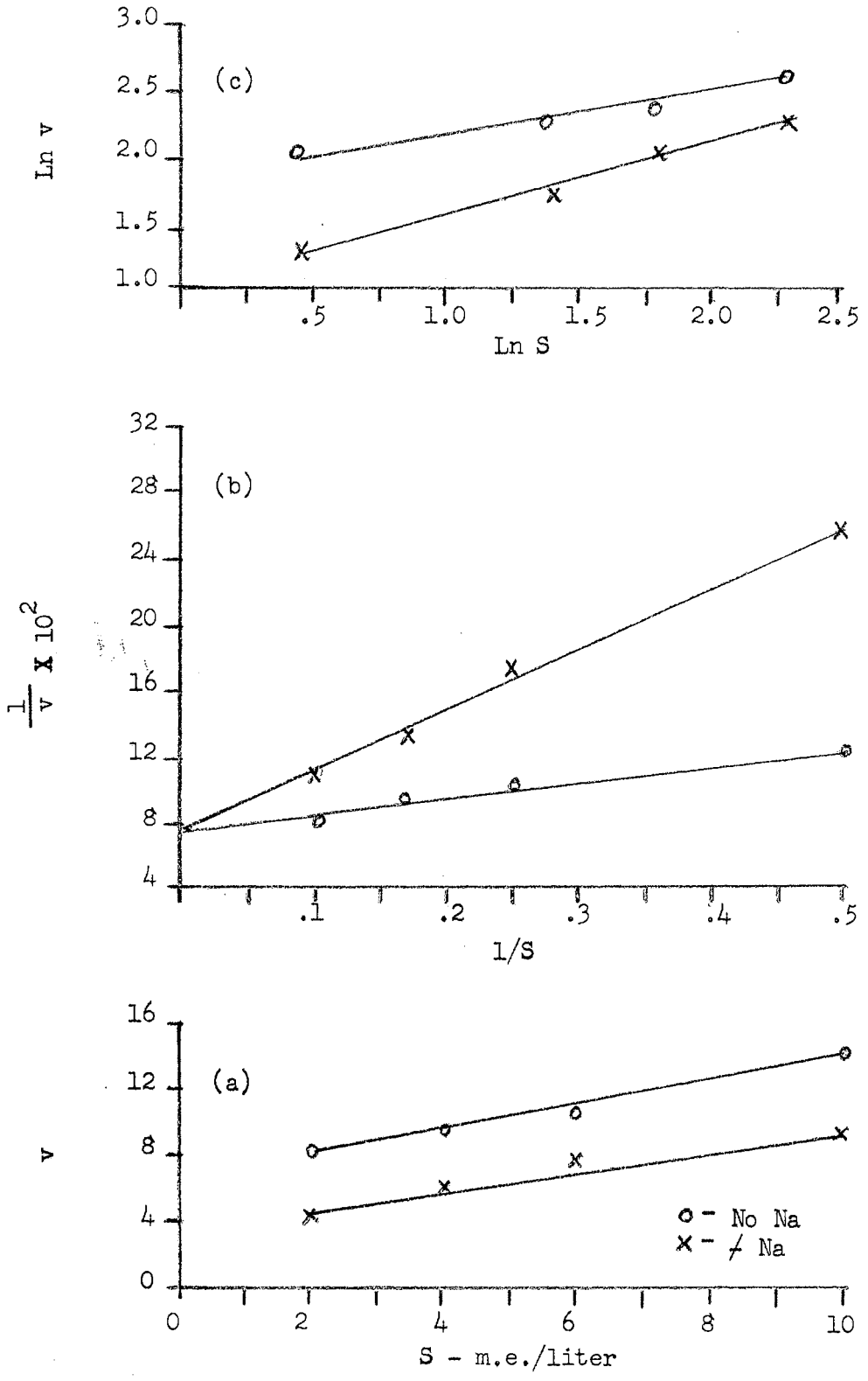

Figure 8. Interference of Two Milliequivalents per Liter of Sodium on Cesium Uptake as a Function of Cesium Concentration. Rep. 1. v = Microequivalents of Cesium per Gram of Fresh Roots per Two Hours.

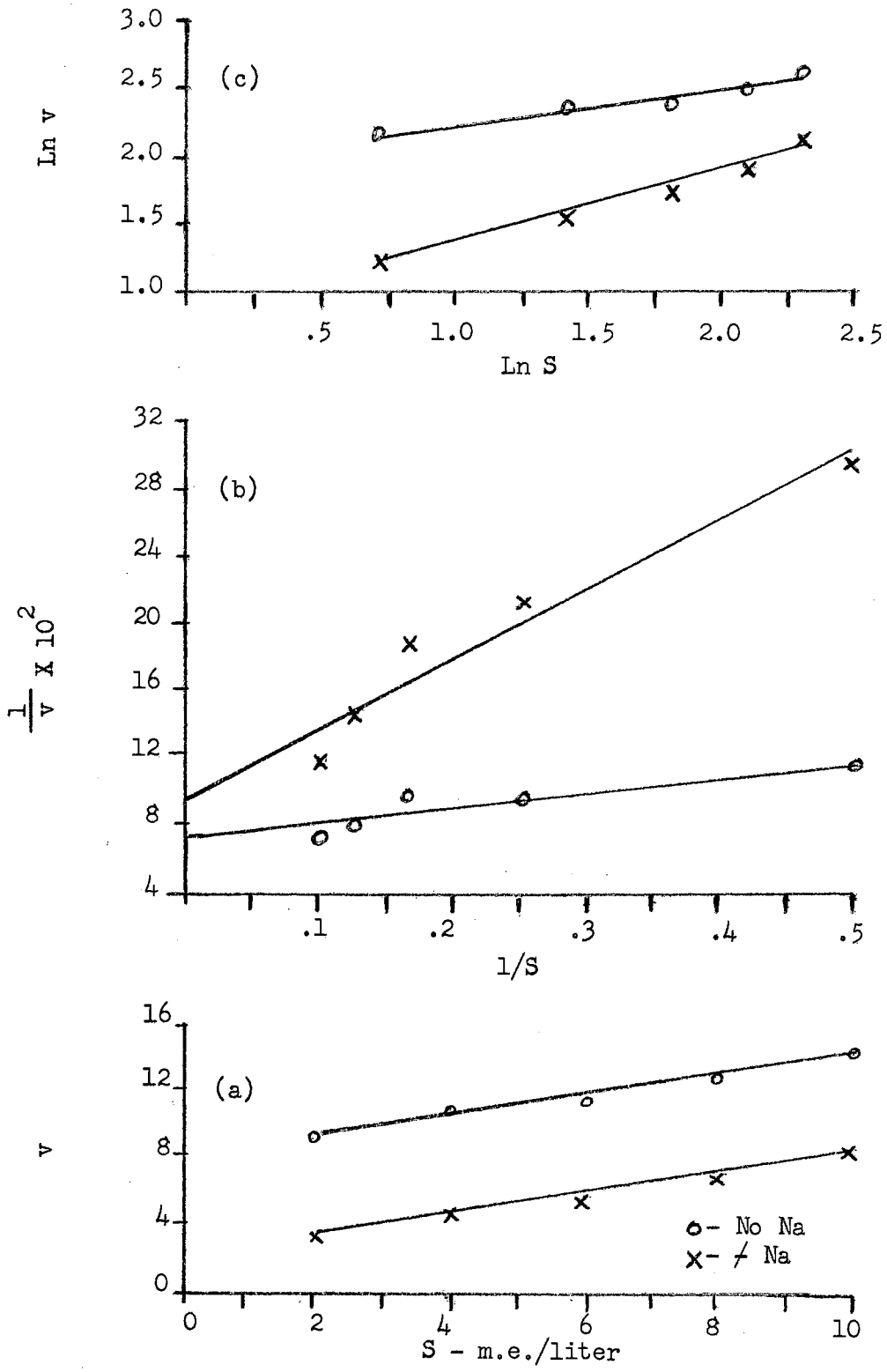

Figure 9. Interference of Two Millequivalents per Liter of Sodium on Cesium Uptake as a Function of Cesium Concentration. Rep. 2. v = Microequivalents of Cesium per Gram of Fresh Roots per Two Hours.

coefficients of determination are given in Table IV.

TABLE IV

REGRESSION EQUATIONS AND r^2 VALUES FOR LINES FITTED TO DATA IN FIGURES 8 AND 9

Fig.	Ion(s)	Plot	Regression Equation	r^2
8	Cs	(a)	$y = 0.702(x) + 6.70$	0.96
8	Cs	(b)	$y = 10.11x10^{-2}(x) + 7.24x10^{-2}$	0.77
8	Cs	(c)	$y = 0.30(x) + 1.87$	0.92
8	Cs + Na	(a)	$y = 0.65(x) + 3.05$	0.94
8	Cs + Na	(b)	$y = 36.44x10^{-2}(x) + 7.36x10^{-2}$	0.98
8	Cs + Na	(c)	$y = 0.54(x) + 1.01$	0.98
9	Cs	(a)	$y = 0.618(x) + 7.48$	0.94
9	Cs	(b)	$y = 9.28x10^{-2}(x) + 7.05x10^{-2}$	0.85
9	Cs	(c)	$y = 0.27(x) + 1.96$	0.90
9	Cs + Na	(a)	$y = 0.628(x) + 2.00$	0.98
9	Cs + Na	(b)	$y = 41.95x10^{-2}(x) + 9.64x10^{-2}$	0.94
9	Cs + Na	(c)	$y = 0.56(x) + 0.78$	0.96

Sodium was present at a concentration of two milliequivalents per liter. In the plot velocity versus concentration the data shown that sodium reduced cesium uptake by a constant amount regardless of the cesium concentration. The slopes of these regression lines were not significantly different. The reciprocal plots show competitive inhibition of cesium by sodium. The slopes of the lines of cesium alone and cesium plus sodium were significantly different at the 1% level. The Freundlich type plots also illustrate the interference of sodium with cesium.

Here again the slopes of the lines were significantly different at the 1% level.

Potassium interference with cesium uptake is illustrated in Figures 10 and 11. Regression equations and coefficients of determination are given in Table V. Potassium was at a constant concentration of two milliequivalents per liter.

TABLE V

REGRESSION EQUATIONS AND r^2 VALUES FOR LINES FITTED TO DATA IN FIGURES 10 AND 11

Fig.	Ion(s)	Plot	Regression Equation	r^2
10	Cs	(a)	$y = 0.58(x) + 6.25$	0.90
10	Cs	(b)	$y = 12.12\text{x}10^{-2}(x) + 7.82\text{x}10^{-2}$	0.85
10	Cs	(c)	$y = 0.30(x) + 1.76$	0.83
10	Cs + K	(a)	$y = 1.04(x) + 0.83$	0.96
10	Cs + K	(b)	$y = 73.48\text{x}10^{-2}(x) + 1.59\text{x}10^{-2}$	0.99
10	Cs + K	(c)	$y = 0.90(x) + 0.35$	0.98
11	Cs	(a)	$y = 0.78(x) + 6.54$	0.96
11	Cs	(b)	$y = 13.54\text{x}10^{-2}(x) + 6.20\text{x}10^{-2}$	0.94
11	Cs	(c)	$y = 0.36(x) + 1.80$	0.96
11	Cs + K	(a)	$y = 0.93(x) + 1.68$	0.96
11	Cs + K	(b)	$y = 60.67\text{x}10^{-2}(x) + 2.75\text{x}10^{-2}$	0.98
11	Cs + K	(c)	$y = 0.79(x) + 0.59$	0.98

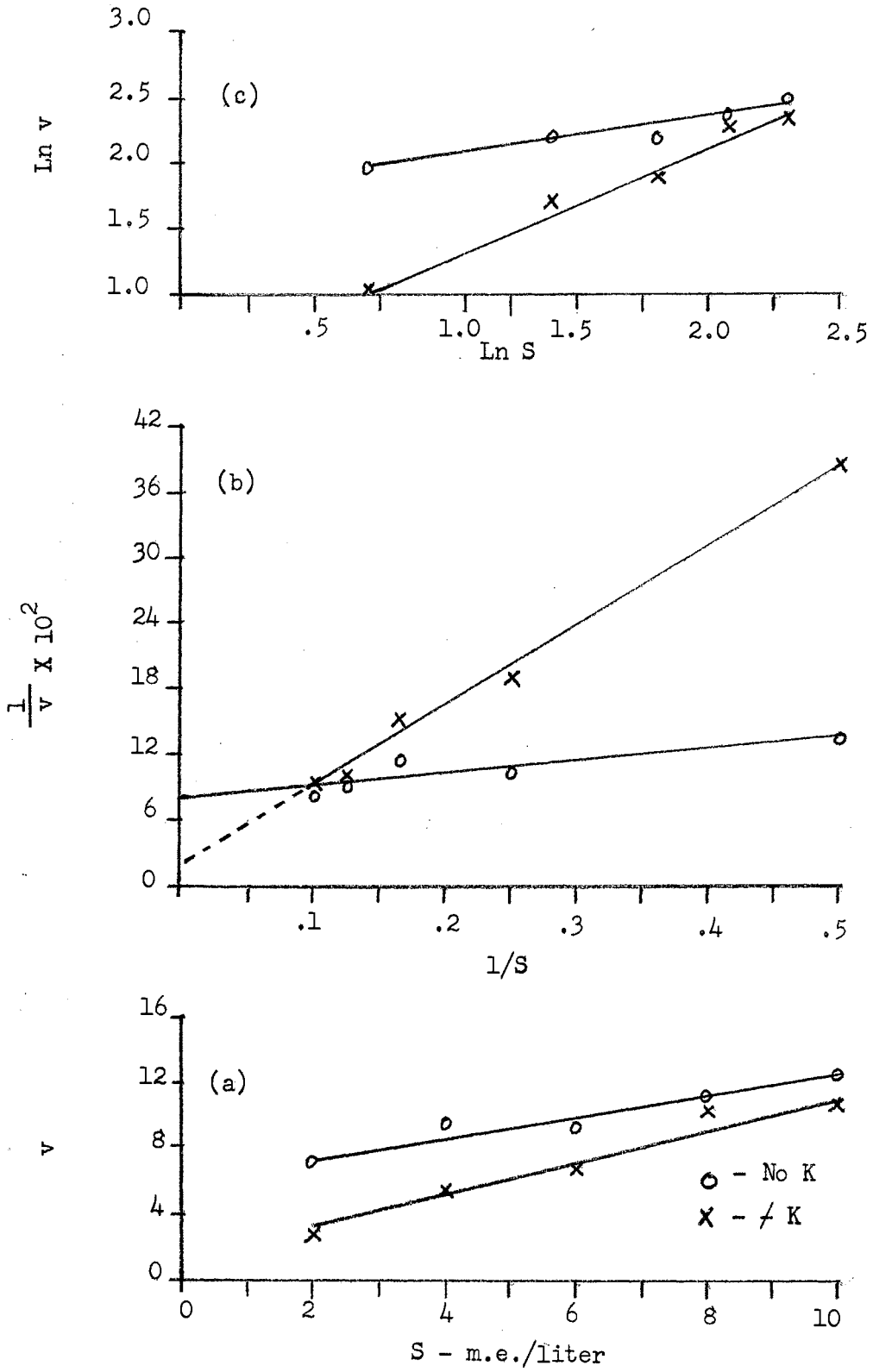

Figure 10. Interference of Two Milliequivalents per Liter of Potassium on Cesium Uptake as a Function of Cesium Concentration. Rep. 1. v = Microequivalents of Cesium per Gram of Fresh Roots Per Two Hours.

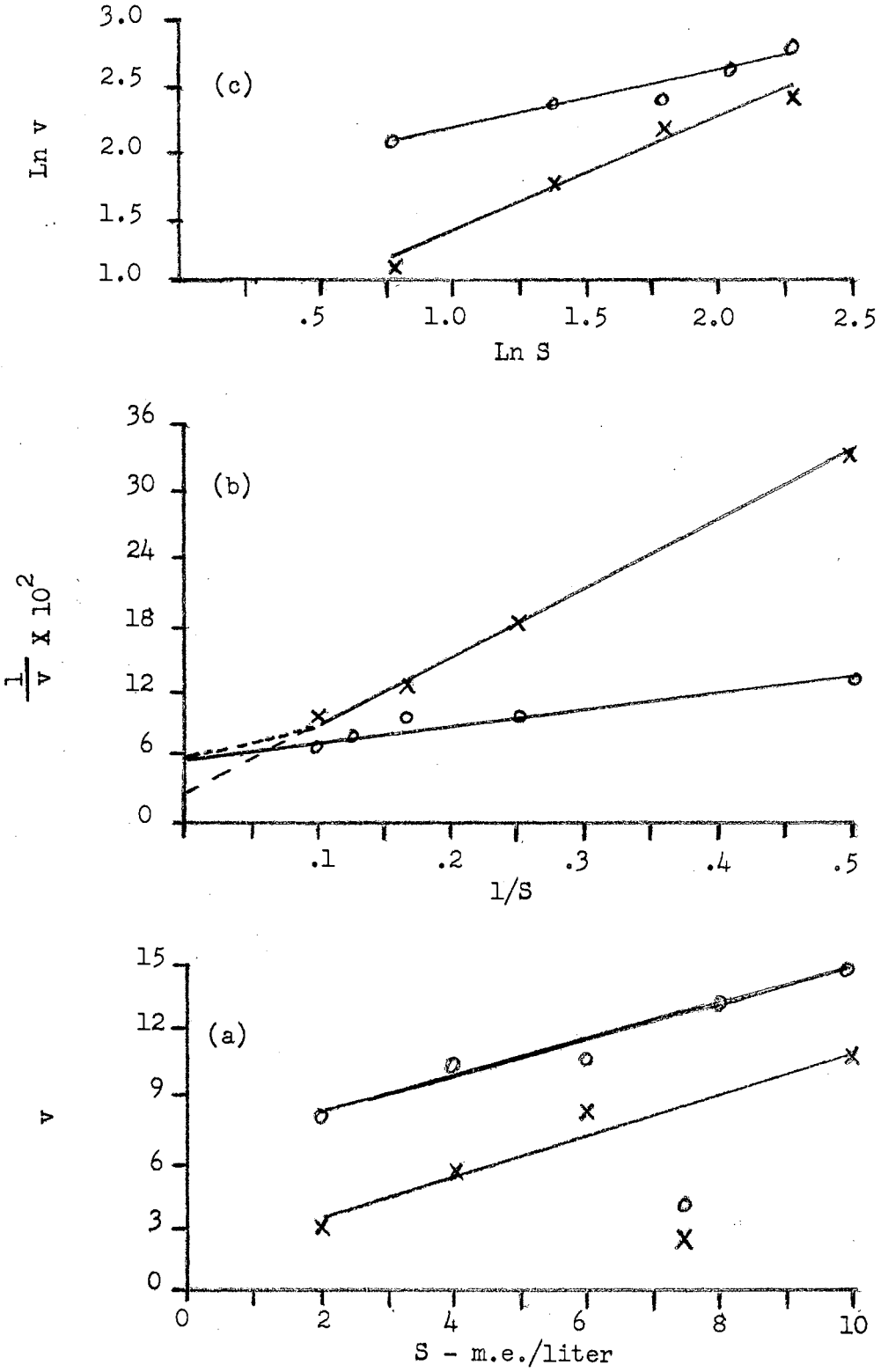

Figure 11. Interference of Two Milliequivalents per Liter of Potassium on Cesium Uptake as a Function of Cesium Concentration. Rep. 2. v = Microequivalents of Cesium per Gram of Fresh Roots per Two Hours.

The slopes of the lines in Figure 10 (a) were significantly different at the 5% level while those in Figure 11 (a) were not significantly different. Thus, in one experiment potassium acted in a manner similar to sodium and in the other it did not. The ln-ln plots tend to converge at higher concentrations and come closer to convergence than the sodium plots. The slopes were significantly different at the 1% level. The reciprocal plots are quite different for potassium than for sodium. In order to adequately interpret these plots, further investigation at higher concentrations would be necessary.

The data presented in Figures 12 and 13 represent the effects of 0.25 milliequivalents of calcium in solution on cesium uptake. Since it has been thought that calcium in low concentrations was necessary for selective ion uptake, a relatively low concentration was used. No calcium effect is illustrated in either figure. Again only one line per graph was plotted, but all regression lines and coefficients of determination are given in Table VI.

Results presented thus far show that cation uptake by non-aerated intact plants fits the mathematical characterizations of the carrier and association-induction theories. Thus, neither theory could be accepted or rejected as the sole mechanism responsible for cation uptake. Therefore, further characterization would be required. It was thought that magnitude measurements of various energies involved in the cation uptake process might be of value for this purpose.

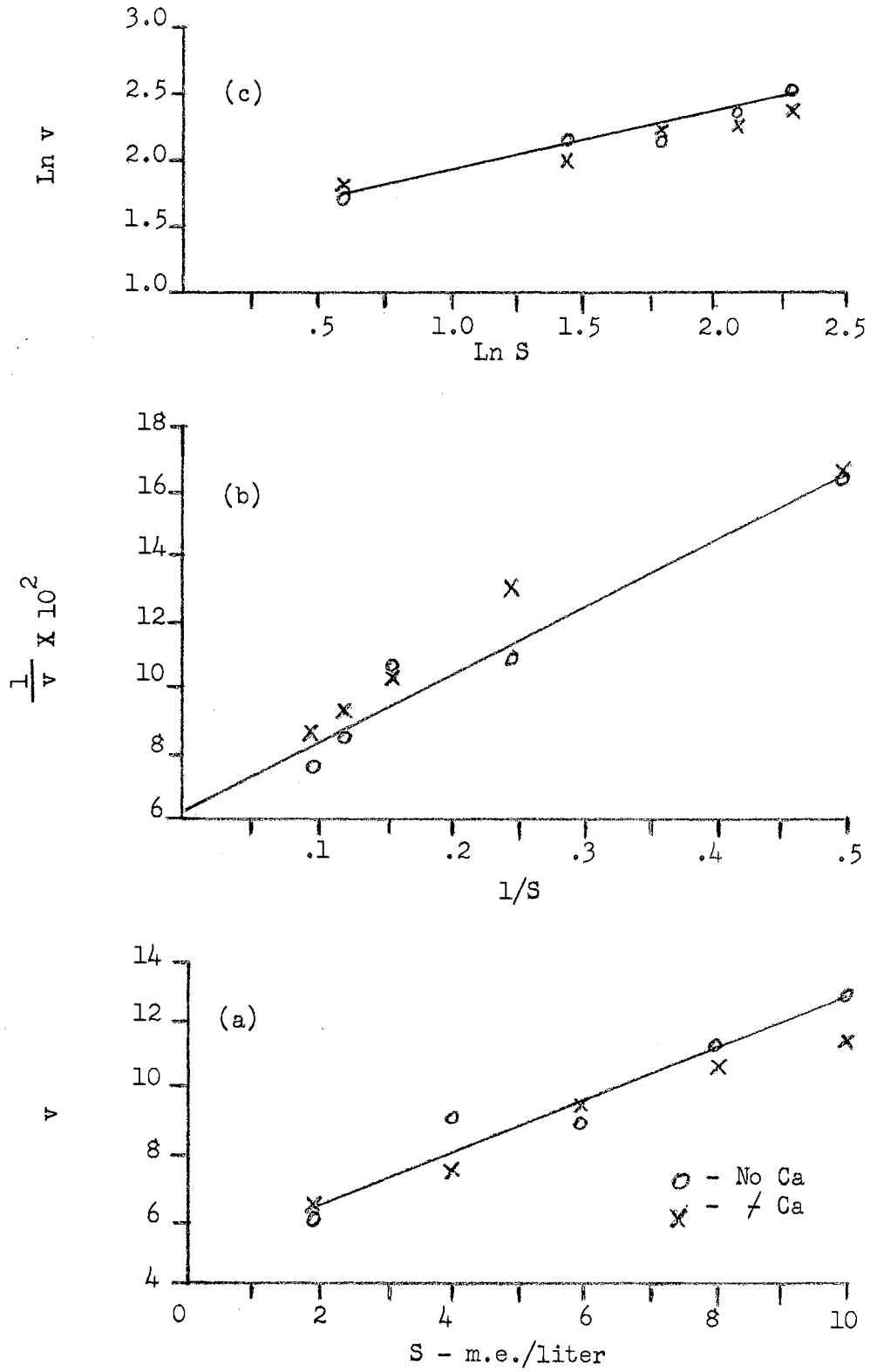

Figure 12. Interference of 0.25 Milliequivalents of Calcium per Liter on Cesium Uptake as a Function of Cesium Concentration. Rep. 1. v = Microequivalents of Cesium per Gram of Fresh Roots per Two Hours.

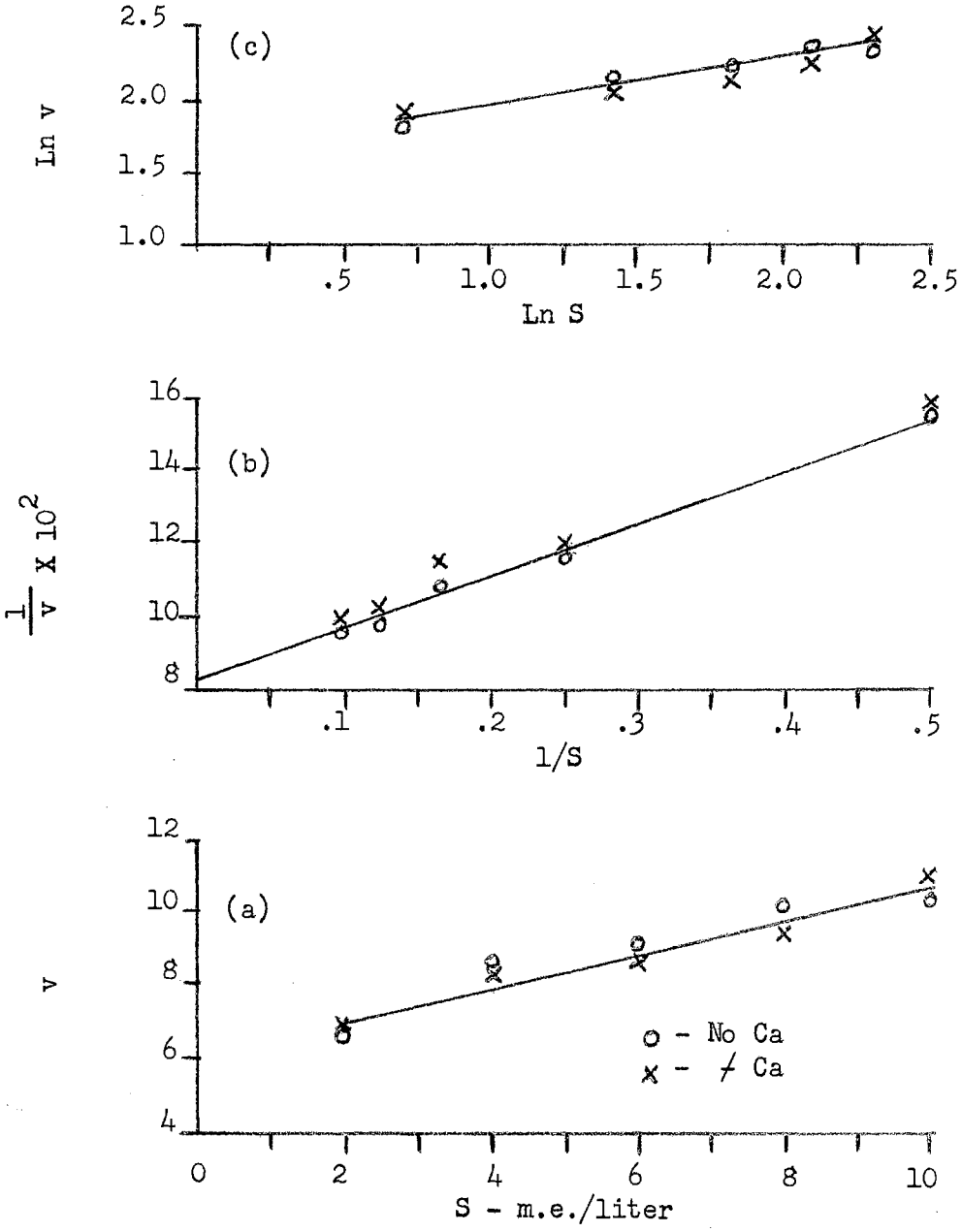

Figure 13. Interference of 0.25 Milliequivalents of Calcium per Liter on Cesium Uptake as a Function of Cesium Concentration. Rep. 2. v = Microequivalents of Cesium per Gram of Fresh Roots per Two Hours.

TABLE VI

REGRESSION EQUATIONS AND r^2 VALUES FOR DATA

IN FIGURES 12 AND 13

Fig.	Ion(s)	Plot	Regression Equation	r^2
12	Cs	(a)	$y = 0.78(x) + 5.01$	0.94
12	Cs	(b)	$y = 20.17 \times 10^{-2}(x) + 6.42 \times 10^{-2}$	0.96
12	Cs	(c)	$y = 0.44(x) + 1.51$	0.92
12	Cs + Ca	(a)	$y = 0.67(x) + 4.88$	0.98
12	Cs + Ca	(b)	$y = 18.75 \times 10^{-2}(x) + 7.54 \times 10^{-2}$	0.94
12	Cs + Ca	(c)	$y = 0.42(x) + 1.47$	0.92
13	Cs	(a)	$y = 0.45(x) + 6.23$	0.86
13	Cs	(b)	$y = 14.30 \times 10^{-2}(x) + 8.27 \times 10^{-2}$	0.98
13	Cs	(c)	$y = 0.30(x) + 1.67$	0.79
13	Cs + Ca	(a)	$y = 0.54(x) + 5.57$	0.96
13	Cs + Ca	(b)	$y = 14.49 \times 10^{-2}(x) + 8.41 \times 10^{-2}$	0.94
13	Cs + Ca	(c)	$y = 0.32(x) + 1.64$	0.90

Energy Magnitude Measurements

It might be useful at this point to discuss further the Briggs-Haldane constant (Km) of the carrier mechanism and the thermodynamic substrate dissociation constant of Ling (Ki). The Briggs-Haldane constant as shown in the Appendix is given by the equation

$$Km = \frac{k_2 + k_3}{k_1}$$

and is a true thermodynamic dissociation constant only when k_3 is negligible with respect to k_2. The rate limiting step is generally considered to be regulated by k_3. The back reaction involving k_4 is considered negligible. Ling's constant (Ki) is a true thermodynamic constant because of the nature of his derivation.

If measurements of the overall reaction enthalpy and energies of association (carrier system) or adsorption (association-induction) are to be meaningful, true thermodynamic constants must be used in their calculation. It is possible by calculation of association or adsorption energies to show whether or not the constant involved is a true thermodynamic constant. This can be accomplished by using the association or adsorption energies for the same ion as a substrate ion and inhibiting ion according to the equation

$$\Delta F = RT\ln K$$

where: ΔF = free energy of association or adsorption in cal./mole

R = gas constant

T = absolute temperature

K = Km, Ka, Ki or Kj

If the energy found is the same, then either the constant K is a true thermodynamic constant as in the theory of Ling or k_3 is negligible with respect to k_2. This would be true since in both cases the dissociation constant for the inhibiting ion is a true thermodynamic constant.

In order to obtain data for the determination of the overall reaction enthalpy, Q_{10}'s and activation energies, an experiment was conducted with temperature and ionic concentration in the absorbing

solution as variables. The results are presented in Figure 14. These data and the data presented in the previous section were used to calculate association or adsorption energies. These are presented in Table VII. All energies were for 24°C except where indicated.

TABLE VII

FREE ENERGIES OF ASSOCIATION OR ADSORPTION FOR CESIUM, SODIUM AND CALCIUM

Ion		ΔF_i [a]	Ion		ΔF_i	ΔF_j [b]
Aerated	Cs	-3.64	Non Aerated	Cs	-3.88	
Non Aerated	Cs [c]	-3.66		Na		-3.28
	Cs [c]	-3.62		Cs	-3.91	
	Cs	-3.56		Na		-3.46
	Cs	-3.82		Na	-3.66	
	Cs	-3.62		Ca	-3.62	
	Cs	-3.75	19°C	Cs	-3.47	
			29°C	Cs	-3.65	

[a] Free energy of association or adsorption as a substrate ion in kcal./mole.

[b] Free energy of association or adsorption as an interfering ion in kcal./mole.

[c] Replicated experiment from single group of plants.

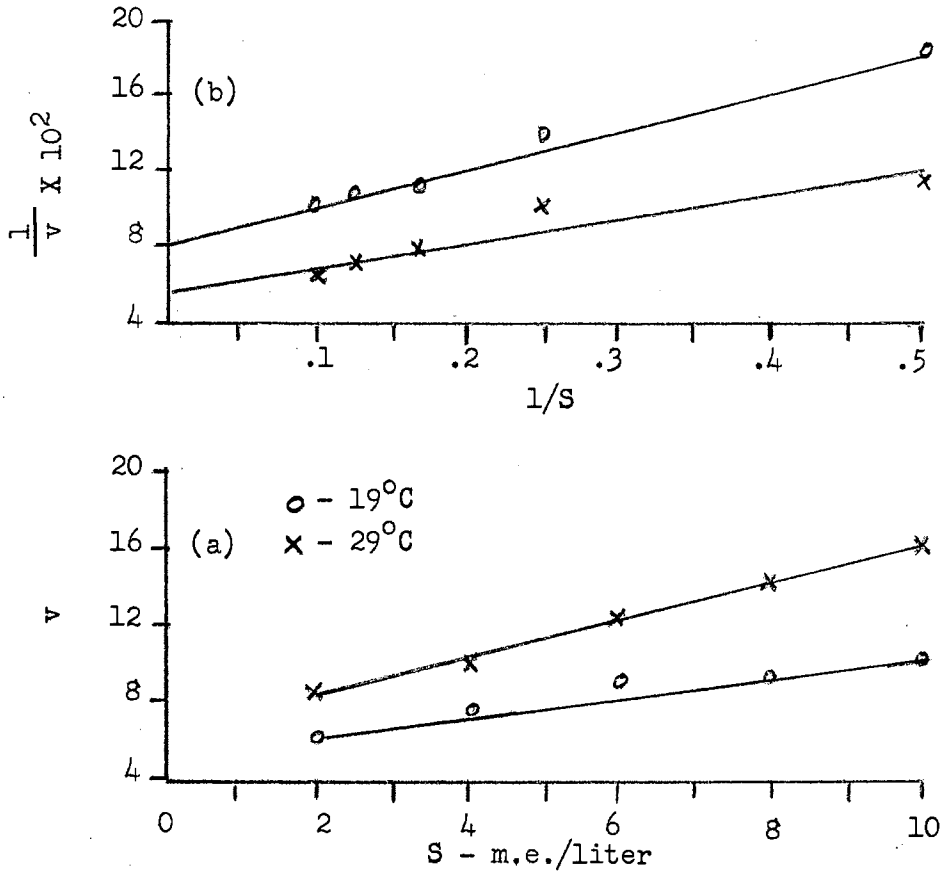

Figure 14. Effect of Temperature on Cesium Uptake as a Function of Cesium Concentration. v = Microequivalents of Cesium per Gram of Fresh Roots per Two Hours.

The association or adsorption energy of cesium at 24°C ranged from -3.62 kcal./mole to -3.91 kcal./mole for plants from different plantings. For the replicated experiment they were very close and were considered to be the same, because the slopes and intercepts of the reciprocal plots were used in these calculations and were found to be not significantly different. By the same reasoning the association or adsorption energies of sodium as an interfering ion was concluded to be different from cesium, since the slopes of the lines involved in the calculations were significantly different. The free energies of association or adsorption for cesium were considered to be the same for the aerated and non-aerated experiments.

It was thought that the energy values for sodium as an interfering ion and substrate ion were sufficiently close to warrent the conclusion that the constant involved was a true thermodynamic constant. Thus, further calculations based on the need for a true thermodynamic constant could be made.

The free energy change for 29°C was greater than that for 19°C. This would be expected to occur within reasonable temperature limits. The values are negative indicating that the reaction could proceed spontaneously. A negative free energy change does not necessarily mean that the reaction will take place, but is merely an indication that the process can occur under proper conditions. The magnitude of the free energy change indicates how large the potentiality is for the reaction to occur. Thus, the need for activation energies is not ruled out.

The association or adsorption energy for calcium would be expected

to be larger than for the monovalent ions in an adsorption system. This was not observed.

The overall reaction enthalpy was calculated using the Arrhenius equation and found to be -1.89 kcal./mole. Generally it is considered that overall heats of reaction for physical processes are less than 2 kcal./mole.

From the velocity versus concentration plot in Figure 14, it can be seen that the regression lines for 19°C and 29°C are not parallel. The slopes were significantly different at the 5% level. Consequently, the activation energies and Q_{10} values varied with concentration. Activation energies and Q_{10}'s were plotted versus concentration in Figure 15. The relationships were found to be linear over the concentration range used. The Q_{10} and energy values obtained were of little value in making a decision between the carrier and adsorption-desorption migration theories. The reason for this is, the values obtained are in the transition zone between enzymatic and physical processes. In view of this, it was decided to conduct selectivity studies to determine if ionic selectivity by plants might be explained on an adsorption basis or if the multiple acceptor system would be necessary.

Investigations of Selective Ion Uptake

For this series of experiments two radioactive isotopes were placed into solution. The amounts of each taken up by the plant were determined as discussed previously. Selectivity coefficients

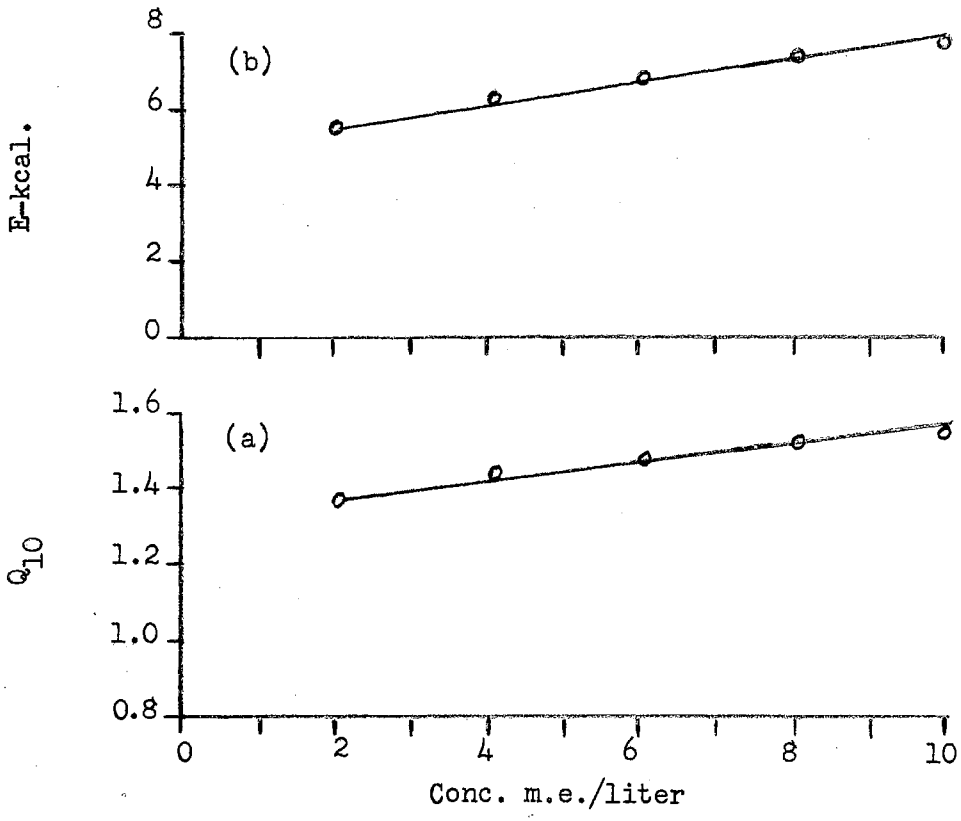

Figure 15. Cesium Uptake Q_{10} and Activation Energy Values as a Function of Cesium Concentration.

were calculated according to the equation (25)

$$K_B^A = \frac{\bar{X}_A^{Z_B} \; X_B^{Z_A}}{\bar{X}_B^{Z_A} \; X_A^{Z_B}}$$

where: K_B^A = selectivity coefficient of ion A

$\bar{X}_A$ = equivalent fraction of ion A in the plant

$\bar{X}_B$ = equivalent fraction of ion B in the plant

X_A = equivalent fraction of ion A in solution

X_B = equivalent fraction of ion B in solution

Z_A = valence of ion A

Z_B = valence of ion B

The results of the experiment for cesium and sodium selectivity are given in Figures 16 and 17. A plot of velocity versus concentration for the solutions with constant total ionic concentration, but varying ratios of cesium and sodium is given in Figure 16 (a). This plot shows that more sodium than cesium was taken up until a ratio of approximately 2:1 of cesium to sodium was reached. This plot also shows that the total equivalents taken up per gram of fresh root tissue were constant for these solutions. This followed the cation equivalent constancy theory of Bear and co-workers (2, 3).

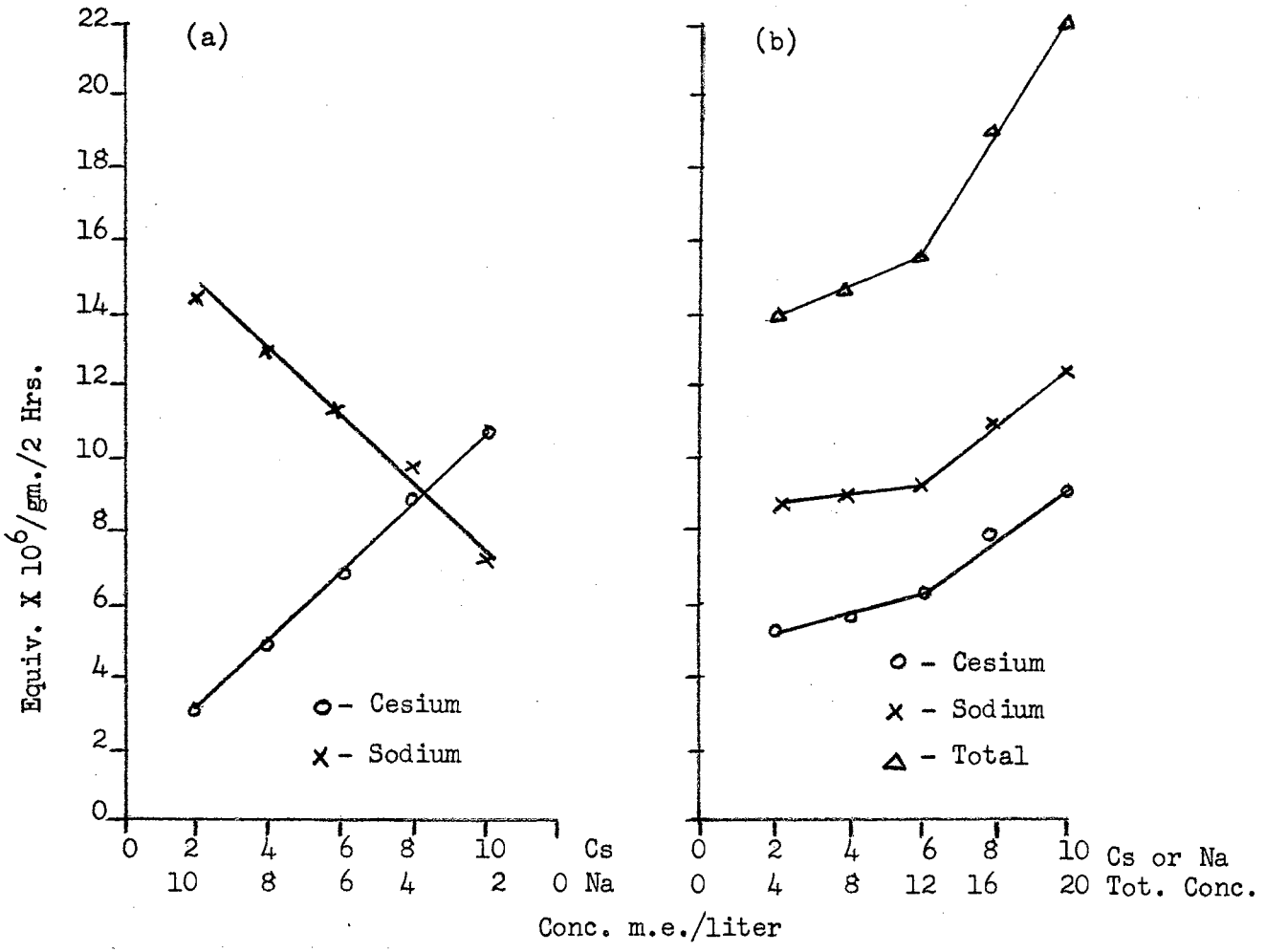

Figure 16. Cesium and Sodium Uptake as a Function of Ionic Ratios at Constant Total Ionic Concentration (a) and as a Function of Total Solution Concentration at Constant Ionic Ratio (b).

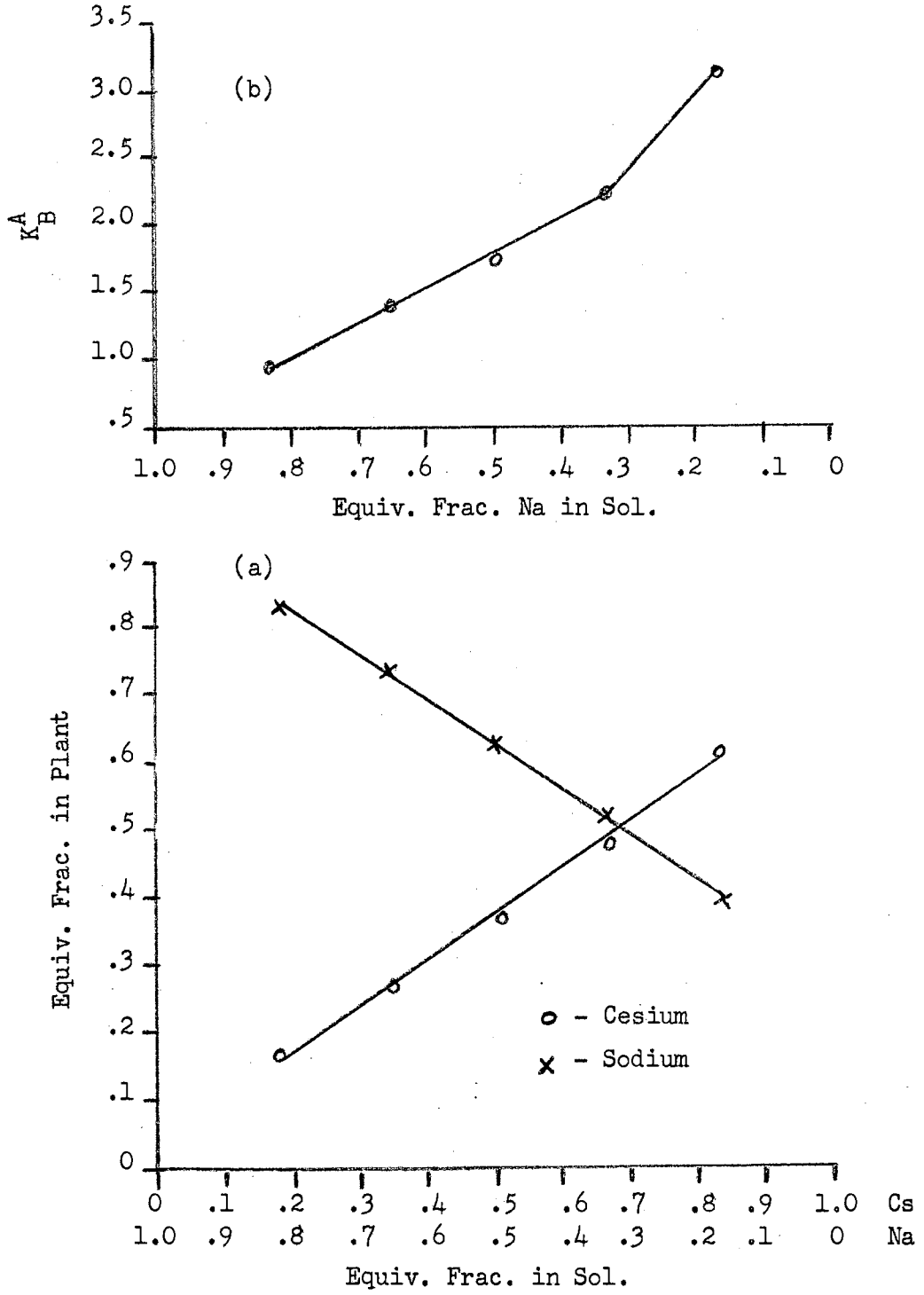

Figure 17. Equivalent Fraction of Cesium and Sodium in the Plants as a Function of the Equivalent Fraction of Cesium and Sodium in Solution (a) and Selectivity Coefficient of Sodium as a Function of the Equivalent Fraction of Sodium in Solution (b).

A plot of velocity versus concentration for the solutions containing a 1:1 ratio of ions, but with increasing total ionic concentration in solution is shown in Figure 16 (b). Here again, more sodium than cesium was taken up, but a total concentration effect is also exhibited. At a total solution concentration greater than twelve milliequivalents per liter, uptake of both ions increased sharply. The linear pattern was always observed previously. Epstein and co-workers (15, 16) noted a similar effect for one ion in the presence of another and hypothesized a dual carrier system.

A plot of equivalent fraction in the plant versus equivalent fraction in solution is shown in Figure 17 (a). This plot also shows that more sodium than cesium was taken up until approximately a ratio of 2:1 cesium to sodium was reached. A plot of the sodium selectivity coefficient versus equivalent fraction of sodium in solution is illustrated in Figure 17 (b). This shows that the plants were more selective for sodium than cesium except where the ratio of cesium to sodium was 1:5. As the sodium concentration became less and less in solution the plants became more and more selective for sodium. The sodium selectivity coefficients for the solutions of equal ionic ratio, but varying total concentration, were relatively constant. These are given in Table VIII. There was a tendency for them to decrease with increasing concentration, but they are all greater than 1.0. This shows that the plant was more selective for sodium than cesium at equal ratios of the two ions regardless of total ionic concentrations.

TABLE VIII

SELECTIVITY COEFFICIENTS FOR SODIUM AND CALCIUM FOR SOLUTIONS OF CONSTANT EQUIVALENT RATIOS

Conc. in m.e. per Liter	Ions Present		
	Na / Cs A = Na	Ca / Cs A = Ca	Na / Ca A = Na
4	1.68	1.26	6.04
8	1.64	1.18	5.49
12	1.51	1.44	4.00
16	1.42	1.16	3.18
20	1.39	1.75	2.49

Data obtained from investigation of cesium and calcium selectivity are given in Figures 18 and 19. The plot of velocity versus concentration in Figure 18 (a) shows that slightly more calcium than cesium was taken up even at an equivalent ratio of 1:1 for the two ions. Also, it again illustrates that the total equivalents of ions taken up were relatively constant compared to situations where solution concentrations varied as illustrated in Figure 18 (b). The data in Figure 18 (b) also shows that more calcium than cesium was taken up at 1:1 equivalent ratio. However, it will be noted that a total solution concentration effect is illustrated in that the two lines are not parallel. Apparently, calcium uptake was stimulated more by total solution concentration than cesium uptake.

A plot of equivalent fraction in the plant versus equivalent fraction in solution is given in Figure 19 (a). It shows that

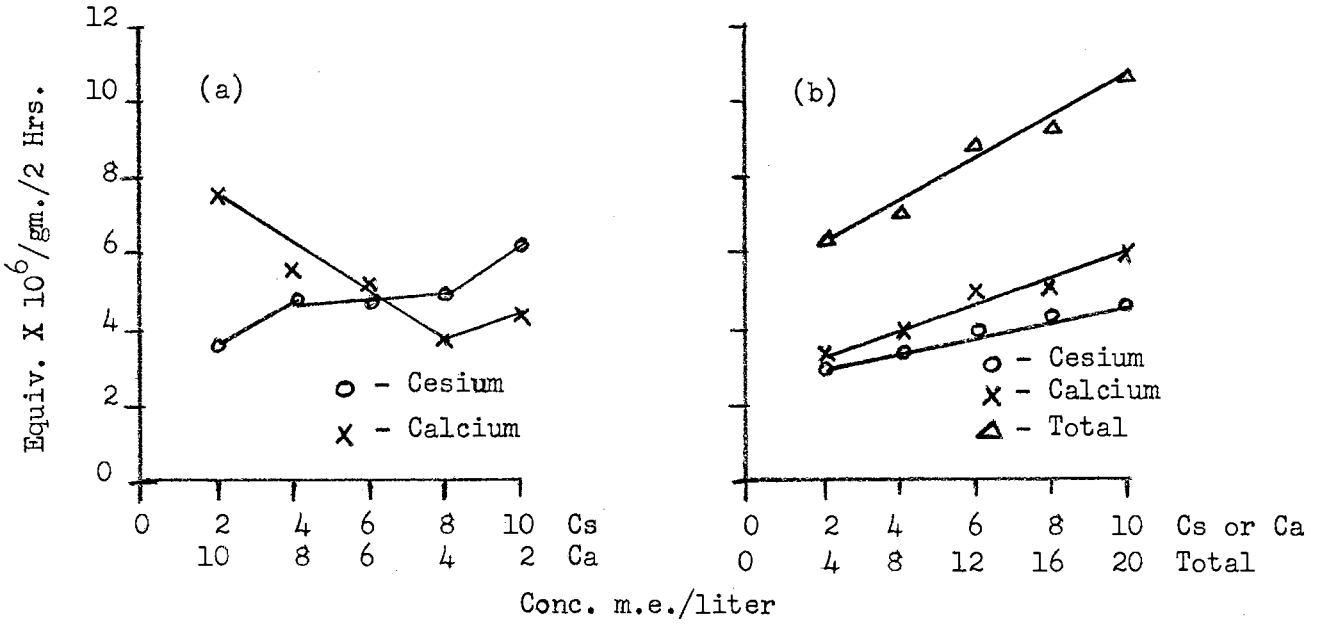

Figure 18. Cesium and Calcium Uptake as a Function of Equivalent Ratios at Constant Total Equivalent Concentration (a) and as a Function of Total Solution Concentration at Constant Equivalent Ratio (b).

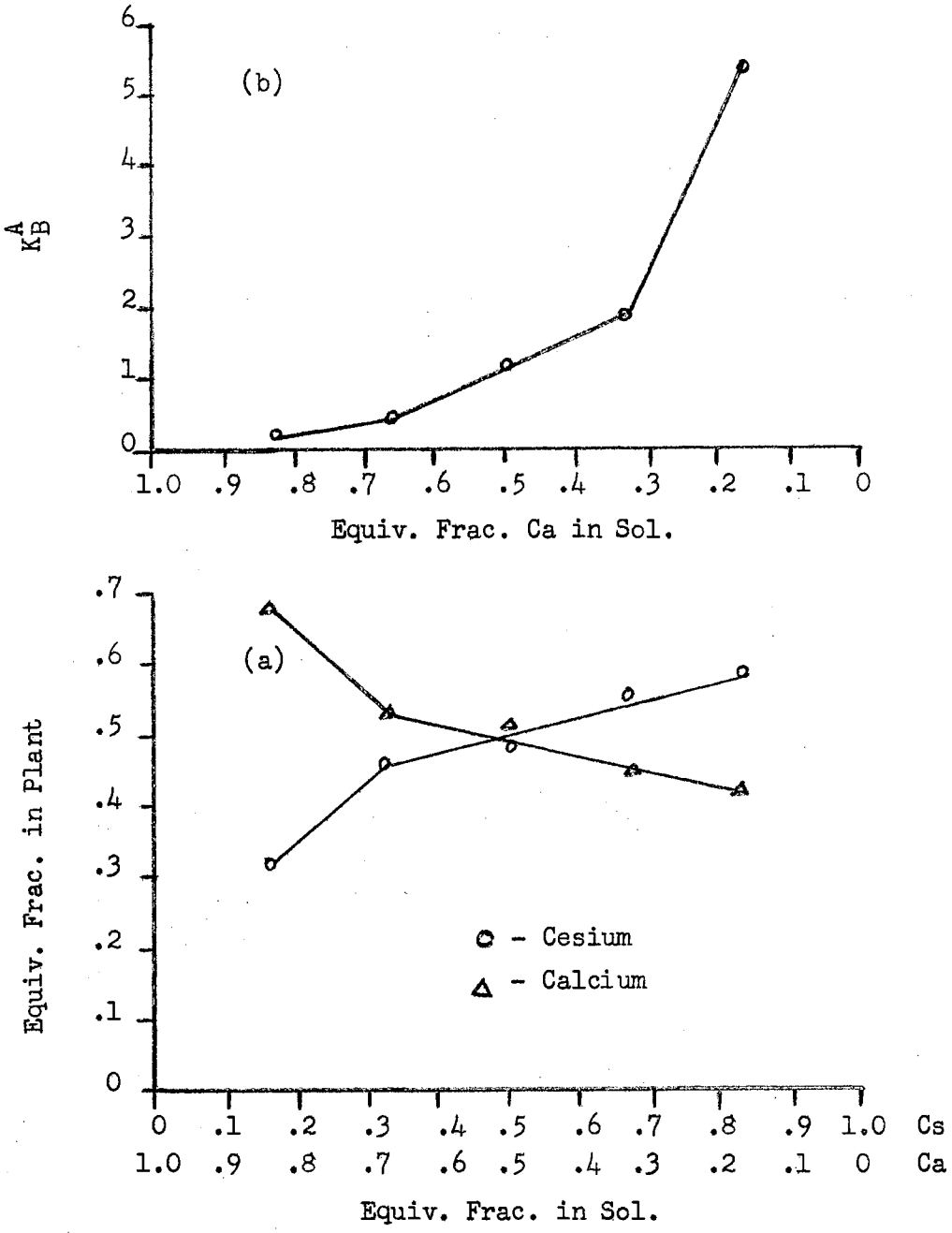

Figure 19. Equivalent Fraction of Cesium and Calcium in the Plants as a Function of the Equivalent Fraction of Cesium and Calcium in Solution (a) and Selectivity Coefficient of Calcium as a Function of the Equivalent Fraction of Calcium in Solution (b).

slightly more calcium than cesium was taken up at a 1:1 equivalent ratio. A plot of the calcium selectivity coefficients versus equivalent fraction of calcium in solution is illustrated in Figure 19 (b). This shows that the plants were more selective for calcium except at low cesium to calcium ratios. The plants became much more selective for calcium as the calcium concentration in solution decreased. The selectivity coefficients for calcium, when the equivalent ratios were constant, were also relatively constant. These are given in Table VIII. There was a tendency for them to increase with increasing total concentration.

Data obtained from investigation of sodium and calcium selectivity are given in Figures 20 and 21.

The plot of velocity versus concentration in Figure 20 (a) shows that more sodium than calcium was taken up at all equivalent ratios. It also shows that total equivalents of ions taken up were relatively constant regardless of the ionic ratio. The plot of velocity versus concentration for the equal equivalent ratio solutions, in Figure 20 (b), shows the same type of total solution concentration effect observed for the sodium and cesium selectivity experiment. The same comments apply here as for that experiment.

A plot of equivalent fraction in the plant versus equivalent fraction in the solution is given in Figure 21 (a). This plot also shows that more sodium than calcium was taken up at all equivalent ratios. A plot of sodium selectivity coefficients versus equivalent fraction of sodium in solution is given in Figure 21 (b). It shows that the plants were more selective for sodium at all ratios of sodium

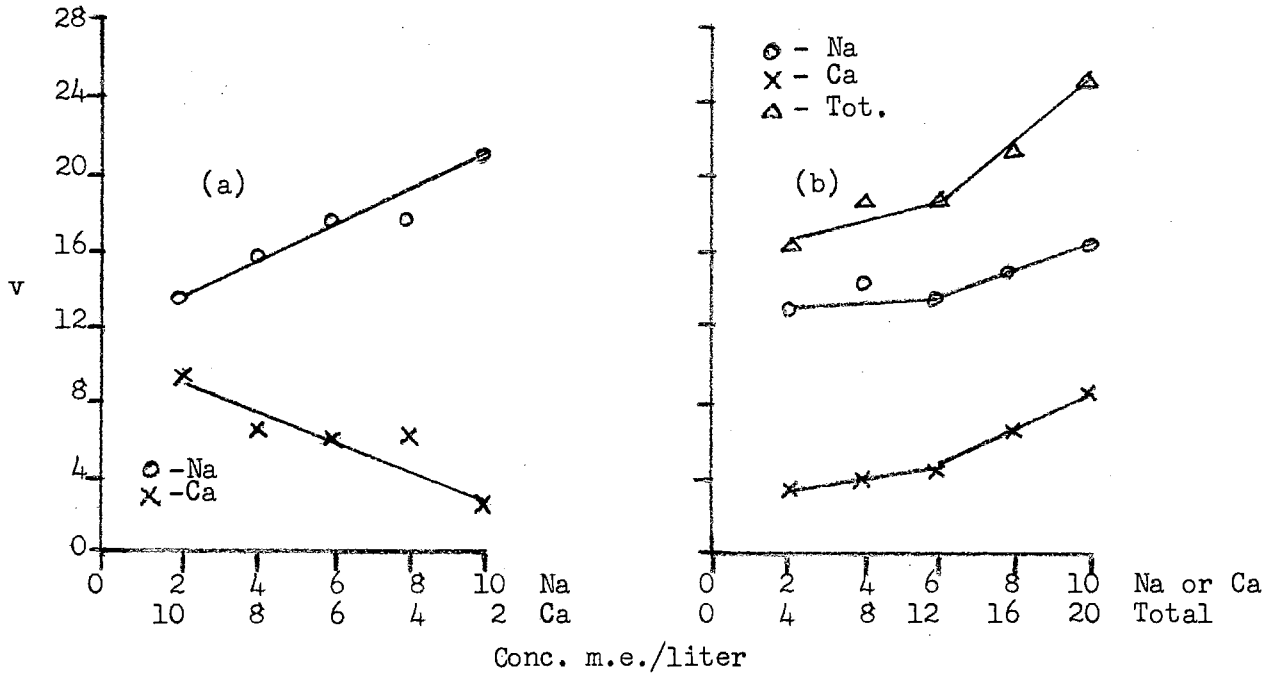

Figure 20. Sodium and Calcium Uptake as a Function of Equivalent Ratios at Constant Total Equivalent Concentration (a) and as a Function of Total Solution Concentration at Constant Equivalent Ratio (b).

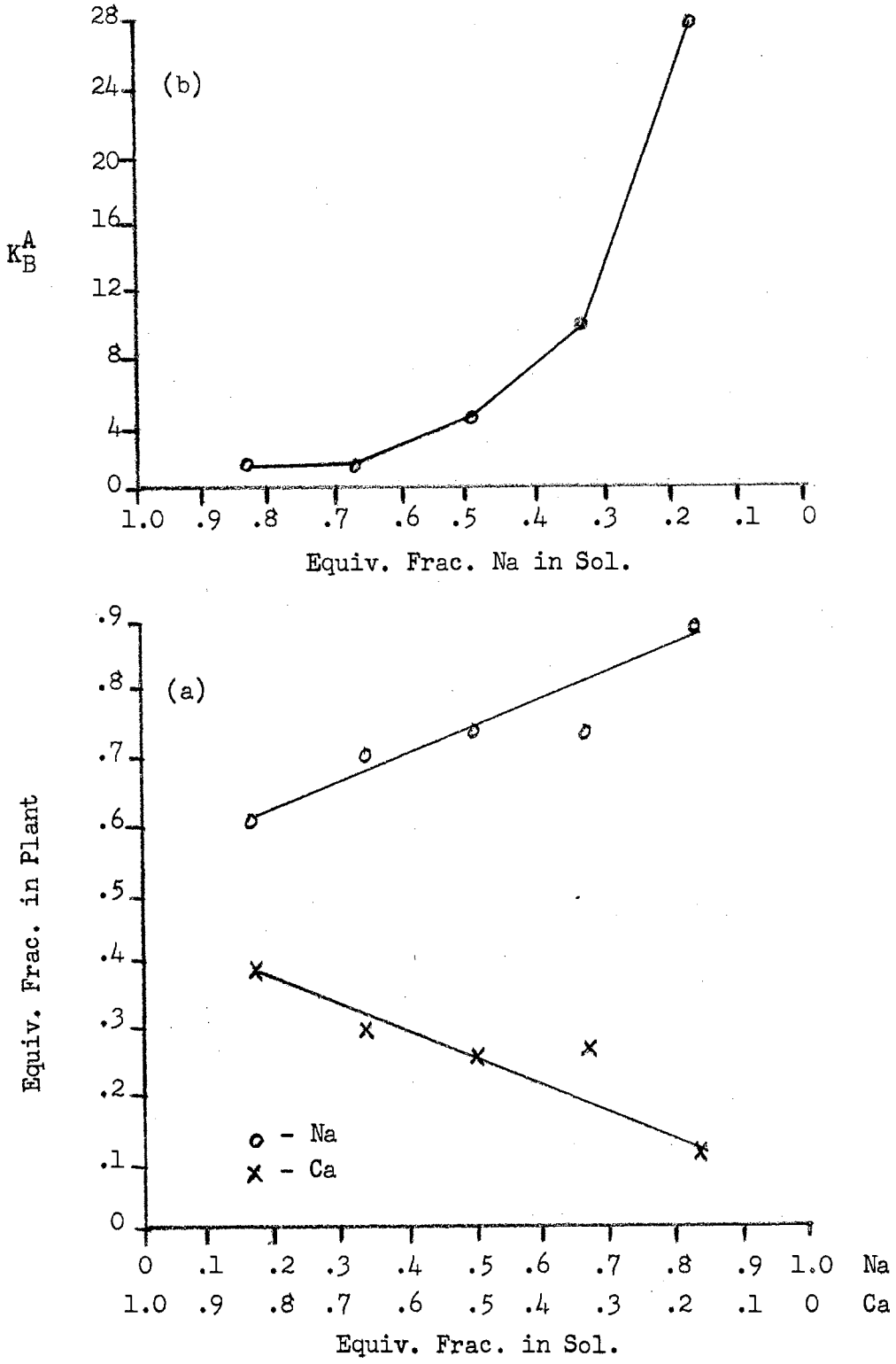

Figure 21. Equivalent Fraction of Sodium and Calcium in the Plants as a Function of the Equivalent Fraction of Sodium and Calcium in Solution (a) and Selectivity Coefficient of Sodium as a Function of the Equivalent Fraction of Sodium in Solution (b).

and calcium. The plants became much more selective for sodium as the sodium concentration in solution decreased. The sodium selectivity coefficients for the solutions of constant equivalent ratios exhibited a definite decrease with increasing total solution concentration. These coefficients are given in Table VIII. Thus the selectivity coefficients for the monovalent ions tended to decrease and the divalent ion selectivity coefficients tended to increase with total solution concentration. Middleton, Handley and Overstreet (40) found a similar effect with potassium and attributed it to a dual carrier system. Helfferich (25) states that this can be expected with exchange phenomena.

Specificity, in both enzyme and exchange phenomena, is a highly complex action and has not been fully investigated in either case. Enzymes are known to be highly specific, but the mode of action has not been clearly defined. For ion exchange phenomena some general trends have been observed for ion specificity. An exchanger tends to prefer: 1) the counter ion of higher valence, 2) the counter ion with the smaller equivalent volume, 3) the counter ion with the greater polarizability, 4) the counter ion which interacts more strongly with the fixed ionic groups or matrix and 5) the counter ion which participates least in complex formation with the co-ion. There are exceptions to these rules, since some of the effects counteract one another.

The data presented would not require a double carrier system to explain the ion selectivity observed. From Table VII, the free energy change for cesium was greater than for sodium indicating that cesium interacted more strongly with the fixed charge sites. With the larger hydrated radius of sodium this is reasonable. However, sodium was

found to be taken up selectively over cesium. Another consideration must be taken into account according to Ling (34). The adsorption-desorption migration process takes place in two steps: First the occupation of a site by an ion which depends directly upon the magnitude of the free energy of adsorption, and second, replacement of this ion by a second counter ion which depends inversely upon the free energy of adsorption of the first ion. An optimal free energy for a maximal rate of entry should exist and probably would be somewhat less than the maximum free energy of adsorption of the ions involved.

Calcium was found to be taken up selectively over cesium when the equivalent ratios were the same. This would follow the trends given above. Calcium exhibited the same free energy of adsorption and has a smaller ionic radius than cesium.

Sodium was found to be taken up selectively over calcium at all equivalent ratios and concentrations used. From the above discussion, this would point out the importance of the second stage of the adsorption-desorption migration on the velocity of uptake.

Although the selectivity observed in these experiments can be adequately explained on the basis of what is known about adsorption, investigation of ion concentrations higher and lower than those used here would have to be conducted before ruling out the multiple carrier mechanism.

Since the data discussed thus far do not afford concrete conclusions with respect to the two uptake mechanisms under investigation, it was decided to conduct an investigation of possible energy sources.

Energy Source Study

In general, it is considered that metabolic energy is required at five places in a carrier system. These are: 1) carrier synthesis, 2) formation of the carrier-ion complex, 3) movement of the carrier-ion complex across the plasmalemma, 4) release of the ion into the cytoplasm and 5) movement of the carrier back across the plasmalemma.

From previous discussion it is evident that formation of the carrier-ion complex would not require metabolic energy, but would be spontaneous under the proper conditions. The activation energy for either uptake mechanism would be thermal in nature. Any of the other four processes mentioned above could require metabolic energy. To release an ion from its carrier would require energy at least equal to the energy of association of that ion with its carrier. This quantity was measured and it was decided to determine if the roots contained sufficient potential metabolic energy to remove the attached ions.

Major anaerobic energy sources are generally considered to be ATP, ADP and creatine phosphate. Creatine phosphate has not been found to occur in plants (54). Thus, it was decided that the formation of ATP and ADP could be stopped with inhibitors and the amounts available determined. This total amount would be considered as being available for removal of the ions from their carrier.

First, the effects of three metabolic inhibitors were investigated using solutions and sets of intact plants as discussed previously. In the first experiment the plant roots were placed in the inhibiting solutions for varying lengths of time, removed and washed for two minutes

in 500 milliliters of deionized water. They were then placed for two hours in a $6x10^{-3}$N CsCl solution tagged with Cs^{137}. The results of this experiment are given in Figure 22. It will be noted that after approximately twenty minutes in the inhibiting solution, no further decrease in uptake resulted. It was found that after two hours in the inhibiting solution and then two hours in the absorbing solution, the plants were wilted. Therefore, one hour in the inhibiting solution was chosen for the next experiment.

After removal from the inhibiting solution and washing, the roots were placed, for varying lengths of time, in a $6x10^{-3}$N CsCl absorbing solution tagged with Cs^{137}. From this point the plants were treated as discussed previously. The data are presented in Figure 23. It will be noted that from two to four hours no increase in cesium uptake occurred. From these two experiments, it was concluded that the major sources of metabolic energy for ion uptake were inhibited by the treatments used. Further, the sources of anaerobic energy were used up at the end of two hours.

In the following experiment the plant roots were subjected to one hour in the inhibiting solution and three hours in a tagged $6x10^{-3}$N CsCl absorbing solution. This experiment was conducted as previously discussed. The data are presented in Table IX. The amount of energy calculated as being necessary was arrived at using an average of all the free energy measurements previously made for cesium, the amount of cesium taken up and a value of 5.3 kcal./mole for ATP $\longrightarrow$ ADP (42). Separation of ATP and ADP could not be accomplished by the method used. The difference in pentose determined was attributed to ATP $\longrightarrow$ AMP in

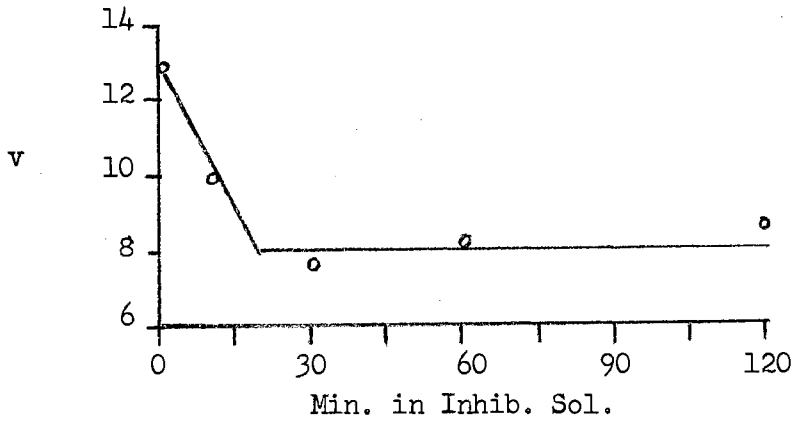

Figure 22. Cesium Uptake as a Function of Time in a Metabolism Inhibiting Solution of $1x10^{-3}$M Sodium Cyanide and $1x10^{-3}$M Iodoacetic Acid. v = Micro-equivalents of Cesium per Gram of Fresh Roots per Two Hours.

order that the maximum amount of potential metabolic energy possible would be used in the comparison of available to needed energy.

The extreme variation noted in the samples can be accounted for by the fact that the small amount of ATP found was at the lower limit of detection by the method used. The method of detection was good for $\pm$0.1 micromoles of ATP per gram of roots. If the amount calculated as being necessary had been present, the sensitivity of the determination method would have been adequate.

The change in calories per gram of roots in Table IX was calculated using the values that would give a maximum. The micromoles of cesium taken up were average values of the plants subjected to the radioactive absorption solution. It was found that approximately four to six times more energy was needed than was found to be used.

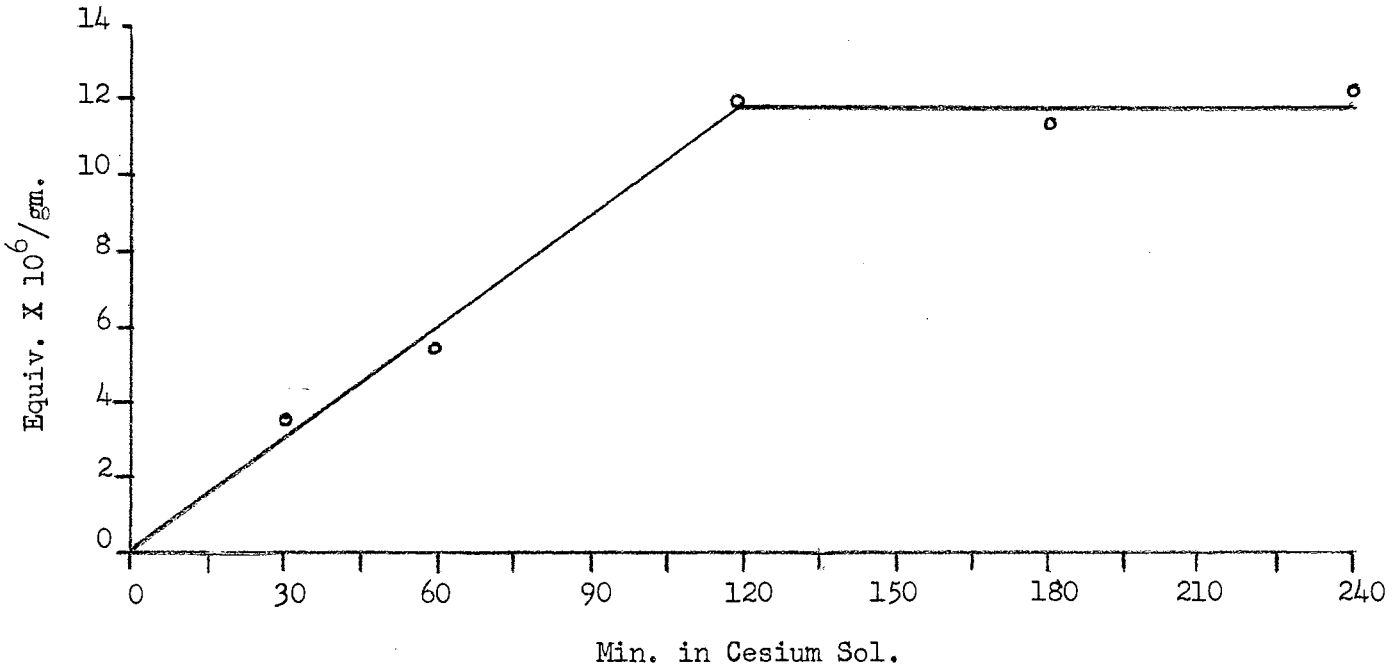

Figure 23. Cesium Uptake as a Function of Time After One Hour in the Inhibiting Solution. Concentration of CsCl-$6x10^{-3}$N.

TABLE IX

COMPARISON OF MAXIMUM ENERGY DELIVERED BY SORGHUM ROOTS

AND ENERGY NEEDED FOR ION UPTAKE

Treat.	$\frac{\Delta \text{ moles ATP}}{\text{gm. roots}}$	$\frac{\Delta \text{ moles}^{a}\ \text{ATP} \longrightarrow \text{AMP}}{\text{gm. roots}}$	(ΔCal./gm.roots)x10^3	$\frac{\Delta \text{ Uptake moles Cs}}{\text{gm.roots}}$	$\left(\frac{\text{Calc.cal.}}{\text{Needed}}\right)$ x10^3
First Trial					
No Cs	0.8				
No Cs	0.1				
Cs	0.3	0.5	5.3	6.9	25.7
Cs	0.2	0.6	6.4	6.9	25.7
Second Trial					
No Cs	0.1				
No Cs	0.6				
Cs	0.2	0.4	4.2	6.5	24.2
Cs	0.2	0.4	4.2	6.5	24.2

[a] Energy release from ATP $\longrightarrow$ AMP was taken as 10.6 kcal./mole. Each pyrophosphate bond releasing 5.3 kcal./mole (42).

An insufficient amount of ATP was found to accomplish only one of the four processes where it might have been used in a carrier system. However, such a system might have been operative. It is possible that other anaerobic sources of energy as important as ATP and ADP could exist in plants.

That energy was necessary for ion uptake was shown by the metabolic inhibition experiments. However, where utilization of this energy takes place is the primary concern. It might be used in the four processes given previously or in protein formation for fixed charge sites of a process such as association-induction. If used for the production of fixed charge sites, once the sites were filled ion uptake would cease. It is known that inhibitors of protein synthesis (e.g. ribonuclease and chloramphenicol) may reduce ion uptake without an appreciable effect on respiration. Thus, it has been considered that active ion uptake might be more closely linked to protein synthesis than other metabolic processes. The mechanism that has been proposed by Sutcliffe is a cycle of ion binding and release accompanying the synthesis and hydrolysis of protein (50). Although the carrier of Epstein and co-workers might well be a protein, the carrier-ion complex of this mechanism is different than that proposed by Sutcliffe and Ling.

CHAPTER V

SUMMARY AND CONCLUSIONS

These investigations were concerned with two objectives. These were: 1. To investigate the mechanism of cation uptake using a system more nearly approaching the natural conditions of the plant and environment than excised roots and forced aeration. 2. To evaluate the proposed mechanisms of cation uptake with measured quantities in addition to kinetic considerations. In order to carry out these objectives, a series of short term absorption experiments were conducted. Intact plants of Sugar Drip forage sorghum nine to twelve days old were used.

Aeration of the absorbing solution was found to have no advantage over non-aeration when 2.5 centimeters of the roots extended above the liquid surface. Cesium, sodium and calcium uptake were found to satisfy the mathematical characterizations of both the carrier and association-induction mechanisms of cation uptake. Interference studies showed that sodium at 2 milliequivalents per liter interfered competitively with cesium uptake, while, calcium at 0.25 milliequivalents per liter had no measurable effect.

By use of association or adsorption energy calculations it was concluded that the dissociation constant measured could be used as a true thermodynamic constant. These calculations also indicated that

this phase of absorption could have been spontaneous. Using this concept and data from absorptions at two temperatures, the overall reaction enthalpy was found to be -1.89 kcal. Activation energy and Q_{10} values were found to vary with absorption solution concentration. Activation energies ranged from 5.52 to 7.68 kcal./mole. Q_{10} values ranged from 1.37 to 1.55.

Sodium was found to be taken up selectively over cesium and calcium. Calcium was found to be taken up selectively over cesium. The selectivity coefficients for the various ions were found to increase with decreasing concentration of that ion in solution. It was not necessary to invoke the multiple carrier hypothesis to explain the selectivity observed.

It was concluded that the major sources of metabolic energy for ion uptake were inhibited by exposure of the plant roots to $1x10^{-3}M$ sodium cyanide and $1x10^{-3}M$ iodoacetic acid. The sources of anaerobic energy were found to be used up at the end of two hours of absorption. The maximum amount of anaerobic energy for ion uptake, as ATP, was measured. It was found to be inadequate by a factor of four to six to accomplish only one of four processes where it might have been used in a carrier system.

The data presented in this thesis does not prove or disprove either the carrier or association-induction hypothesis of ion uptake. It does show that measurements other than kinetics must be made in order to adequately describe the ion uptake mechanism. Some of the magnitudes of measurements made were in the transition zone between enzymatic and adsorption phenomena, while others tended to indicate

an adsorption process. However, additional measurements using wide ion concentration ranges and absorption times will have to be made before the cation uptake mechanism can be adequately characterized.

LITERATURE CITED

1. Adams, Fred and J. B. Henderson. Magnesium Availability as Affected by Deficient and Adequate Levels of Potassium and Lime. Soil Sci. Soc. of Amer. Proc. 26:65-68. 1962.

2. Bear, F. E. and A. L. Prince. Cation-equivalent Constancy in Alfalfa. Jour. Amer. Soc. Agron. 37:217-222. 1945.

3. _____ and Stephan J. Toth. Influence of Calcium on Availability of Other Soil Cations. Soil Sci. 65:69-74. 1948.

4. Bange, G. G. J. Interactions in the Potassium and Sodium Absorption by Intact Maize Seedlings. Plant and Soil 11:17-29. 1959.

5. _____ and R. Overstreet. Some Observations on Absorption of Cesium by Excised Barley Roots. Plant Physiol. 35:605-608. 1960.

6. Bartlett, R. J. and E. O. McLean. Effect of Potassium and Calcium Activities in Clay Suspensions and Solutions on Plant Uptake. Soil Sci. Soc. of Amer. Proc. 23:285-289. 1959.

7. Becking, J. C. On the Mechanism of Ammonium Ion Uptake by Maize Roots. Acta. Botan. Neerland. 5:1-79. 1950.

8. Bennet-Clark, T. A. Salt Accumulation and Mode of Action of Auxin. A Preliminary Hypothesis. 284-291. The Chemistry and Mode of Action of Plant Growth Substances. Ed. R. L. Wain and F. Wightman. Butterworths, London. 1956.

9. Boszormenyi, Z. and E. Cseh. The Uptake of Halide Ions and their Relationships in Absorption. Physiol. Plant. 14: 242-252. 1961.

10. _____ and _____ Studies of Ion-Uptake by Using Halide Ion Changes in the Relationships Between Ions Depending on Concentration. Physiol. Plant. 17:81-90. 1964.

11. Dainty, Jack. Ion Transport and Electrical Potentials in Plant Cells. Annual Review of Plant Physiology. 13:379-402. 1962.

12. Dixon, Malcom and Edwin C. Webb. Enzymes. Academic Press Inc. New York. 1958.

13. Drake, M. and G. P. Scarseth. Relative Abilities of Different Plants to Absorb Potassium and Effects of Different Levels of Potassium on the Adsorption of Calcium and Magnesium. Soil Sci. Soc. of Amer. Proc. 4:201-204. 1940.

14. Epstein, E. The Essential Role of Calcium in Selective Cation Transport by Plant Cells. Plant Physiol. 36:437-444. 1961.

15. _____ and C. E. Hagen. A Kinetic Study of the Absorption of Alkali Cations by Barley Roots. Plant Physiol. 27:457-474. 1952.

16. _____ and James E. Leggett. The Absorption of Alkaline Earth Cations by Barley Roots. Kinetics and Mechanism. Amer. Jour. Bot. 41:785-791. 1954.

17. Etherton, Bud and N. Higinbotham. Uptake of Cations and Charge in the Mesoplasm. Science 131:409-410. 1960.

18. Foy, C. D. and S. A. Barber. Magnesium Deficiency and Corn Yield on Two Acid Indiana Soils. Soil Sci. Soc. of Amer. Proc. 22:145-148. 1958.

19. Fried, Maurice and J. C. Noggle. Multiple Site Uptake of Individual Cations by Roots as Affected by Hydrogen Ion. Plant Physiol. 33:139-144. 1958.

20. _____, J. C. Noggle, and C. E. Hagen. The Relationships Between Adsorption and Absorption of Cations. Soil Sci. Soc. of Amer. Proc. 22:495-499. 1958.

21. Fruton, Joseph S. and Sofia Simmonds. General Biochemistry. John Wiley and Sons Inc. New York. 1958.

22. Gray, B., M. Drake, and W. G. Colley. Potassium Competition in Legume-Grass Associations as a Function of Root Exchange Capacity. Soil Sci. Soc. of Amer. Proc. 17:235-239. 1953.

23. Handley, R., Vidal R. Dios, and R. Overstreet. Metabolic and Non-Metabolic Uptake of Sodium in Roots of Zea mays. Plant Physiol. 35:907-912. 1960.

24. _____ and R. Overstreet. Uptake of Calcium and Chloride in Roots of Zea mays. Plant Physiol. 36:766-769. 1961.

25. Helfferich, Friedrich. Ion Exchange. McGraw-Hill Book Co., Inc. New York. 1962.

26. Huffaker, R. C. and A. Wallace. Possible Relationships of Cation Exchange Capacity of Plant Roots to Cation Uptake. Soil Sci. Soc. of Amer. Proc. 22:392-394. 1958.

27. _____ and _____ Sodium Absorption by Different Plant Species at Different Potassium Levels. Soil Sci. 87: 130-134. 1959.

28. Hunter, A. S. Yield and Composition of Alfalfa as Affected by Variations in Calcium-Magnesium Ratios in the Soil. Soil Sci. 67:53-62. 1949.

29. Jenny, Hans and Karl Grossenbacker. Root-Soil Boundary Zones as Seen in the Electron Microscope. Soil Sci. Soc of Amer. Proc. 27:273-277. 1963.

30. _____ and R. Overstreet. Cation Interchange Between Plant Roots and Soil Colloids. Soil Sci. 47:257-272. 1939.

31. Lagerwerff, J. V. Comparable Effects of Adsorbed and Dissolved Cations on Plant Growth. Soil Sci. 86:63-69. 1958.

32. _____ and Michael Peech. Relation Between Exchange Adsorption and Accumulation of Calcium and Rubidium by Excised Barley Roots. Soil Sci. 91:84-93. 1961.

33. Le Clerg, Erwin L., Warren H. Leonard, and Andrew G. Clark. Field Plot Technique. Burgess Publishing Co. Minneapolis. 1962.

34. Ling, Gilbert N. A Physical Theory of the Living State. Blaisdell Pub. Co. New York. 1962.

35. Lucas, R. E. and G. D. Scarseth. Potassium, Calcium and Magnesium Balance and Reciprocal Relationship in Plants. Jour. Amer. Soc. of Agron. 39:887-896. 1947.

36. Lundegardh, H. Leaf Analysis. Hilger and Watts Ltd. Hilger Div. London. 1951.

37. Maron, Samuel H. and Carl F. Prutton. Principles of Physical Chemistry. The Macmillan Co. New York. 1958.

38. McLean, E. O. Uptake of Sodium and Other Cations by Five Crop Species. Soil Sci. 82:21-28. 1956.

39. Mehlich, A. and J. F. Reed. The Influence of Degree of Saturation, Potassium Level and Calcium Additions on Removal of Calcium, Magnesium and Potassium. Soil Sci. Soc. Amer. Proc. 10:87-93. 1945.

40. Middleton, L. J., R. Handley, and R. Overstreet. Relative Uptake and Translocation of Potassium and Cesium in Barley. Plant Physiol. 35:913-918. 1960.

41. Moore, David P., Roy Overstreet, and Louis Jacobson. Uptake of Magnesium and its Interaction with Calcium in Excised Barley Roots. Plant Physiol. 36:290-295. 1961.

42. Mortimer, C. T. and H. D. Springall. Modern Thermochemistry. Endeavour 23:22-26. 1964.

43. Olsen, R. A. and Michael Peech. The Significance of the Suspension Effect in the Uptake of Cations by Plants From Soil-Water Systems. Soil Sci. Soc. Amer. Proc. 24:257-261. 1960.

44. Overstreet, R. and H. Jenny. Studies Pertaining to the Cation Adsorption Mechanism of Plants in Soil. Soil Sci. Soc. of Amer. Proc. 4:125-130. 1939.

45. Perkins, H. F. and Matthias Stelly. Calcium and Magnesium Content of Oats and Crimson Clover Grown on Norfolk Sandy Loam as Affected by Sodium and Potassium. Soil Sci. 86:305-309. 1958.

46. Rains, D. W., Walter E. Schmid, and Emanuel Epstein. Absorption of Cations by Roots. Effects of Hydrogen Ions and Essential Role of Calcium. Plant Physiol. 39:274-278. 1964.

47. Romney, E. M., G. V. Alexander, W. A. Rhoads, and K. H. Larson. Influence of Calcium on Plant Uptake of Sr^{90} and Stable Strontium. Soil Sci. 87:160-165. 1959.

48. Specht, Alston W. Evidence for and Some Implications of a Variation-Control Mechanism in Plant Composition. Soil Sci. 89: 83-91. 1960.

49. Steward, F. C. and F. K. Millar. Salt Accumulation in Plants: A Reconsideration of the Role of Growth and Metabolism. Symp. Soc. Exp. Biol. 8:367-406. 1954.

50. Sutcliffe, J. F. Mineral Salts Absorption in Plants. Pergamon Press. New York. 1962.

51. Tanada, T. Effect of Ribonuclease on Salt Absorption by Excised Mung Bean Roots. Plant Physiol. 31:251-253. 1956.

52. Tanada, T. Localization and Mechanism of Calcium Stimulation of Rubidium Absorption in the Mung Bean Root. Amer. Jour. Bot. 49:1068-1072. 1962.

53. Tromp, J. Interactions in the Absorption of Ammonium, Potassium and Sodium Ions by Wheat Roots. Acta. Bot. Neerland. 11:147-192. 1962.

54. Umbreit, W. W., R. H. Burris, and J. F. Stauffer. Manometric Techniques. Burgess Pub. Co. Minneapolis. 1957.

55. Van Itallie, Th. B. Cation Equilibrium in Plants in Relation to the Soil. Soil Sci. 46:175-186. 1938.

56. Viets, Frank G., Jr. Calcium and Other Polyvalent Cations as Accelerators of Ion Accumulation by Excised Barley Roots. Plant Physiol. 19:466-481. 1944.

57. Wallace, Arthur. Solute Uptake by Intact Plants. Arthur Wallace, Los Angeles, Cal. 1963. 155-158.

58. _____ and C. R. Carmack. Solute Uptake by Intact Plants. Arthur Wallace, Los Angeles, Cal. 1962. 40-41.

59. _____ and S. M. Sufi. Solute Uptake by Intact Plants. Arthur Wallace, Los Angeles, Cal. 1962. 19-26.

60. Woodruff, C. M. Cation Activities in the Soil Solution and Energies of Cationic Exchange. Soil Sci. Soc. of Amer. Proc. 19: 98-99. 1955.

APPENDIX

Kinetics of Enzyme Action

Consider the enzymatic reaction process as follows: (21)

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} \text{Products} + E$$

The following designations will be used:

(E) = Total concentration of enzyme E

(S) = total concentration of substrate, so chosen that (S) is much greater than (E)

(ES) = concentration of enzyme-substrate complex

(E) - (ES) = concentration of free enzyme

If $E + S \rightleftharpoons ES$ is reversible then the dissociation constant of ES, defined as Km, can be written as,

$$Km = \frac{[(E) - (ES)](S)}{(ES)}$$

solving for (ES)

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

If the velocity constant for the decomposition of ES is k_3, and the measured velocity is v, then $v = k_3(ES)$, and

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

The maximal velocity V will be attained when the concentration of ES is maximal, i.e., when all the enzyme is bound by substrate, and (ES) = (E). Then,

$$V = k_3(ES) = k_3(E)$$

If V is substituted for $k_3(E)$, the Michaelis-Menten equation is obtained.

$$v = \frac{V(S)}{Km + (S)}$$

By taking reciprocals the Lineweaver-Burk equation is obtained

$$\frac{1}{v} = \frac{Km}{V}\left[\frac{1}{(S)}\right] + \frac{1}{V}$$

Briggs and Haldane (21) pointed out that Km represents the true dissociation constant of ES only if the velocity of the dissociation of ES is much greater than the rate of its conversion to products and E. The rate of formation of ES may be denoted by the term $k_1[(E) - (ES)](S)$ and the rate of decomposition of ES by the term $[k_2(ES) + k_3(ES)]$.

The overall rate of change in the concentration of ES is therefore

$$\frac{d(ES)}{dt} = k_1[(E) - (ES)](S) - k_2(ES) - k_3(ES)$$

So long as the rate of the reaction (v) is constant, then (ES) is constant and the term d(ES)/dt = 0. Under these circumstances,

$$(ES) = \frac{k_1(E)(S)}{k_1(S) + k_2 + k_3} = \frac{(E)(S)}{(S) + [k_2 + k_3/k_1]}$$

If the quotient $(k_2 + k_3)/k_1$ is set equal to Km, the expression is the same as derived by Michaelis. Therefore, Km is a true dissociation constant of ES only when k_3 is negligible with respect to k_2.

For the application of the Michaelis-Menten equation to the competitive inhibition of enzymes, the equilibrium

$$E + I \rightleftharpoons EI$$

where I denotes the inhibitor, must also be considered. For a competitive inhibitor the concentration of the free enzyme becomes

$$[(E) - (ES) - (EI)]$$

and the dissociation of the enzyme-inhibitor complex is

$$Ka = \frac{[(E) - (ES) - (EI)](I)}{EI}$$

The overall rate of formation of ES is

$$\frac{d(ES)}{dt} = k_1 [(E) - (ES) - (EI)](S) - k_2(ES) - k_3(ES)$$

At the steady state of the reaction, $d(ES)/dt = 0$ and

$$(ES) = \frac{(S)[(E) - (EI)]}{(S) + Km}$$

Substituting for (EI)

$$(ES) = \frac{(E)(S)Ka}{KmKa + Km(I) + Ka(S)}$$

Then as before

$$v = \frac{V(S)Ka}{KmKa + Km(I) + Ka(S)}$$

Modification by the method of Lineweaver and Burk gives

$$\frac{1}{v} = \frac{1}{V}\left[Km + \frac{Km(I)}{Ka}\right]\left[\frac{1}{(S)}\right] + \frac{1}{V}$$

Kinetics of Competitive Entry of Ions by Association and Induction According to Ling (34)

Adsorption-desorption migration for the ith external ion depends fundamentally on the mole fraction of fixed ionic sites occupied by the ith ion. There is only a finite number of such fixed ionic sites; the mole fraction of the ith ion-fixed-ion doublets depends on the ith-ion adsorption energy, and on the adsorption energy and concentration of counter ions competing for the same sites. The dissociation constant, Ki, for the ith ion adsorption is defined by

$$Ki = \frac{(p.f.)_i^{fr.}}{(p.f.)_i^{ads.}} \quad \exp. \left(\frac{\Delta Ei}{RT} \right)$$

where: $(p.f.)_i^{fr.}$ = partition function of ith ion in free state

$(p.f.)_i^{ads.}$ = partition function of ith ion in adsorbed state

ΔEi = adsorption energy of the ith ion

R = gas constant

T = absolute temperature

Assume that there are only two major external ions, pi and pj, competing for fixed-charge sites, f. At equilibrium, of $(f)_0$, the total number of fixed-charge sites per unit surface area, $(f)_v$ are vacant. Thus,

$$(f)_0 = (f)_v \neq (pi^{ex} \cdot f) \neq (Pj^{ex} \cdot f)$$

However,

$$(pi^{ex} \cdot f) = \lceil pi \rfloor_{ex} (f)_v \frac{1}{Ki}$$

$$(pj^{ex} \cdot f) = \lceil pj \rfloor_{ex} (f)_v \frac{1}{Kj}$$

where $\lceil pi \rfloor_{ex}$ = external concentration of ith ion.

Since the rate of ith-ion permeation is proportional to the number of ith-ion-fixed-site pairs, one may expect that a theoretical maximum rate of entry, Vi, should be reached when $(pi^{ex} \cdot f) = (f)_0$ and that the actual rate of ith-ion permeation, vi, should be related to Vi in such a way that

$$\frac{Vi}{vi} = \frac{(f)_0}{(P_i^{ex} \cdot f)}$$

by substitution

$$(f)_0 = (f)_v + (f)_v \frac{1}{Ki} \lceil pi \rfloor_{ex} + (f)_v \frac{1}{Kj} \lceil pj \rfloor_{ex}$$

$$= (f)_v \left(1 + \frac{\lceil Pi \rfloor_{ex}}{Ki} + \frac{\lceil Pj \rfloor_{ex}}{Kj} \right)$$

Then,

$$\frac{Vi}{vi} = \frac{Ki}{\lceil pi \rfloor_{ex}} \left(1 + \frac{\lceil pi \rfloor_{ex}}{Ki} + \frac{\lceil Pj \rfloor_{ex}}{Kj} \right)$$

and

$$\frac{1}{vi} = \frac{1}{Vi} \left(Ki + \frac{Ki \lceil pj \rfloor_{ex}}{Kj} \right) \frac{1}{\lceil pi \rfloor_{ex}} + \frac{1}{Vi}$$

VITA

Arthur Blake Onken

Candidate for the Degree of

Doctor of Philosophy

Thesis: INVESTIGATIONS CONCERNING PROPOSED CATION UPTAKE MECHANISMS IN PLANTS

Major Field: Soil Science

Biographical:

Personal Data: Born August 13, 1935, in Alice, Texas, son of Arthur R. and Iley Belle Onken.

Education: Graduated from William Adams High School, Alice, Texas in 1953. Undergraduate work at the Agricultural and Mechanical College of Texas from 1953 to 1955 and Texas College of Arts and Industries from 1955 to 1959. Received Bachelor of Science degree, with a major in Agricultural Engineering in 1959; received a Master of Science degree from Oklahoma State University with a major in Soil Science in 1960; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in August, 1964.

Experience: Worked during summers of 1950 through 1953 in sheet metal and plumbing shop. Employed by Texaco as roustabout, draftsman and rod and chainman during summers of 1954 and 1955. Worked for Chiles Drilling Company during summers of 1956, 1958 and 1959. Part time employment as laboratory technician for Bureau of Reclamation during school years of 1957 through 1959 and summer of 1957. Graduate assistant at Oklahoma State University 1959 to 1964.

Member: Alpha Tau Alpha, Alpha Chi, Phi Kappa Phi, Society of Sigma Xi, American Society of Agronomy and Soil Science Society of America.

Date of final examination: July, 1964