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Cation absorption by excised Barley roots in relation to root cation exchange capacity.

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CATION ABSORPTION BY EXCISED BARLEY ROOTS IN RELATION TO ROOT CATION EXCHANGE CAPACITY

by

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I. Introduction

Many investigators believe that uptake of cations by plants consists of two consecutive processes characterized by the passive entry, mainly cation exchange of ions into tissues, and their active accumulation within tissues in contact with the ionic environment (27,30,53,61,86).

However, there is considerable discrepancy of opinions on the function of cation exchange adsorption in cation uptake. It has been proposed, on the one hand, that the exchange adsorption of cations from the environment is a predominant process in the cation uptake by plant roots resulting in the differential uptake of monovalent and divalent cations. This proposal has been theorized by the Donnan principle (20,42,62,77,78,91,95). On the other hand, it has been suggested by many investigators that exchange adsorption has little or no effect on the accumulation of cations by plants (14,25,52,53).

In spite of considerable research in recent years on these processes, the question whether, and to what extent, the initial exchange adsorption of cations by plant roots determines their subsequent accumulation remains unsolved. Because of the considerable ecological and agricultural importance of the subject, much more information about this problem is required.

The difficulty of evaluating the role of cation-exchange capacity (CEC) of plant roots in the cation uptake process comes from the complexity of the uptake process and the general nature of the root CEC. The relationship of CEC to both quantitative and qualitative or ratio of mono/divalent cation uptake must be considered. In addition to these difficulties, the methods for determining the root CEC have served to complicate studies of the relationship of CEC to the cation uptake process.

Although large differences in root CEC among some plant species and their concomitant effect on cation absorption have been reported, (20,28,42,77,78), there are probably other genetical, physiological or cytological differences among species that influence uptake. The root CEC values have been altered by some nutrio-environmental factors, especially by source and level of nitrogen (14, 18,35,65,77,95). This method of inducing differences appeared to be a means of providing roots genetically alike but differing in root CEC. Such roots could be used to examine the effect of CEC on cation uptake without introducing variables arising from different plant species.

In this study, efforts were made to improve the method to determine the root CEC and to characterize the non-metabolic absorption by excised barley roots. The effects of N induced levels of root CEC, on the ratio of monovalent to divalent cations as well as total cations absorbed by

the root were studied.

II. Terminology

In order to avoid ambiguity, a number of words will be defined as follows:

Absorption and uptake are used synonymously, and imply every kind of net accumulation of ions from external solutions by the root. Desorption implies outward net movement from the root to the external solution. Metabolic absorption is used for absorption requiring metabolic energy. Non-metabolic absorption is used for exchange adsorption and diffusion.

A tissue of the root into which ions move passively is defined as the Free Space. The Free Space consists of two main components, the Water Free Space (WFS), where the concentrations of ions quickly become equal to those of the external solution, and the Donnan Free Space (DFS), containing a high concentration of exchange adsorption sites or non-diffusible anions.

Native cation refers to Na, K, Ca, Mg, etc., normally occuring in the plant root as opposed to Li, Rb, Ba, Sr, etc., not normally found in the root.

III. Review of Literature

1). The mechanism of cation uptake by plant roots and the function of cation exchange in this process

Recognition that inorganic salts are taken up by plant roots and other biological systems led naturally to speculation about the mechanism of uptake. From the latter half of the nineteenth century, considerable effort has been made to elucidate the mineral uptake process and many hypotheses on this process have been proposed.

With the early extensive studies dealing with the time course of ion uptake into plant tissues, there has been recognition of the fact that a relatively brief interval of rapid intake is normally followed by a slower but more prolonged period of absorption (7). In addition to the distinctions regarding the duration and velocity of the two phases, several features distinguish uptake as it occurs during the periods. Thus, steady-state uptake, rigidly dependent on concomitant respiratory metabolism, is in most instances an obligatory aerobic process, has a high temperature coefficient, and is most often characterized by the absorption of both members of an ion pair. On the other hand, the initial phase appears non-metabolic, may occur anaerobically, has a temperature coefficient typical of a physical process, and is predominantly concerned with

cation absorption (53).

It is known that some cations may be non-metabolically absorbed by plant roots. Moore et al. (69) reported that the Ca uptake by 6-day-old barley roots appeared to be largely non-metabolic because the Ca uptake at pH 5 was found to be insensitive to low temperature and dinitrophenol. However, the view that cation uptake involves both non-metabolic and metabolic absorption is generally accepted in most cases of cation uptake (27,30,53,61,86). The outstanding question is whether, and to what extent, the non-metabolic uptake is related to metabolic uptake. The answers to this question has been offered by many investigators, but still they are far from the point of agreement. Before the literature on non-metabolic as related to metabolic uptake is reviewed, the concept of "free space" and "carrier theory" will be briefly reviewed.

Early investigators of ion absorption viewed the cytoplasm simply as a membrane across which transport into the central vacuole occurs. The observations about the complexity of cell structure, however, made it difficult to presume a simple membrane which separates the external and internal solution. Attempts have been made to determine more exactly the location of permeability barriers in plant cells by estimating the volume of tissue which is passively penetrated by salts or organic solutes (3,4,8,9, 51,71). These attempts introduced a concept of "free space"

in the study of salt uptake mechanism (9). In order to define a tissue into which ions move passively to a concentration equalling that of the medium, the terms "water-free space" and "outer space" have been applied. If the freely accessible space within a cell or tissue contains immobile electrical charges or adsorption sites, Donnan equilibria will be established in the space. In this case, the actual volume of a cell or tissue penetrated passively by ions may be different from that calculated by assuming that ions reach the same concentration in the cell tissue as in the medium. For this reason, it is often necessary to refer to the "apparent" free space rather than actual free space of a tissue or cell.

From measurements of passive ion uptake from salt solutions of different concentrations, it is possible to deduce that the "apparent free space" consists of two components, the "water-free space" and a "Donnan-free space." Attempts to determine the concentrations of immobile anions in Donnan-free space have been made (4). According to Briggs (4), penetration of salt into the roots occurs by free diffusion through the free space. Hope and Stevens (40) and Butler (8) have regarded the initial flux as a diffusion process from external solution into free space.

The early findings that plant roots can absorb mineral salts against a concentration gradient and that the absorption process is characterized by a considerable degree of

selectivity led to the "carrier theory." General reviews of the "carrier theory" include those by Ussing (89), Robertson (73), Steinbach (79), Laties (53), and Epstein (27). Osterhout (70) and Jacobson *et al.* (45) have outlined several properties which the ion binding compounds must possess to account for characteristic features of the absorption process. These workers also stress the need for assuming that the complex is labile and breaks down again, releasing the ions. This absorption process then has been pictured as follows. At the outer surface of a membrane, impermeable to the free ions, the ions combine with metabolically produced binding compounds or carriers, traversing the membrane in this form. Upon reaching the inner surface, the binding compound or carrier is chemically altered by metabolic processes so that the ions are set free. These assumptions can be expressed in the following equations:

$$1).\quad R + M \underset{\text{outside } k_2}{\overset{k_1}{\rightleftharpoons}} MR$$

$$2).\quad MR \underset{k_4}{\overset{k_3}{\rightleftharpoons}} R' + M_{\text{inside}}$$

where R and R' represent different chemical states of the metabolically produced carrier, M the ion, MR the unstable carrier-ion complex and k the rate constant for each reac-

tion indicated. The overall reaction is essentially irreversible (23). Although kinetic studies of cation uptake have supported this concept (23,24,25,26,54), the process remains a "carrier theory" in that no carriers have been identified.

It has been widely held that cation exchange adsorption at the cell surface is the initial step in ionic entry (23,24,44,45,59,60,74,85). However, there is a discrepancy of opinions on the possible function of cation exchange in the cation uptake process.

Mattson (62) and Elgabaly and Wiklander (20) have made application of the Donnan equation to explain the differential cation uptake by plant roots. According to the Donnan theory of membrane equilibria, the distribution of ions of different valence in a Donnan system is governed by the relative activity of both inside and outside solutions. The distribution of monovalent and divalent cations between the colloid and the outside solution is generally given by the following equation:

$$\frac{(M^{+})i}{(M^{+})o} = \frac{(M^{++})i^{\frac{1}{2}}}{(M^{++})o^{\frac{1}{2}}}$$

where $(M^{+})i$ and $(M^{+})o$ represent the activity in the inside and outside solution, respectively, of the ion M^{+}. From the square root relation, it follows that divalent ions

will accumulate to a greater degree than monovalent ions in the phase with the higher activity. Furthermore, it follows that a relatively concentrated inside solution and a dilute outside solution would favor the adsorption of divalent ions (20). This is referred to as the "valence effect" (19). Thus, when ion activity in soils is very low, this valence effect is larger, but diminishes with increased ion activity in the soil solution.

On the basis of the Donnan theory described here, Elgabaly and Wiklander (20) studied the effect of the cation exchange capacity (CEC) of the plant root on the uptake of Na and Ca from bentonite suspensions. Their results showed a higher Ca/Na ratio in the pea roots with the higher CEC and a lower ratio in barley roots with the lower CEC. They stated that these results were in good qualitative agreement with those expected according to the Donnan theory, and that the Donnan distribution of ions of different valence would tend to be reflected in the composition of the plants. Elgabaly (22) and Wiklander (21) have applied the Donnan principle to differential anion uptake by plant roots. They found that the roots with higher CEC absorbed a lower amount of Cl^-. These results were explained on the assumption that roots with higher negative charges may tend to repel Cl^-.

Vervelde's observations (91) on the surface potentials of plant roots showed that the ionic distribution across

the exterior walls of the outer cells of the roots was in accordance with the laws of the Donnan equilibrium. He summarized his observation as follows. As the Donnan distribution may be regarded as adsorption, these observations confirm the idea that the first step of ion uptake is an adsorption on to cell constituents. The adsorptive forces are not restricted to surface layers. Therefore, though entire organs do not reach a state of equilibrium with the medium, the tendency to reach such an equilibrium is indicated from the occurrence of ionic compositions of plant roots or plant organs which are in agreement with the requirements of the Donnan theory. An example of this is to be seen in the preferential uptake of divalent ions by plant roots with high CEC contents.

Wallace and his associates (76,77,78) have reported a positive relationship between the root CEC and the plant content of Ca or K. Huffaker and Wallace (42) found that the K, Ca, and Mg contents of different plant species grown under similar conditions could be related in a majority of cases by $CEC_1 / CEC_2 = K_2 / K_1 = (Ca_1 + Mg_1 / Ca_2 + Mg_2)^{\frac{1}{2}}$ where the subscripts 1 and 2 represent different species.

Heintze (37) also reported positive relationships between CEC values and relative uptakes of mono- and divalent cation by plant species grown under controlled nutrient solutions. High CEC values were associated with low K/Ca ratios of plant tops or of the whole plant when comparisons

were restricted to the same K level of the growing medium.

White, Drake and Baker (95) reported the effect of induced changes on root CEC and Ca adsorption from benonite systems by excised barley roots. Calcium gains increased directly with N-induced increases in CEC of barley roots when the excised roots were reacted 24 hours with 100% Ca saturated bentonite. The excised barley roots with high CEC gained more Ca than the low CEC roots from the 94 and 100% Ca clay suspensions.

Recently Franklin (28) studied the possible relations between the CEC of plant roots and the uptake of ions. In his study, plants were pretreated to alter the root CEC. The amounts of Ca and K absorbed by the excised roots after a one-hour uptake period in 10^{-3}-N chloride solution were displaced by a series of successive rinses, the pH ranging from 2 to 12. The results showed a significant linear correlation between the total uptake of both Ca and K and the exchangeable ions which were not removed by the first acid rinse, but which were removed with subsequent rinses.

McLean (66) found no systematic relationship between root CEC (or per cent N) with the composition of Ca, Mg, P, K, and Na when various crops were grown in 1/5 Hoagland solution. He explained that this might not be unexpected in specific media devoid of competing colloids where the Donnan effects are at a minimum.

Cunningham and Nielsen (14) offered evidence against

relationship between root CEC and cation uptake by plants. Their experimental results on Italian rye grass showed that the differential uptake of mono- and divalent cations was not related to root CEC.

Fried, Noggle, and Hagen (29) applied steady-state analysis to the absorption of cations by excised barley roots. Their results indicated that ions were adsorbed and absorbed by specific ion binding compounds of the roots and that only negligible amounts of the cations tested were absorbed nonspecifically.

Wallace (92) introduced a concept of "effective" root CEC rather than total root CEC to explain the discrepancies in the effect of the root CEC on both the total cation absorption and on the ratio of divalent to monovalent cations in the plants. As one approach to this problem, he attempted to saturate the root CEC sites by pretreatment with cations not readily exchangeable and then to determine this effect on cation accumulation, but failed to produce any definite conclusion.

Lagerweff and Peech (52) conducted experiments in which excised barley roots were exposed to solutions containing RbCl and $CaCl_2$, in different combinations of the activity ratio $(Rb^+) / (Ca^{++})^{\frac{1}{2}}$ of the ionic strength. They concluded that exchange adsorption and metabolic accumulation of cations are two independent co-existing processes. A comparison of the calculated per cent saturation of the

exchange sites with Rb^{+} and Ca^{++} with the amounts of these two cations absorbed by the roots, showed that the changes in the uptake of Rb^{+} by the roots reflected only a small fraction of the sharp changes in the per cent saturation of the exchange sites with Rb^{+}, whereas the uptake of Ca^{++} reflected more nearly the slight changes in the percentage saturation of the exchange sites with Ca^{++}. They stated 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.

Epstein and Leggett (25), in a study of the time course of absorption of Sr^{89} by excised barley roots, distinguished between adsorption exchange and true accumulation by the simple experiment of removing all exchangeable Sr^{89} at the end of each absorption interval by exposing the tissue to nonradioactive Sr. When this was done, absorption proved a linear function of time from the beginning of the absorption period. Thus, while the adsorption-exchange reaction proceeds for at least half an hour, the accumulation rate is clearly independent of the saturation state of the adsorption-exchange sites. In the same experiment, however, they stated that cations exchangeably adsorbed on the exchange surfaces of the root, in the absence of an external reservoir of the ions in the solution, are absorbed very slowly by the active mechanism. In the absence of a reservoir of ions in the external solution,

the exchange sites effectively competed with the carriers for the limited supply of ions.

Laties (53) demonstrated in his review of salt transport that the root CEC has little or nothing to do with metabolic accumulation mechanism.

2). <u>The origin and nature of cation exchange sites of plant roots</u>

At the beginning of the twentieth century, Devaux (16) noticed that plant cellular walls and pectose of these cellular walls could fix an appreciable amount of cations in salt solutions. A prolonged washing with distilled water could not remove the cations that were fixed, but the immersion for a short time in a solution of another cation released completely the cation from the cellular wall. His findings on the cation exchange phenomenon between plant roots and a salt solution led to his early hypothesis that the cation exchange reaction may play a vital role in the nutrient uptake process.

Although the plant component responsible for cation exchange reactions is not completely understood, several studies of this component have been reported.

Mattson and his co-workers (63) have shown by staining methods with methylene blue that the most active acidoids of the root are localized in the surface layer and most of

all in the growing tip. It was also pointed out by them that the root acidoid content could be attributed to pectic substances, and that these can function as cation exchangers.

Williams and Coleman (92) studied the cation exchange properties of plant root surfaces. They reported that the rapidity of the exchange reactions between plant roots and salt solutions indicated the existence of a cation double layer associated with root surface and that the cation exchange phenomena did not depend directly upon root metabolism, since the same results were obtained at 0° C and 25° C, with living roots, and with ether killed roots.

More recently, using ether killed plant roots, Keller and Deul (48) were able to demonstrate that 70 to 90 per cent of their CEC was due to their pectic content. Crooke and his associates (11) studied the relationship between CEC and pectin content of leek roots. They found that the root CEC, pectin content, respiration and nitrogen content were highest at the root tip and decreased with increasing distance from the root tip.

Knight et al. (49,50) estimated the uronic acid content of plant roots by a micro-decarboxylation method and chromatographically identified the extracted uronic acids. They concluded that CEC is, in general, accounted for by uronic acid content.

These findings indicate that carboxylic groups of pec-

tin may be some of the main acidic groups of plant roots. Heintze (37) studied the origin of the negative charges on root membranes by comparing the titration curves of plant roots. The buffer curves of root suspensions showed that at least some of the acidic groups of root surfaces are derived from carboxylic groups of pectic acids. This result is in accordance with the results which were shown by Mehlich (68) for wheat and alfalfa by the same method as Heintze's.

3). <u>The effect of nitrogen on the root CEC</u>

It has been reported that plant root CEC is affected by several factors: age of plant, metabolic activity of plant roots, and environmental circumstances in which plants are grown (35,37). Among these factors, the effect of nitrogen on root CEC has been under relatively intensive investigation, because of the possibility that high CEC induced by high nitrogen level may cause differential uptake of mono- and divalent cations.

McLean, Adams and Franklin (65) investigated the relationships between the root CEC and the root nitrogen content of a number of agronomic crops grown in gravel cultures to which 1/5 Hoagland solution was applied. Some of these crops were grown at three different nitrogen levels in the culture media. The root CEC was determined by using elec-

trodialysis. They found high correlation (R = 0.866) between the CEC of plant roots and their nitrogen content. They stated that an increased level of nitrogen was generally accompanied by a corresponding increase in the nitrogen content and the CEC of the plant roots.

Smith and Wallace (77) studied the influence of nitrogen fertilization on root CEC and their concomitant effects on cation uptake by plants. Cucumbers and a fescue grass were grown in pot culture with a mixture of synthetic ion exchange resins, bentonite and sand for 140 days. Nitrogen was supplied at the rates of 4 and 48 meq./ pot (300 g..clay and 3,500 g. sand). The results showed nitrogen fertilization increased the root CEC of at least some plant species, and that the N influence on CEC of the roots was most marked on plants with a low root CEC. As a result of the nitrogen application, the following relationship was found between the root CEC and the cation content.

$$\frac{CEC_1}{CEC_2} = \left[\frac{Ca_1}{Ca_2}\right]^2 = \frac{K_2}{K_1}$$

where the subscripts 1 and 2 refer to two different species. They stated that these results indicated a possible nitrogenous nature of cation exchange sites and help to substantiate the inverse K and Ca ratio square relationships for

explaining differential cation uptake by plants. Furthermore, this helps to explain nitrogen fertilizer influence on differential absorption by plants.

The reports of the effect of nitrogen on root CEC and of the concomitant effect of differential cation uptake by plants have been made by several other investigators (18, 35,37,95).

On the other hand, it was also reported that higher application of nitrogen did not result in higher root CEC (14,28,43,93).

Cunningham and Nielsen (14) showed evidence against the reported relationship between root CEC and nitrogen application. In their experiment, six levels each of NO_3-N or NH_4-N (0-500 ppm.) were thoroughly mixed into the clay loam soil in which Italian ryegrass was grown from seed for 9 weeks. The experimental results showed that the root CEC was constant at different N levels and did not vary with increased nitrogen content in the grass.

Since Heintze (37) found no close relationship between CEC of various species and the nitrogen content of their roots, he did not support the suggestion that cation exchange sites of roots are of a nitrogenous nature. Although there is no clear explanation as to the mechanism of the nitrogen effects on root CEC, increased exchange sites resulting from increased carboxyl groups and/or amino groups has been suggested (19).

4). The methods to measure the root CEC

It is essential to estimate accurately the value of the CEC of roots in order to evaluate the function of cation exchange adsorption in the process of cation uptake. Many methods have been employed to estimate the root CEC (1,2, 10,13,17,31,34,64,67,97). They are mainly divided into two methods: estimating exchange acidity of roots and exchange reactions between roots and salt solution.

When the root CEC is determined by estimating the exchange acidity of the roots, the roots are saturated with hydrogen ions by electrodialysis or acid washing. The amount of the hydrogen ion replaced with salt solution is determined by potentiometric titration with standard base solution.

The method using electrodialysis is essentially the same as described by Drake et al. (17). In this method, fresh roots were placed in Visking bags and electrodizlyzed at 120 volts for 90 minutes. After dialysis, five grams of centrifuged roots were placed in a beaker containing 200 ml. 1 N-KCl solution. The root-KCl system was titrated with KOH to pH 7.0 as measured by the glass electrode. A 5-minute interval was adopted as an arbitrary titration period. The root CEC of the dicotyledonaus plants investigated using this technique were roughly double the values for monocots. They stated that these fundamental differences

help to explain the relatively high content of divalent cations in plant materials of legumes and other dicots with high CEC roots.

Crooke (10) used an acid washing technique to measure root CEC. In this technique, 2, 5-gram samples of roots were placed twice in 400 ml. of 0.01 N HCl for 5 minutes. After the acid washing and water rinse, the roots were placed in 200 ml. N-KCl for titration. The roots-N-KCl system was titrated to pH 7.0 over a 5-minute period with 0.01 N-KOH. The amount of KOH consumed was expressed as the CEC value.

Crooke (13) modified his fresh root acid-washing method to study dry roots. In this method, dried milled root material was washed with 0.01 N-HCl for 5 minutes and rinsed with water. The root-N-KCl suspension was titrated to pH 7.0 with 0.01 N-KOH during the arbitrary five-minute titration time. He stated that this method was used successfully for measuring of higher and lower plants and gave values which correlated well with their uronic acid content.

Williams and Coleman (97) determined the CEC of roots by saturating them with Ba^{++} or NH_4^+ or H^+ by means of a 10-second immersion in neutral salt or acid solution, followed by a 10-second immersion in a replacing solution. Their preliminary experiments involving varying times of contact between roots and saturating and replacing solutions showed that exchange capacities for a given set of roots

were relatively constant as long as contact with the two solutions was of the order of 30 seconds or less. With longer times of contact, the amount of ions taken up and released became larger.

An exchange procedure was developed by Bartlett (1) for the simultaneous measurement of cation- and anion-exchange capacities of roots, using an unbuffered salt solution. In this method, intact roots were immersed with agitation for 5 minutes in 50 ml. 0.2 N-NaCl, and then rinsed five separate times in about 50 ml. distilled water. The sodium absorbed by the roots was replaced by placing the roots for 1 minute with agitation in 25 ml. N-HNO_3. The amount of sodium replaced was expressed as the root CEC. He stated that the procedure has the advantage of not requiring (a) titration involving an arbitrary end point or (b) electrodialysis or prewashing in acid, both of which affect the measured CEC of roots.

Bell and Walker (2) studied the relationship between the measurement of CEC on surface area-basis and a dry weight-basis. Cation exchange values which were determined by the standard base titration of the electrodialyzed roots were found to have a straight line relationship with surface area when only surface exchange was measured.

IV. Materials and Methods

1). Plant Materials

In all experiments excised roots of Erie barley were used and prepared essentially as described by Jacobson et al. (47). Fifty grams of seed were activated and sterilized with 75 ml. of 10 percent H_2O_2 for 20 minutes. After the H_2O_2 treatment, the seeds were rinsed with distilled water and soaked 24 hours in 1.5 liters of distilled water with continuous aeration. The seeds were then thoroughly washed with distilled water and evenly distributed on two sheets of cheese cloth supported by a nylon screen. The end of the cheese cloth was dipped into the nutrient solution. The seedlings were grown in the dark using a dilute nutrient solution consisting of 0.1 m mole/liter each of $Ca(NO_3)_2$, $MgSO_4$, and KH_2PO_4. The composition of the nutrient solution was subjected to certain treatments to meet the special requirements to be described briefly under each experiment. The nutrient solution was gently aerated. Three days after transferring the seed to the nylon screen, the plant roots were rinsed with distilled water and the nutrient solution was renewed. Two days after the renewal of the nutrient solution, it was replaced with distilled water and 24 hours later, the roots were excised just below the seed.

2). Condition of Cation Absorption Experiments

The excised roots were washed twice with distilled water and the adhering water was removed by pressing soft tissue paper to the roots. A ratio of one gram of root material to 500 ml. of solution was employed in the absorption experiments, normally conducted at 24° C.

Uptake of cations was studied under both aerobic and anaerobic conditions of solution. The aerobic conditions of the solution were provided by gentle aeration and anaerobic conditions were provided by gently bubbling N_2 gas before and during the uptake experiment. The N_2 gas was first passed through 3 per cent alkaline pyrogallol solution to remove contaminating O_2 and then was washed with distilled water before bubbling in the solution. Before the uptake experiments, the N_2 gas was bubbled for 90-120 minutes to lower the O_2 in the solution to 0.2-0.3%. The oxygen concentration of the solution was measured using a Beckman Laboratory Oxygen Analyzer, Model 777. The mouth of the solution container, 500 ml. erhlenmyer flask, was covered with two sheets of polyethylene film in order to lower efficiently the O_2 percentage in the solution.

3). Determination of Cation Content of the Sample

After the cation uptake experiments, the excised roots

were washed six times with 50 ml. of distilled water, dried first at 90° C. for 60 minutes and then at 75° C. in an oven over night. The weighed dry root materials were ashed at 500° C. in a muffle furnace. The ashes were dissolved into 2 ml. of 1:1 HNO_3 solutions which were diluted to proper concentrations of cation contents. The cations studied were determined using a Perkin Elmer Model 214 Atomic Absorption Spectrophotometer. When Ba or Rb was determined, 1,000 ppm K was added to the solution to eliminate the effect of other alkaline cations. In the case of Ba, the acetylene-N_2O flame was used (15,90). For other cations, the acetylene-air flame was used.

V. Results and Discussion

1). Study on the Root CEC Assay

Much confusion exists on the role of root CEC on the cation absorption process and the effect of the environmental factors on the root CEC. This confusion can be partially explained by the methodology of the root CEC assay. Although many methods to determine the root CEC have been proposed, the values of the root CEC obtained by different methods are not always the same for the same plants while remaining consistent within themselves (1,13, 37). In spite of the necessity of accurately determining the root CEC in these studies, it is very difficult to decide which method is most accurate.

Those methods proposed are roughly divided into two groups. One group is based on the estimation of exchange acidity of the roots which are saturated with hydrogen ions by electrodialysis or acid washing. The other is a method to use direct exchange reactions between roots and a salt solution. Although the electrodialysis method has been intensively employed in the studies of the root CEC, this method is a drastic process and is open to some criticism on the grounds that bases and organic materials of the roots may be released from the interior of the roots (1,13). Bartlett (1) studied the effect of electrodialysis

on the root CEC value which was measured by his Na exchange method. He found that electrodialysis removed amino acids and other organic compounds from the roots and abruptly increased the measured CEC, the inceeases in the CEC being closely correlated to the severity of the treatment.

The acid washing method proposed by Crooke (10,13) appeared not to seriously injure the roots and gave values which were well correlated to the uronic acid content (13). The acid washing method is based on the assumptions that the plant roots are almost completely saturated with H^+ by the acid washing and that the H^+ adsorbed is almost completely replaced by a salt solution, such as N-KCl. However, there is room for doubt in whether these assumptions are correct. Plant roots usually contain much larger amounts of cations than the root CEC values reported. Therefore, it may become difficult to saturate the root exchange sites with H^+ if acid washing causes cation leakage from the inside of the root cells. Moreover, it has been reported that cation exchange between a salt solution and exchange sites lasted about half an hour (54). If a short period of acid washing is employed to avoid cation leakage, the roots will not be saturated with H^+.

From the standpoint of the information mentioned above, an experiment was conducted to study whether acid washing of the roots causes cation leakage from the interior of the root cells. Five grams of excised barley roots obtained

as described above (p. 23) were mixed with 500 ml. of various concentrations of HCl for 5 minutes and then rinsed with 300 ml. of distilled water. The acid washing and rinsing procedures were repeated with new solutions. This acid washing technique is essentially the same as the one proposed by Crooke (10). The amounts of K, Ca and Mg lost into the acid and water and remaining in the roots were determined. The results are reported in Table I.

When 10^{-2} N-HCl was employed to rinse the roots, the resulting solutions contained about 30 meq./100 g. dry roots. This value is much higher than the reported value of the root CEC. Therefore, this result indicates that not only cations on the exchange sites but also cations in the root cells are removed by the acid washing procedure. The amounts of cations removed by the acidic rinsings decreased with decreasing concentrations of HCl. When lower concentrations of acid are employed, it may be difficult to sufficiently remove the cations on the exchange sites. Moreover, almost equal amounts of cations were removed by the first acid washing and water rinse as were removed by the second treatment. This result indicates that the cation removal by this treatment is continuously taking place, and that such a continuous leakage of cations disturbs the root exchange sites that are to be saturated with H^+ by the acid washings.

Crooke (10) adopted this method of 10^{-2} N-HCl washing

Table I. Successive Cation Losses from the Excised Barley Roots by Acid Washing with HCl

Conc. of HCl, N	The amounts of cations removed*			The amount* of cations remained in the roots
	Cation	1st acid** washing	2nd acid** washing	
10^{-2}	K	6.42	7.25	21.54
	Ca	1.27	1.49	4.15
	Mg	5.26	7.90	39.37
10^{-3}	K	2.24	3.12	31.91
	Ca	0.71	0.60	5.74
	Mg	3.00	1.85	45.21
10^{-4}	K	0.77	0.74	35.76
	Ca	0.52	0.29	6.10
	Mg	2.24	1.20	46.71
H_2O	K	0.43	0.27	37.22
	Ca	0.26	0.15	6.56
	Mg	0.10	0.79	49.75

* meq./ 100 g. dry roots. mean of three values

** 5 min. acid washings and 5 min. rinses with distilled water

to saturate the roots with H^+ because these treatments gave comparable values with those obtained by electrodialysis. However, the results in Table I indicate that any procedure of acid washing of the roots is unable to completely saturate the roots with H^+.

Even though electrodialysis or acid washing were able to completely saturate the roots with H^+, another problem arises from the titration of such a H^+-saturated root with standard base. Some investigarors pointed out that the titration involves an arbitrary end point (1,13,17,37). Similar results were obtained in this study. (Figure 1). The dried and milled barley roots were treated with 10^{-2} N-HCl in the same manner as described by Crooke (13). The root KCl suspension was titrated with 0.01 N-KOH to restore and maintain the pH at 7.0 for a 5-minute titration time. An acidic drift was observed after the titration, therefore the titration was repeated 15 minutes after the first titration until no more acidic drift was observed. The amount of 0.01 N-KOH required for each titration was plotted as a function of time (Figure 1).

The acidic drift continued as long as 3 hours. The total amount of 0.01 N-KOH required to maintain the dry roots KCl suspension at pH 7.0 for 5 minutes without more acidic drift was twice as much as the value for the first 5-minute titration period, expressed as the root CEC.

As this titration was done without eliminating the ef-

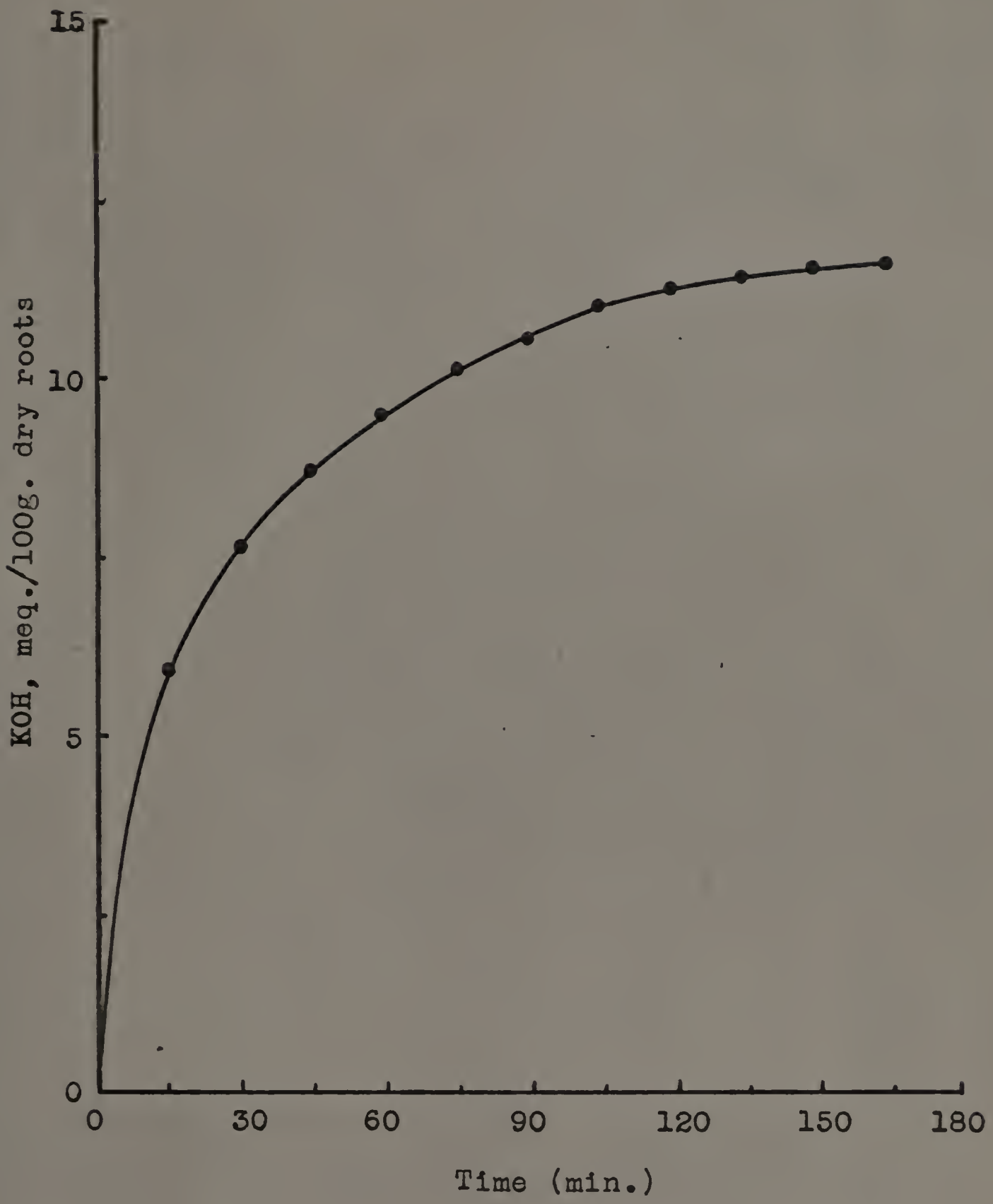


Fig. 1. Titration curve of HCl washed barley roots in N-KCl suspensions with 0.01 N-KOH.

fect of atmospheric CO_2 on the suspension, CO_2 may be partially responsible for the acidic drift. With fresh living roots an acidic drift has been observed due to metabolic uptake of K and the consequent leakage of H^+ (13), and the release of H^+ following enzymatic breakdown of pectin to pectic acid and methanol (37). Crooke (13) stated that the acidic drift in the titration of dried, milled root material is much smaller and is due simply to diffusion of H^+ from the interior of this material. However, Figure I shows that a respectable degree of acid drift took place in the titration of dried, milled root material. The results obtained in this study indicate that an estimation of the acidity of "hydrogen ion saturated roots" cannot give a quantitatively accurate value for the root CEC, although it may represent the relative value of the root CEC.

Another group of methods to determine the root CEC is based on the direct exchange reaction between the roots and a salt solution. The amount of the cation adsorbed by the roots is expressed as the root CEC. Many modifications of this method have been reported (1,28,43,67,97). Bartlett (1) recently developed an exchange procedure for the simultaneous measurement of the cation and anion exchange capacities of roots, using 0.2 N-NaCl solution. In this method, intact roots were immersed with agitation for 5 minutes in 50 ml. of 0.2 N-NaCl, and then rinsed five separate times with about 50 ml. distilled water. The sodium absorbed by

the roots was removed by agitating them for 1 minute with 25 ml. N-HNO_3. The amount of sodium removed was expressed as the root CEC. It is generally accepted that the cation exchange reaction itself is completed very rapidly on the surface of clays and synthetic exchange resin particles. Although the locus of the cation exchange sites in the roots is in or on the cell walls (53), the structure of the cell walls appears complicated enough to require much longer times for the completion of the cation exchange reaction. Leggett and Epstein (54) found that Sr^{++} exchange between nonradioactive $SrCl_2$ and radioactive Sr^{++} on the barley root exchange sites lasted for half an hour. This result indicates that the short exchange period of 5 minutes suggested by Bartlett will not be able to complete the exchange reaction between the salt solution and cations adsorbed on the root exchange sites.

In order to study the cation exchange reaction as a function of time, the following experiment was conducted. Excised barley roots (2 g. fresh weight) were mixed with 500 ml. of 0.1 N-NaCl solution for 5 to 60 minutes. This mixture was aerated gently during the absorption period. A second identical mixture was bubbled with N_2 gas before and during the absorption period. The O_2 partial pressure of the solution bubbled with air or N_2 gas was found as 17.5% and 0.3, respectively, at the beginning of the experiment. The N_2 gas employed to prevent the metabolic

absorption of Na by the roots. The effects of the anaerobic condition on the cation uptake will be discussed in detail later.

Although the berley seedlings were grown without Na in the nutrient solution, the roots contained about 5 meq./ 100 g. dry weight. The amount of Na absorbed by the roots under both aerobic and anaerobic conditions (in addition to the native content of Na) was plotted against time (Figure 2).

Sodium absorption under the aerobic condition increased rapidly with time, but the increase of Na absorption under anaerobic conditions was slower and stopped within 30 minutes. As will be explained later, metabolic uptake is almost completely eliminated under anaerobic conditions. Therefore, the Na uptake under anaerobic conditions represents the non-metabolic absorption of Na and the difference between Na absorption under the N_2 gas and under aeration represents metabolic absorption which increases linearly with time.

If Na absorption under anaerobic conditions is largely exchange adsorption as discussed later, it indicates that it takes 30 minutes to complete the Na exchange between the roots and NaCl solution. This is in agreement with Leggett and Epstein (54). Therefore it is obvious that the 5-minute exchange period proposed by Bartlett (1) is too short to complete the exchange reaction.

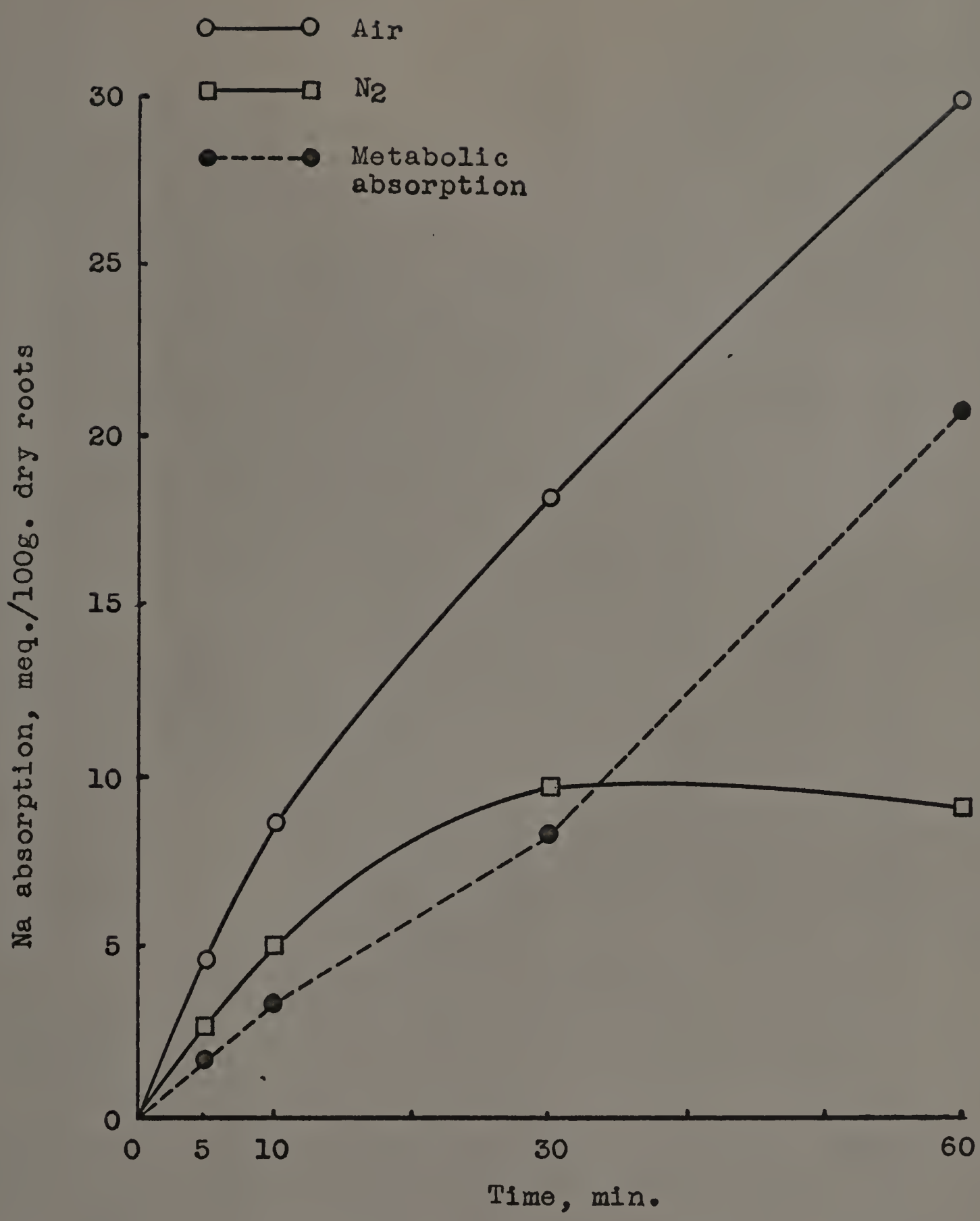


Fig. 2. Effect of O_2 on Na absorption by excised barley roots from 10^{-1}N-NaCl.

During the first five minutes, the roots absorbed 4.8 meq. of Na per 100 g. dry root basis under the aerobic condition and 2.6 meq. of Na under the anaerobic condition. At least 2.2 meq. of Na was metabolically absorbed during the exchange period. Since almost all of the Na in the roots is easily extracted by nitric acid, the CEC values obtained by Bartlett's method include not only exchangeable Na, but also Na which originated from the seeds and absorbed metabolically. Therefore, it is impossible to get an exact value of the root CEC using Bartlett's method.

The result in Figure 2 also indicates that the following conditions must be provided for the quantitative measurement of the root CEC by a direct exchange method. First, a proper exchange period must be provided. Second, the cations which are not normally contained in the roots must be employed as exchange cations to avoid cation leakage from within the root cells. Third, metabolic absorption causing erroneous high values of CEC must be eliminated.

2). Cation Absorption under Anaerobic Conditions

In the paragraph above, it was concluded that metabolic absorption must be eliminated in order to measure accurately root CEC using a direct exchange method.. As metabolic absorption of catinns is greatly dependent on the root

metabolism, especially oxidative metabolism, metabolic absorption is largely prevented by anaerobic conditions, low temperature, or a metabolic inhibitor. These techniques are commonly employed to distinguish non-metabolic absorption from metabolic absorption. Among them dinitrophenol (DNP, uncoupler of oxidative phosphorylation) and other metabolic inhibitors have been extensively used in the study of cation absorption by roots.

It was shown, however, in my preliminary work, that pretreatment of barley roots by DNP resulted in the removal of an appreciable amount of cations from these roots. This appears reasonable because relatively low pH, 4-5, must be provided for DNP to work effectively (80). Other metabolic inhibitors which contain cation in the molecule were excluded from this consideration in order to avoid the concomitant effect of the cation on cation adsorption. Therefore, efforts were concentrated to characterize cation absorption under anaerobic conditions and to determine whether a drastic reduction of O_2 in an exchange solution can completely eliminate metabolic absorption.

Earlier studies by Hoagland (38,39), Steward (81,82, 83,84), and others on the effect of oxygen on ion accumulation revealed that oxygen concentrations which limit respiration are also those which limit ion absorption, and that aerobic and not anaerobic metabolic processes are related to ion accumulation. Extensive investigations on

ion absorption by excised roots and tissue discs have confirmed general dependence of respiration and ion accumulation on aerobic metabolism.

The experimental results in Figure 2 also show that Na absorption is drastically reduced by an anaerobic condition. A question arises, however, as to whether metabolic absorption is completely eliminated under such anaerobic conditions, and what is the nature of the absorption under anaerobic conditions. Moreover, it has been reported that the degree of response of individual cations to anaerobic conditions is not always the same. Letey et al. (55,56, 57,58) applied varied soil oxygen level treatments to plant roots and found that the Na content of the plants increased with decreasing O_2, while K and P content decreased with decreasing O_2. From these observations, Letey suggested that the normal relative exclusion of Na from the shoots of these plants is due to an oxygen-requiring metabolic mechanism. Similar results were obtained by Jackson and Adams (44). Their experimental results showed that K absorption from 10^{-5} M-KCl by excised barley roots was reduced to one-third of the absorption by the aerated roots under 1% oxygen level in the solution. On the other hand, Na absorption was not inhibited by such a low O_2 level. However, 10^{-4} N-DNP depressed both K and Na absorption, indicating that the cation absorption of both is dependent on respiratory metabolism.

The experimental data in Figure 2 clearly shows that Na absorption by excised barley roots under such a low oxygen level as 0.3% in the solution ended within 30 minutes. This result is in agreement with Epstein and Hagen (23), who reported that aerated excised barley roots absorbed three or four times as much Na as the N_2 gas-bubbled roots. The difference in the effect of anaerobic conditions on Na absorption may be explained by a small difference in O_2 concentration in the medium. From these results mentioned, it is obvious that individual cation absorption differs in sensitivity to anaerobic conditions. Therefore, considerable caution was taken to keep a low O_2 level (0.3%) in the solution of this study as was described above.

The time course of Li absorption over 60 minutes under both aeration and N_2 gas bubbling is shown in Figure 3. The mode of Li absorption was similar to Na absorption. The rapid Li absorption under N_2 ended within 30 minutes and was followed by subsequent gradual absorption. The estimated metabolic uptake of Li increases almost linearly with time.

As discussed previously, metabolic ion absorption is dependent upon temperature, as well as aerobic conditions. Therefore, if the metabolic process is involved in Li absorption under anaerobic conditions, low temperature must result in lower absorption of Li. The effect of temperature and oxygen on Li absorption from 0.1 N-LiCl is shown

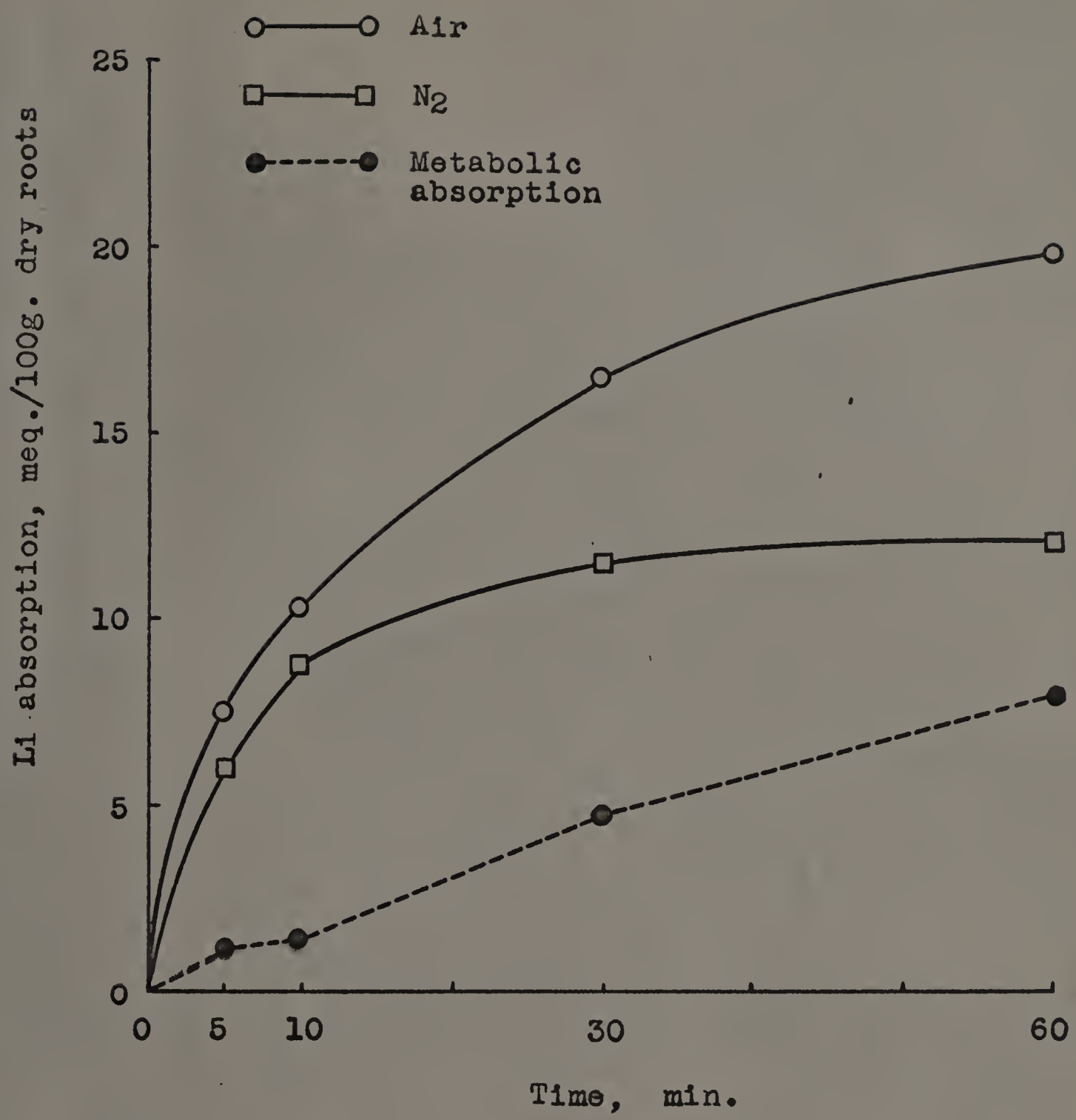


Fig. 3. Time course of Li absorption by excised barley roots from 10^{-1}N-LiCl.

in Table 2.

Table 2. The effect of O_2 and temperature on Li absorption from 0.1 N-LiCl

Temperature	0.5° C		20.5° C	
O_2%	0.2% (N_2)	17.5% (air)	0.2% (N_2)	17.5% (air)
Li-absorption (meq/100g dry roots)	10.03	10.83	10.60	15.68

Absorption period : 60 minutes

Low temperature (0.5° C.) markedly reduced Li absorption by aerated roots, but the effect of low temperature on Li absorption by N_2-bubbled roots was slight. Moreover, either low temperature or anaerobic conditions or both resulted in a very similar amount of Li absorption. The insensitivity of Li absorption by N_2-bubbled roots to low temperature indicates that metabolic absorption was almost completely eliminated under anaerobic conditions.

If it be so, what is the nature of Li absorption under the anaerobic condition?

Figure 4 shows Li absorption by N_2-bubbled barley roots as a function of time and of the concentration of LiCl solution. Li absorption increased with time and with increasing concentration. For each Li concentration, the

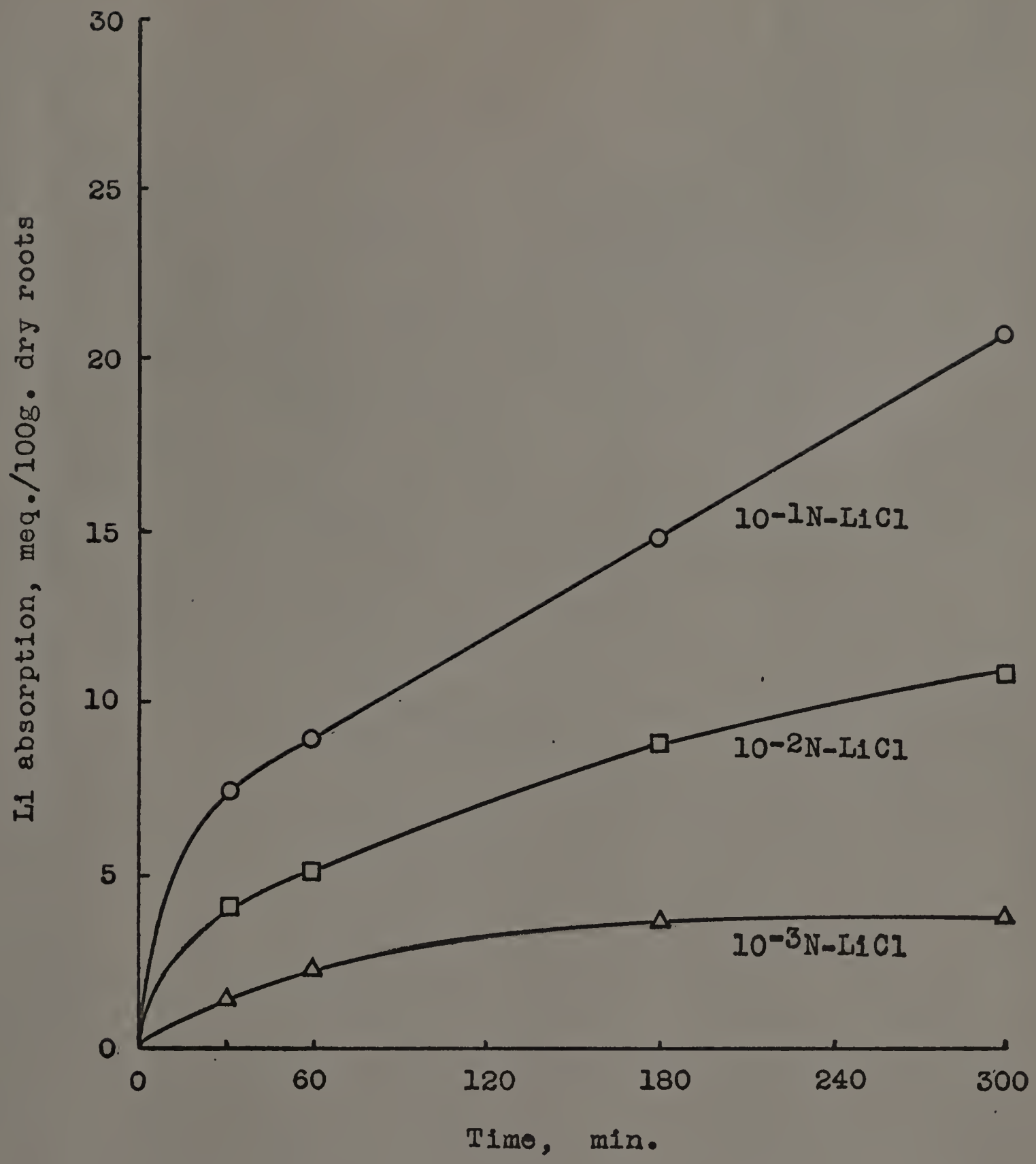


Fig. 4. Li absorption under anaerobic conditions as a function of LiCl concentration and time.

first rapid Li absorption which ended within 30 minutes was followed by gradual and linear increases in Li absorption. The slope of the steady-state Li absorption became steeper as the Li concentration increased from 10^{-3} to 10^{-1} N. This result may be explained by one or more of the following mechanisms: a) cation exchange adsorption, b) diffusion, and c) metabolic absorption which is not prevented by anaerobic conditions.

When the concepts of "water-free space (WFS)" and "Donnan free space (DFS)" are introduced, the mode of cation absorption may be explained as follows (4,53): cations in the external solution enter in accordance with diffusion expectations into the WFS which in essence constitutes an extension of the environment. Penetration of cations to the sites of exchange adsorption occurs by free diffusion through the free space. Then, the cation may be actively transported inside of the cell across permeation barriers from either the WFS or the DFS or both.

In this study, the treated roots were rinsed six times with about 50 ml. aliquots of distilled water immediately after the absorption period. Because the last rinsing water did not indicate Cl^- when it was detected by 1% $AgNO_3$. It is reasonable that almost all cations in the WFS would be removed by these successive rinses. It was also demonstrated that metabolic absorption by N_2-bubbled roots during 60 minutes was negligible (Table 2). From these con-

siderations, it is concluded that at least the first rapid cation absorption by N_2-bubbled roots is primarily the result of cation exchange adsorption between cations in the external solution and cations on the exchange sites in the DFS.

Further attempts were made to elucidate the prolonged and gradual cation absorption by N_2-bubbled roots. Briggs et al. (4) reported an analogous instance; the exchange between inactive Rb-saturated beet disks and 1 meq./ l. of Rb^{86} in the external solution at low temperature has at least two phases. One phase was about 90% completed in 40 minutes and was nearly completed in about 90 minutes. The other phase which has a very much slower exchange lasted more than 20 hours. However, they did not mention any definite evidence that the slow Rb absorption was due to slow exchange.

The second stage of the absorption will be explained by one or more of the following mechanisms:

a). metabolic absorption which is not prevented by the anaerobic condition.

b). slow diffusion of cations across permeation barriers.

c). slow exchange between cations in external solution and cations bound tightly to the exchange sites in the DFS or inside the permeation barrier.

Figure 5 shows the effect of temperature (0.5° C. vs. 23.5° C.) on Li absorption over 300 minutes. Both anaerobic

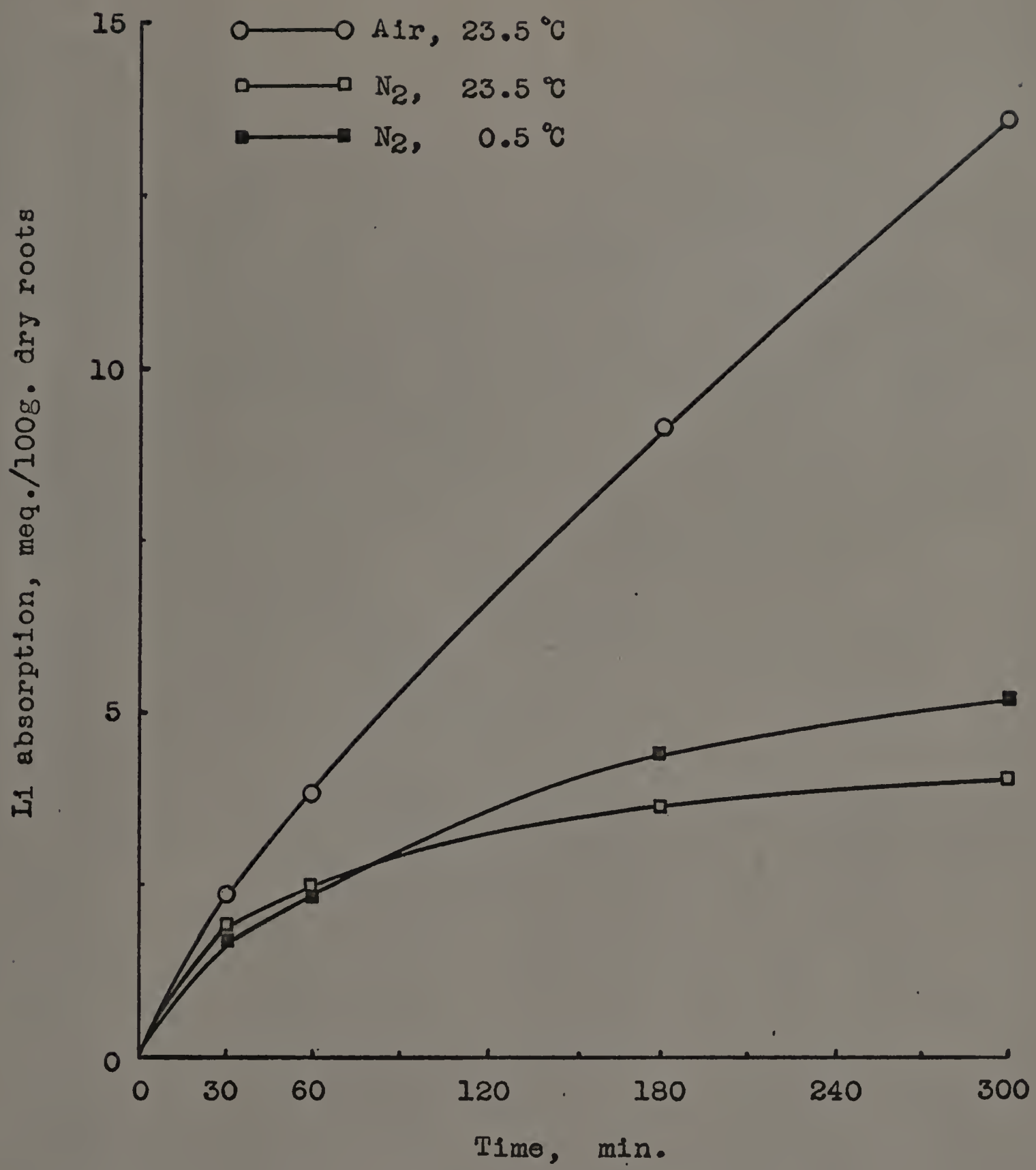


Fig. 5. Effect of oxygen and temperature on Li absorption from 10^{-3}N-LiCl.

conditions and low temperature resulted in a great reduction of Li absorption. Although the difference between Li absorption by N_2-bubbled roots at 0.5° C. and 23.5° C. is slight, the excised barley roots absorbed even larger amounts of Li under low than high temperature after a 60-minute absorption period. These results indicate again that metabolic absorption under anaerobic conditions is almost negligible, and also that the subsequent gradual absorption after the rapid absorption is of a non-metabolic nature.

Absorption of Ba, Sr, Rb and Li from 10^{-3} N single chloride solution under anaerobic conditions is shown in Figure 6. One of the striking characteristics of these cation absorptions is that the four different cations showed quite similar absorption patterns. The major difference among them occurred in the first 60 minutes (the first rapid absorption shoulder) which was in the order of a lyotropic series. This result is analogous to the results obtained by Epstein and Leggett (25), who showed that exchangeable Sr was replaced by other cations in the order of lyotropic series.

Straightforward ion exchange is controlled primarily by the valence and the position in the lyotropic series of the exchanging ions, therefore these results indicate again that the first rapid absorption is mainly due to exchange adsorption.

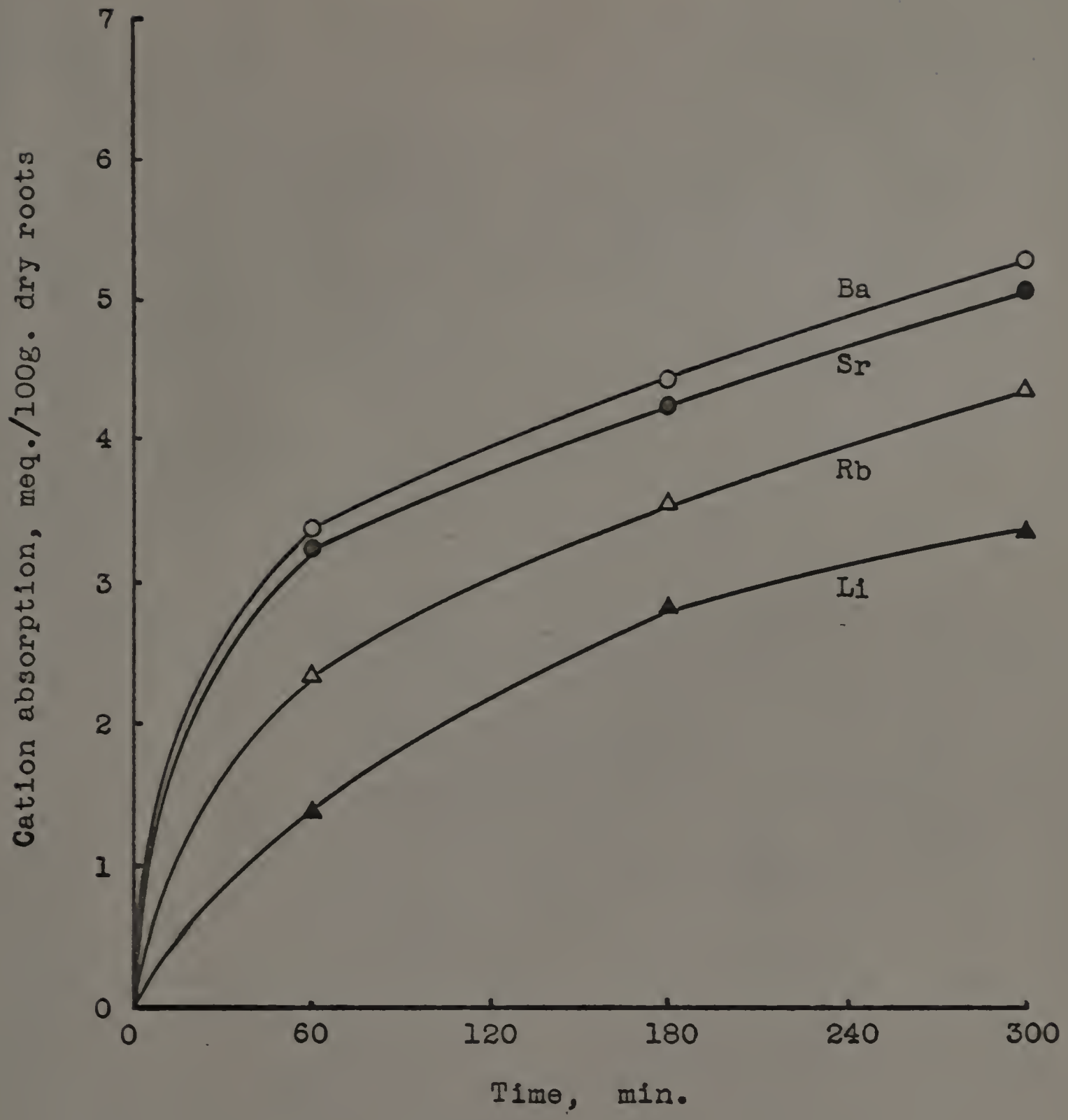


Fig. 6. Cation absorption under anaerobic conditions.

According to Epstein and his associates (23,24,25), competition among ions for combination with ion carriers is characterized by considerable ion specificity. If this metabolic absorption process is involved in cation absorption under anaerobic conditions, cationic specificity must be reflected in the absorption under N_2 gas.

It is clearly shown in Figure 7 that such a specificity is not reflected in Rb and Sr absorption from 10^{-3} N single chloride solution under the anaerobic condition. Therefore, from these data it may be postulated that the site of the carrier molecule is not involved in the exchange adsorption site in DFS.

It is noteworthy that N_2-bubbled roots absorbed slightly higher amounts of Sr than aerated roots. Although no statistical analysis was applied on these data, it appears reasonable to conclude that Sr absorption by excised barley roots is mainly non-metabolic or independent from oxidative metabolism. This result is quite analogous to Moore's and his associates' report on the behavior of Ca (69). They concluded that Ca absorption by excised barley roots is largely non-metabolic, because of the insensitivity of the Ca absorption to low temperature and DNP.

The data described above indicates that metabolic absorption is excluded under anaerobic conditions. However, the question of the nature of such a non-metabolic absorption, exchange adsorption or diffusion, remains unsolved.

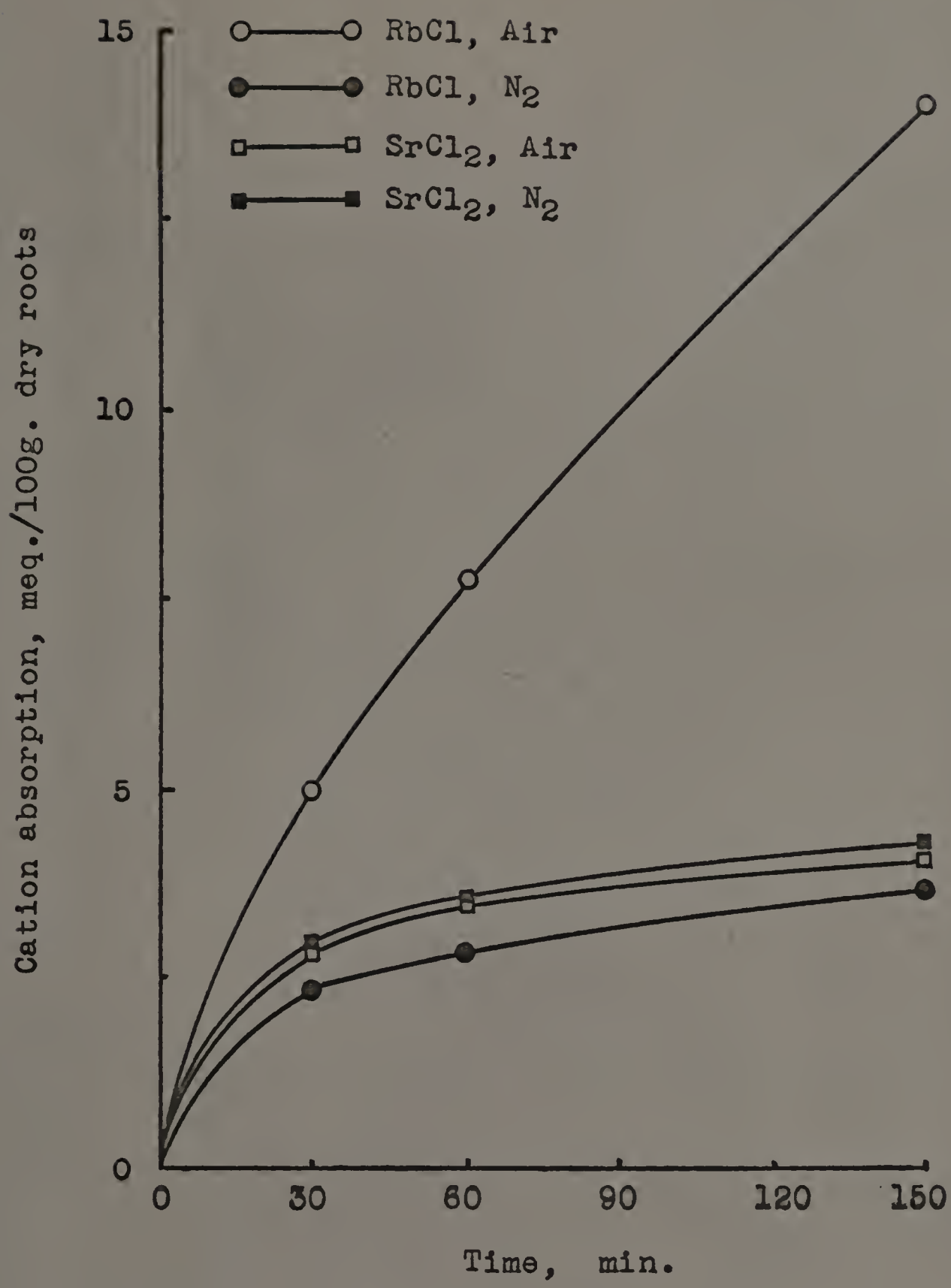


Fig. 7. Effect of oxygen on Rb and Sr absorption from single 10^{-3}N chloride solution.

This question is related, to a large extent, to the following questions: where is the location of the permeation barriers and what is the degree of permeability of the barriers? Also, to what extent is exchange across the permeation barrier possible? This subject is thoroughly reviewed by Laties (53). Although no further attempt to distinguish exchange adsorption and diffusion during the prolonged absorption period was made, an indication may be given by the following consideration.

If diffusion is the rate limiting process in the cation absorption, the entry of one cation will not be effected by the presence of another cation in low concentration. However, if the presence of sites on which exchange adsorption occurs is the controlling factor, interionic competition is to be expected.

On the basis of the idea mentioned above, some evidence has been reported that cationic entry by diffusion is very small (74,87). Figure 8 shows the effect of Sr (10^{-3} N-$SrCl_2$) and oxygen on Rb absorption from 10^{-3} N-RbCl. Rb absorption by N_2-bubbled roots in the presence of Sr is very small. This result is in agreement with Russell (74) and Tadano (87), and indicates that Rb metabolic absorption is inhibited by the anaerobic condition and that Rb exchange adsorption is interfered with by Sr. Even though Rb absorption by N_2-bubbled roots in the presence of Sr involved the absorption by diffusion, the absorption

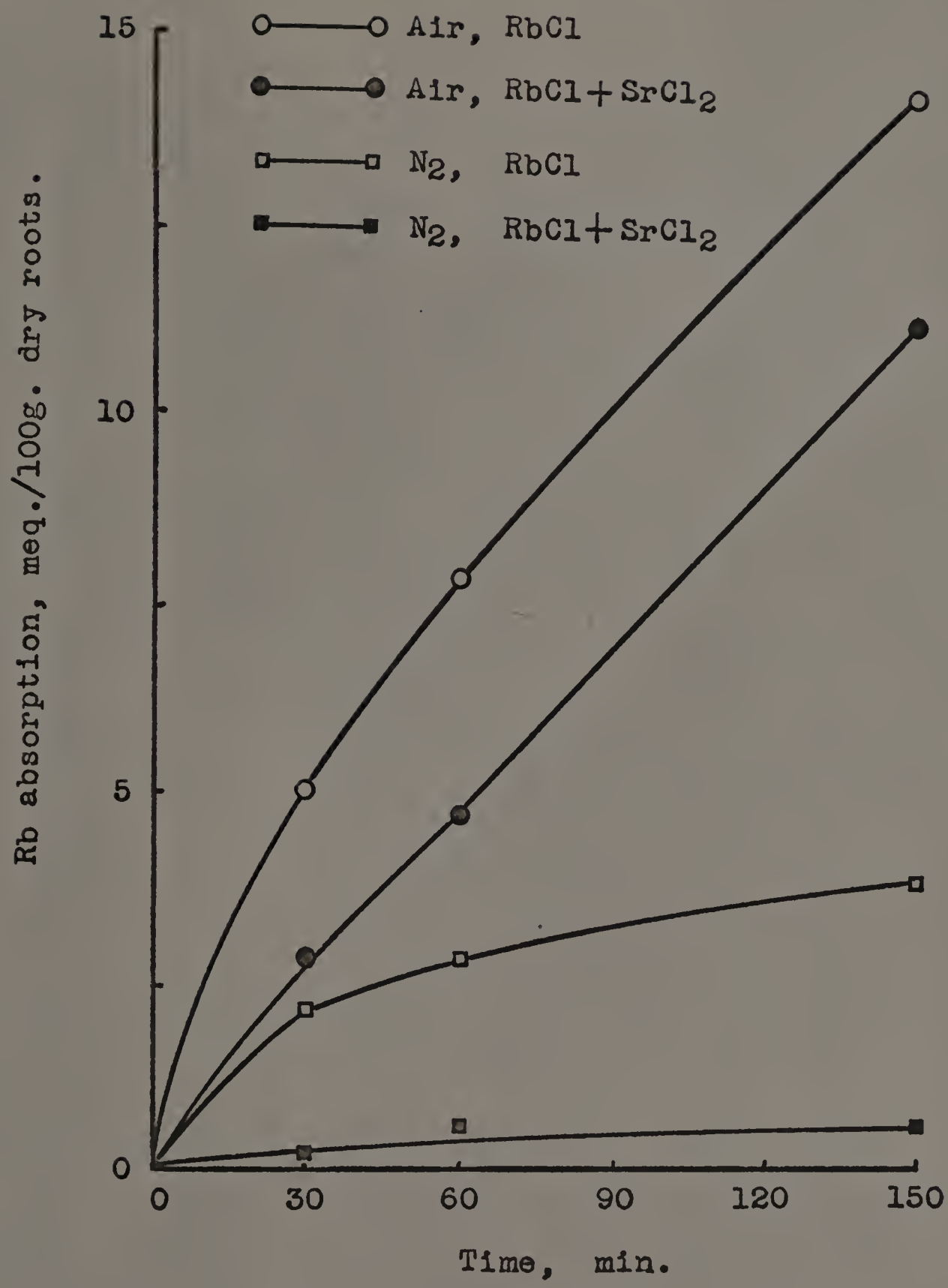


Fig. 8. Effect of oxygen and 10^{-3}N-$SrCl_2$ on Rb absorption from 10^{-3}N-RbCl.

by diffusion will be very small.

These results lead to the conclusion that cation absorption by N_2-bubbled roots from dilute solution (10^{-3} N) is exclusively due to exchange adsorption.

The reduction of Rb absorption by Sr took place at almost the same degree under both aerobic and anaerobic conditions. These results indicate that regardless of the O_2 concentration, Sr occupied exchange sites which were occupied by Rb in the absence of Sr. The relationship between mono- and divalent cations on the exchange sites is best explained by "valence effect" which will be discussed in detail later.

It was briefly discussed previously that individual cations may differ in sensitivity to anaerobic conditions. The responses of Na, Li and Rb to anaerobic conditions were essentially the same (Figs. 2, 3 and 6) and the absorption of these cations by N_2-bubbled roots was found to be mainly by exchange absorption. However, the behavior of Sr under anaerobic conditions is different from the other cations (Fig. 7), because the anaerobic condition was favorable for a very small extent of Sr absorption. Handley et al. (33) reported that uptake of Sr is a non-metabolic process in the meristematic portion of the root tip of Zea may L. and that this Sr absorption was markedly increased under anaerobic conditions. They explained this phenomenon, in that the permeation barrier for Sr is metabolically maintained

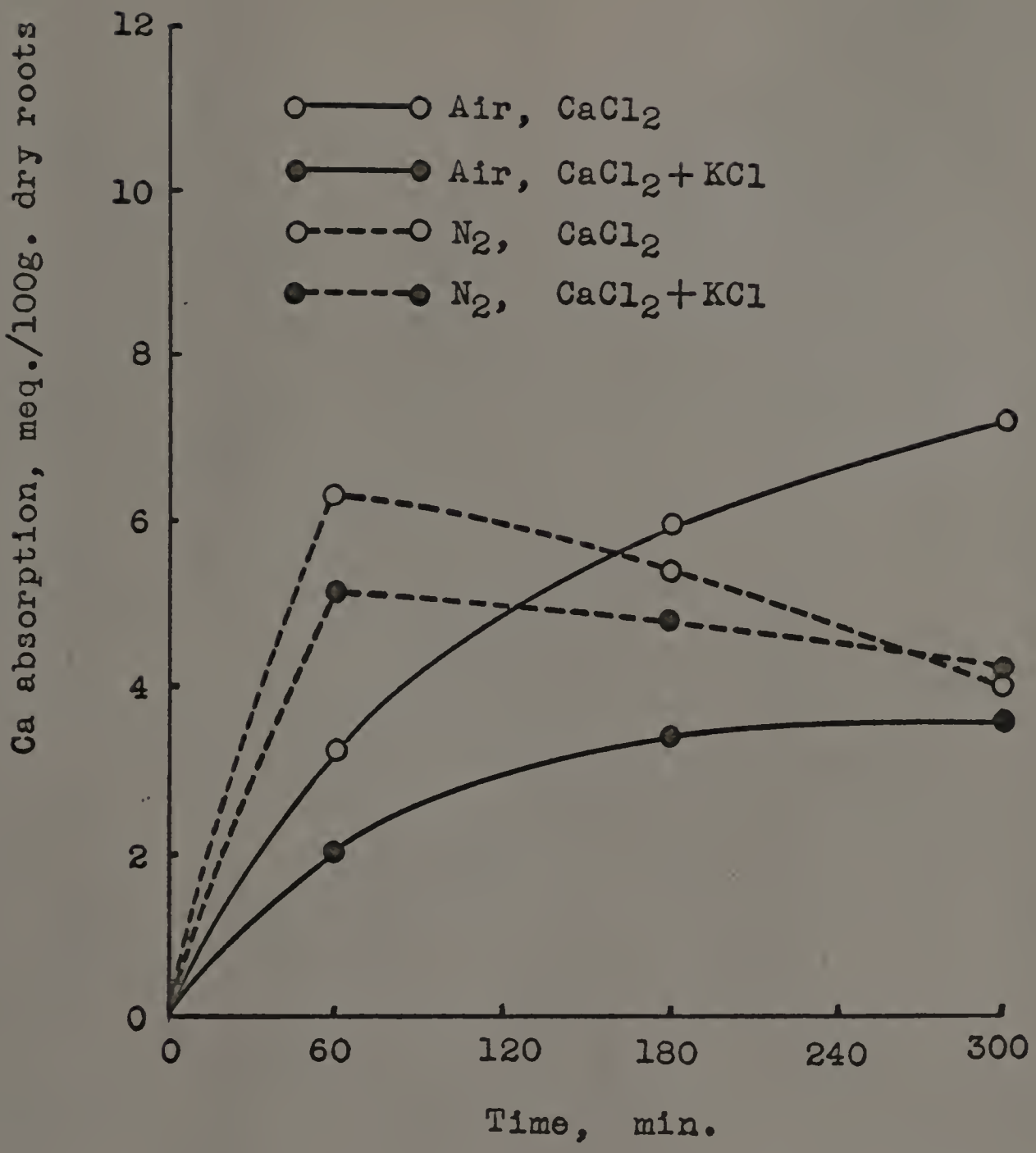


Fig. 9. Effect of oxygen and 10^{-3}N-KCl on Ca absorption from 10^{-3}N-$CaCl_2$

and is readily destroyed by anaerobiosis, permitting larger amounts of Sr to enter the tissue. It is suggested that under anaerobic conditions, destruction of the permeation barrier for Sr may have caused a slight increase of Sr absorption by N_2-bubbled roots.

The effect of oxygen and KCl on Ca absorption from 10^{-3} N-$CaCl_2$ or 10^{-3} N-$CaCl_2$ and 10^{-3} N-KCl mixed solution is shown in Figure 9 where Ca absorption over the native Ca content, 4.19 meq./100 g. dry roots, was plotted against time. The amount of Ca absorbed by aerated roots increased with time, while the Ca absorbed by N_2-bubbled roots reached a maximum within 60 minutes and then gradually decreased. The depressing effect of KCl on Ca absorption by aerated roots is obvious, while the effect is not clear under the anaerobic condition. If the destruction of permeation barriers by anaerobiosis permitted large amounts of Ca to enter the tissue, as suggested by Handley et al. (33) for Sr absorption by corn roots, it appears very difficult to explain why the gradual decrease of Ca took place after 60 minutes. Handley et al. (32,33) reported that $CaCl_2$ and $SrCl_2$ stimulated the respiration of corn roots. Although Moore et al. (69) concluded that Ca absorption by excised barley roots is largely non-metabolic, the behavior of Sr and Ca under anaerobic conditions may be best explained in relation to anaerobic metabolism of the roots.

The behavior of K under aerobic and anaerobic conditions

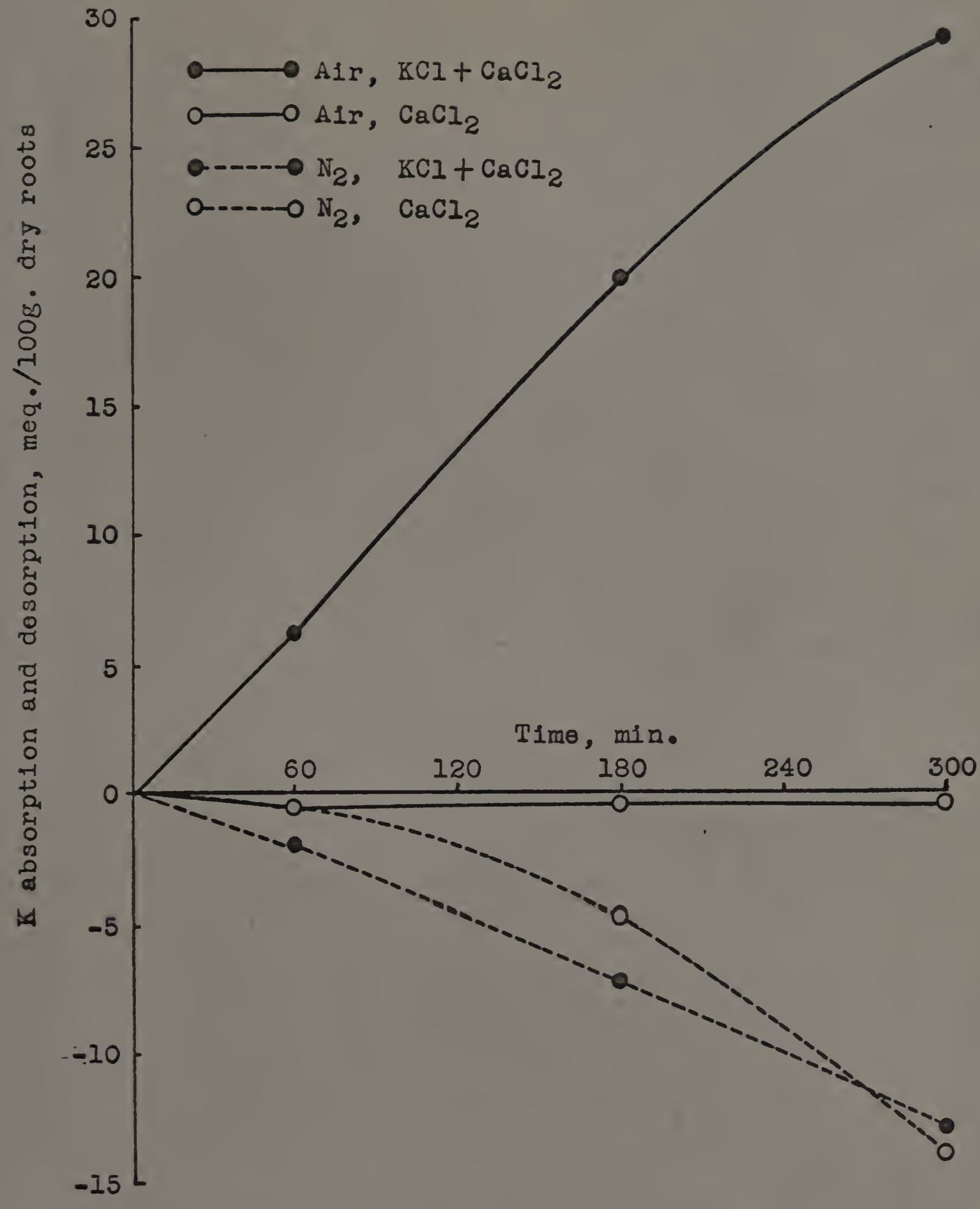


Fig. 10. Effect of oxygen and 10^{-3}N-$CaCl_2$ on K absorption and desorption

is contrasted with Ca (Figure 10). The amounts of K absorption over and desorption from the native K content of the roots, 30.76 meq./l, was plotted against time. Potassium absorption by aerated roots from 10^{-3} N-KCl and 10^{-3} N-$CaCl_2$ mixed solution was similar to Rb absorption from 10^{-3} N-RbCl and 10^{-3} N-$SrCl_2$ mixed solution (Fig. 8). However, K desorption took place regardless of the presence of K under the anaerobic condition. On the other hand, the aerated roots did not lose K into the $CaCl_2$ solution. These results indicate great dependence of K absorption on aerobic metabolism. Although no further attempts to elucidate these phenomena were made, it appears that the interaction between the native cations in the roots and those in the external solution must be taken into consideration in order to study the behavior of cations under anaerobic conditions.

The effect of anaerobic conditions on cation absorption by excised barley roots was discussed in the previous paragraph. It will be necessary to examine the effect of the anaerobic condition on the cation absorbability of the roots themselves. A simple experiment was conducted to determine whether the roots lose their cation absorbability under anaerotic conditions. The first and second set of the roots were allowed to absorb Rb from 10^{-3} N-RbCl under aerobic and anaerobic conditions, respectively. The third set of the roots was exposed to 10^{-3} N-RbCl solution under

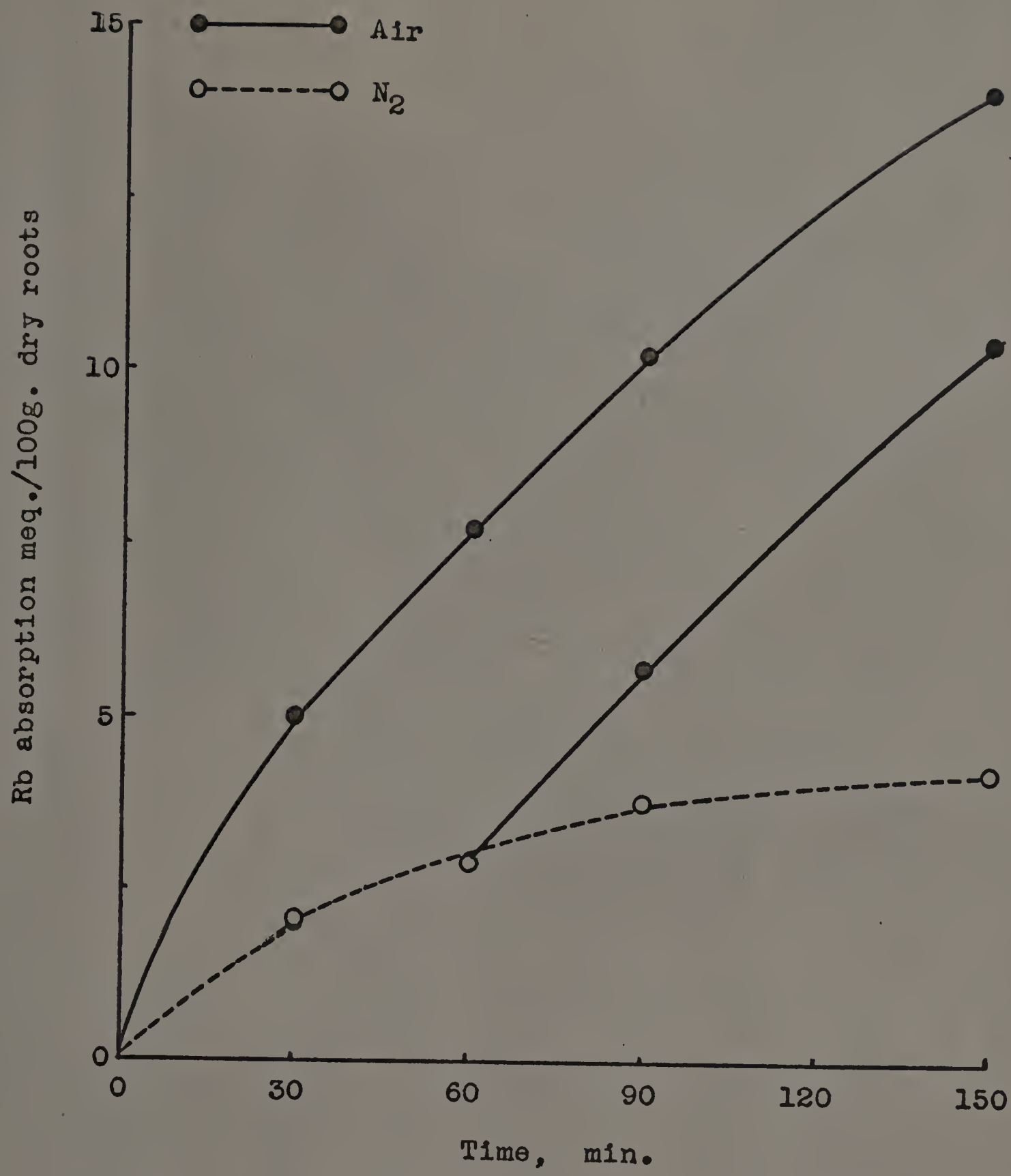


Fig. 11. Effect of the anaerobic condition on Rb absorbability of the roots.

the anaerobic condition for 60 minutes and then transfered to the aerated 10^{-3} N-RbCl solution. The results shown in Figure 11 clearly demonstrate that metabolic absorption of Rb is completely recovered by the aerobic condition. From this result, it is concluded that the anaerobic condition for a period of 60 minutes has no effect on the Rb absorbability of the roots.

3). Proposal of a new method to determine the root CEC and the effect of nitrogen on the root CEC

It was pointed out in the studies of the methodology of the root CEC assay that the following conditions must be provided for the quantitative measurement of the root CEC by a direct exchange method. First, a proper exchange period must be provided. Second, cations which are not normally contained in the roots must be employed as exchange cations. Third, metabolic absorption must be eliminated. It was indicated in the subsequent studies on the characterization of cation absorption by excised barley roots under anaerobic conditions that Li absorption from 10^{-1} N-LiCl under anaerobic conditions is exclusively cation exchange adsorption which is completed in about 30 minutes. Because Li is not contained in the roots, a technique using Li as an exchange cation under anaerobic conditions to prevent metabolic absorption for a 30-minute

exchange period with enough rinses with distilled water to remove a diffusible portion of cation appears to be able to satisfy the requirements for the quantitative measurements of the root CEC by a direct exchange method. From the considerations described above, a new method was developed to determine more exactly the root CEC. The procedure of the method is described as follows:

1) 2 g. of fresh excised roots were immersed for 30 minutes in 250 ml. of 0.1 N-LiCl solution in which N_2 gas was bubbling (O_2 % in the solution should be less than 0.3% at the beginning of the treatment.)

2) The roots were transfered to a small funnel and then rinsed with distilled water immediately after the exchange period until the rinse was free of Cl^-. The presence of Cl^- in the rinse was examined by the 1% $AgNO_3$ test. Six 50 ml. aliquot rinses with distilled water are usually adequate.

3) The roots were dried at 90° C. for 30 minutes in an oven and then placed at 75° C. in an oven overnight.

4) The dried roots were weighed and then ashed at 500° C. for three hours in a muffle furnace. The cooled ashes were wet with a drop of distilled water and dissolved into 2 ml. of 1:1

HNO_3. The nitric acid solution was gently warmed for 20 minutes on a hot plate.

5) Lithium content of the nitric acid solution which were properly diluted were measured using the Perkin Elmer atomic absorption spectrophotometer.

6) The content of Li of the roots was expressed as the root CEC in meq./100 g. dry weight.

A comparison of the root CEC values measured for barley roots by this Li exchange method with the ones measured by three other methods for the same roots is shown in Table 3. As predicted previously, the acid washing method proposed by Crooke (13) and the Na exchange method proposed by Bartlett (1) gave lower and higher values, respectively. Although the electrodialysis gave a similar value, this is a complexed result of the positive error by the drastic electrodialysis treatment and the negative error by the arbitrary titration period.

The opinions of the effects of nitrogen level on the root CEC is not always consistent. Although there has been reported many instances that higher nitrogen level in the medium resulted in the higher root CEC, some investigators have reported that there was no relation between the root CEC and nitrogen level in the medium.

The discrepancy of these results may be partially explained by the methodology of the root CEC assay and by

Table 3. Comparison of four methods for root CEC determination of barley roots

Method	Root CEC (meq./100 g. dry roots)
Electrodialysis method	12.9*
Acid washing method proposed by Crooke (13)	7.9
Na-exchange method proposed by Bartlett (1)	27.6
Li-exchange method	12.8

*After White, Drake and Baker (95)

growth conditions other than nitrogen level in the medium. An experiment was conducted to measure the effect of nutrient nitrogen on the root CEC of barley roots. The nitrogen level in the nutrient solutions was varied from 0 to 5 m. mole/l. of NH_4NO_3. The other nutrients, 0.1 m. mole/l. of $CaSO_4$, $MgSO_4$ and KH_2PO_4, were the same at each nitrogen level. The CEC of the roots obtained from these seedlings was measured by the Li-exchange method described above. The result, given in Figure 12, shows that N level up to 2.5 m. mole/l. of NH_4NO_3 increased the root CEC.

4). Function of the root CEC in cation absorption process

There is considerable discrepancy of opinions on the function of cation adsorption in the cation accumulation process. Laties (53) concluded that exchange adsorption was not related to accumulation, a view that was shared by Epstein (27) and Lagerwertt and Peech (52). There is, however, a long-standing view that the root CEC system is related to the mineral composition of the plants. Crooke and Knight (12) have evaluated published data on the mineral composition of plants in the light of the root CEC and concluded that CEC of the roots is positively correlated with the content in the tops of (a) the total cations, (b) the ash, (c) the excess base, and (d) the total elements. Brown (5) argues that too much evidence exists showing

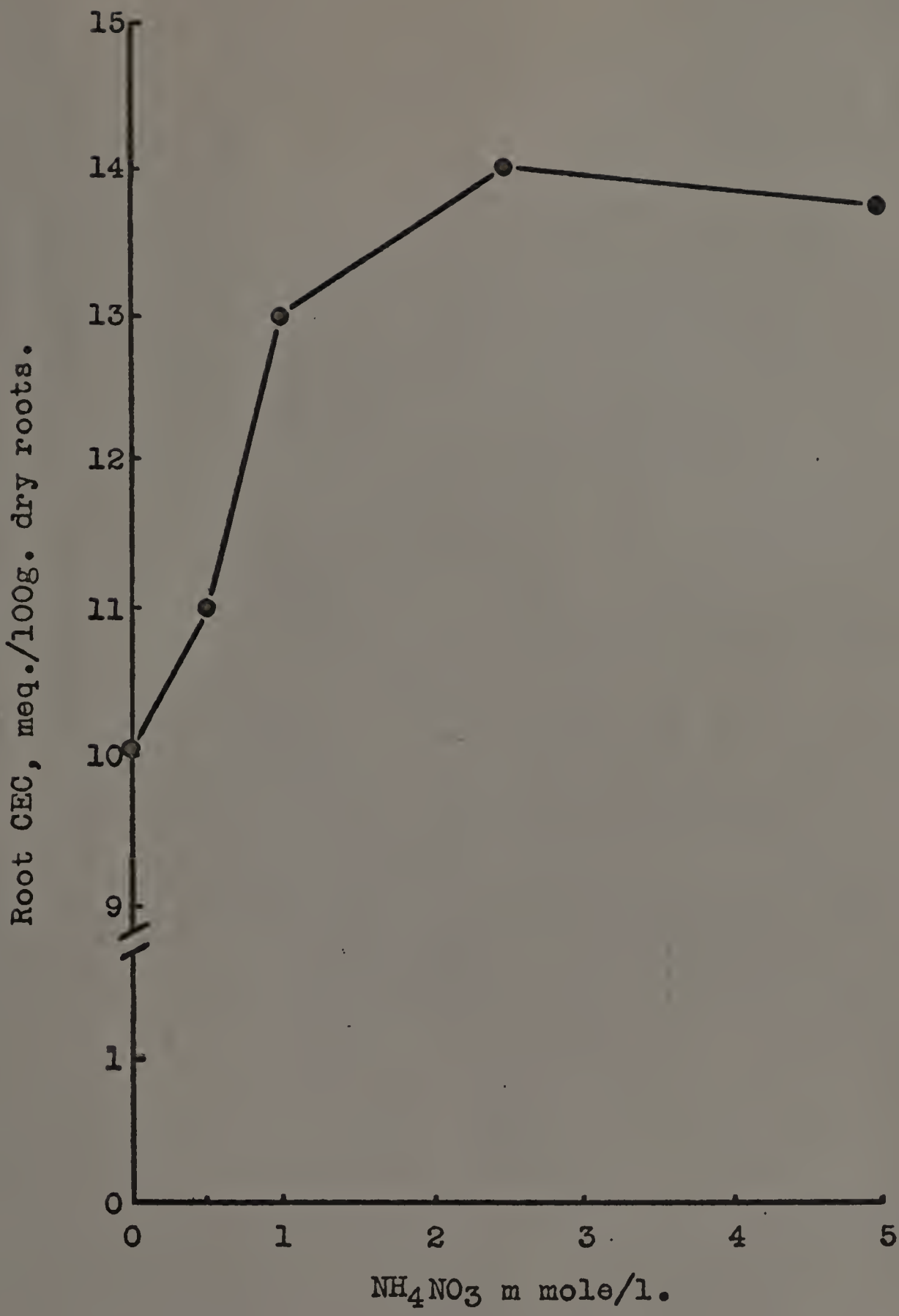


Fig. 12. Effect of N-level in the nutrient solution on the CEC of barley roots.

interactions involving soil colloids, cation exchange in roots, and nutrient elements to dismiss the root CEC as having no influence on the nutrient status of plants.

In order to prove that cation exchange adsorption is a predominant process in the cation absorption by plant roots and results in differential accumulation of monovalent and divalent cations, at least two assumptions must be satisfied. One is an assumption that cation exchange adsorption on the roots satisfies the Donnan expectation and the other is that cation exchange adsorption is a rate limiting factor of cation accumulation. From the standpoint mentioned above, this study was conducted to evaluate the function of the root CEC in cation absorption process.

The first interest of this study is to clarify the relationship between the ionic composition of the external solution and the relative amounts of the different cations absorbed on the exchange sites in DFS of the roots. This relationship is based on the postulate regarding the existence of electro-chemical equilibrium between the adsorbed and solution ions. If this equilibrium is assumed to be reached, the relationship between adsorbed (subscript i) and solution ions (subscript o) in the systems containing monovalent (superscript +) and divalent (superscript ++) cations can be described by Donnan equation:

$$\frac{(A^{+})_o}{(A^{++})_o^{1/2}} = \frac{(A^{+})_i}{(A^{++})_i^{1/2}} \qquad (1)$$

where (A) represents the activity of the cation.

Equation (1) is further described by equation (2):

$$\frac{[M^+]_o^2 \cdot (\gamma_o^+)^2}{[M^{++}]_o \cdot \gamma_o^{++}} \doteq \frac{[M^+]_i^2 \cdot (\gamma_i^+)^2}{[M^{++}]_i \cdot \gamma_i^{++}} \qquad (2)$$

where $[M]$ is molar concentration of cation, γ is activity coefficient.

Equation (2) is arranged into Equation (3).

$$\frac{[M^+]_o^2}{[M^{++}]_o} \doteq K \cdot \frac{[M^+]_i^2}{[M^{++}]_i} \qquad (3)$$

where

$$K \doteq \frac{(\gamma^+)_i^2 \cdot \gamma_o^{++}}{(\gamma^+)_o^2 \cdot \gamma_i^{++}}$$

If the exchange sites in DFS are saturated with M^+ and M^{++}, the following equation will be given:

$$[M^+]_i + 2[M^{++}]_i = Ec \qquad (4)$$

where Ec is concentration of the exchange sites in DFS in equiv./l. of DFS. Equation (4) holds so long as the mobile anion concentration in the Donnan phase is negligible compared with Ec.

From Equations (3) and (4)

$$2k[M^+]_i^2 + R[M^+]_i - REc = 0 \qquad (5)$$

where

$$R = \left[M^{+}\right]_o^2 / \left[M^{++}\right]_o$$

If k is assumed to be unity, the following equation will be obtained from (4) and (5):

$$\frac{\left[M^{++}\right]_i}{\left[M^{+}\right]_i} = \frac{1}{4}\left\{\left(1 + \frac{8Ec}{R}\right)^{\frac{1}{2}} - 1\right\} \qquad (6)$$

The ratio of divalent to monovalent cation adsorbed on the exchange sites in the DFS varies as a function of Ec and R. This relation is visualized in Figure 13 and Figure 14, where the ratio of $\left[M^{++}\right]_i / \left[M^{+}\right]_i$ increases with decreasing R or increasing Ec.

Accordingly, if the concentration of external solution containing a certain ratio of divalent and monovalent cations decreases, the ratio of divalent to monovalent cation absorbed on the exchange site increases. If the concentration of exchange sites in the DFS increases, the ratio of divalent to monovalent cations adsorbed on the exchange sites in the DFS should increase.

If the exchange adsorption which occurs on the root exchange sites in the DFS in accordance with the Donnan distribution is a rate limiting factor of cation accumulation, such a differential cation exchange adsorption must be reflected in total cation accumulation. From the consideration described above, experiments were conducted to

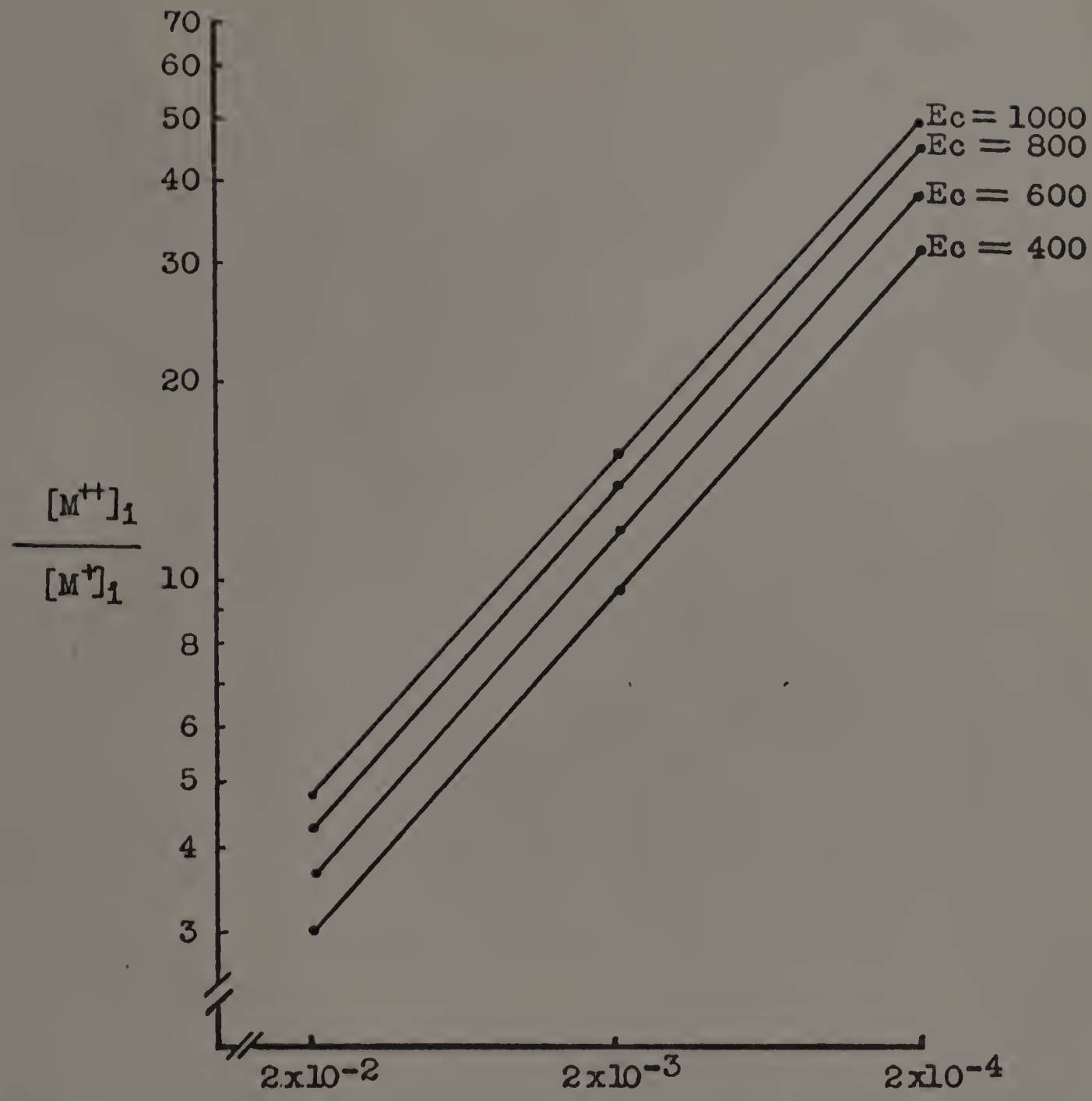


Fig. 13. Relation between $[M^{++}]_i/[M^{+}]_i$ and R

$[M^{++}]_i/[M^{+}]_i$: ratio of divalent to monovalent cations adsorbed on the exchange sites in DFS.

R: $[M^{+}]_o^2/[M^{++}]_o$ in molar concentration.

Ec: concentration of the exchange sites in the DFS in meq./l. of the DFS.

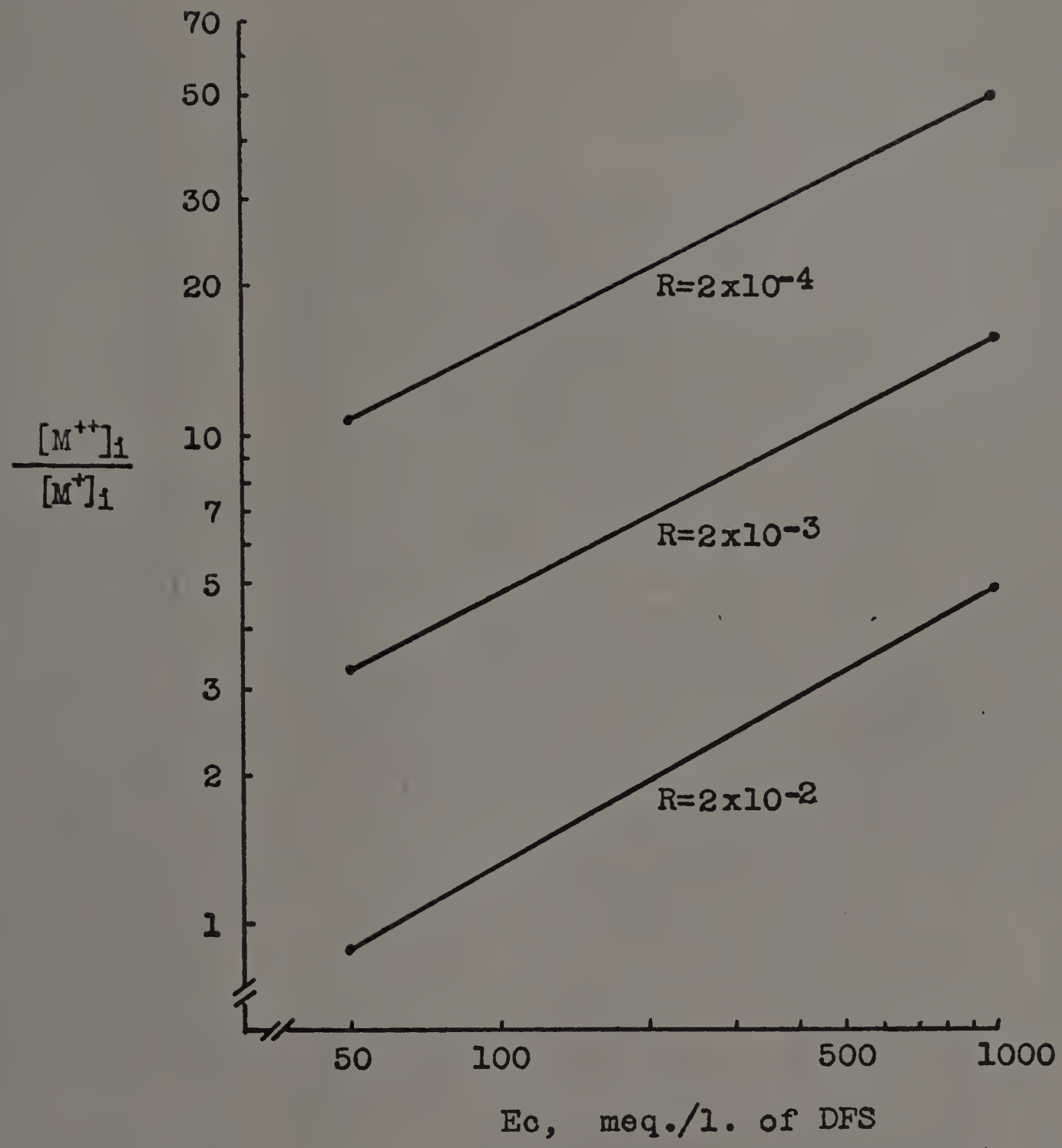


Fig. 14. Relation between $[M^{++}]_i/[M^+]_i$ and Ec.

determine whether exchange adsorption on the root exchange sites follows the Donnan distribution and whether the exchange adsorption is a rate limiting factor of cation accumulation.

Barley seedlings were grown in nutrient solutions containing two different concentrations of nitrogen, 0 and 1 m. mole/l. of NH_4NO_3. The other nutrients, 0.1 m. mole/l. of $CaSO_4$, $MgSO_4$ and KH_2PO_4, were the same at both nitrogen levels. The other aspects of the growth condition was the same as described previously. The CEC of the roots obtained from these seedlings was measured by Li-exchange method and found to be 7.31 and 9.44 meq./100 g. dry roots in the absence and presence of nitrogen in the nutrient solutions, respectively.

The previous discussions on characterization of cation absorption under anaerobic conditions revealed that the absorption of Li and Sr by N_2-bubbled roots during a short absorption period is mainly due to exchange adsorption and that cations not normally found in the roots must be employed in the study of cation exchange adsorption in order to avoid the interaction between native cations in the roots and cations in absorption solutions. Therefore, one gram of the excised barley roots were exposed to 500 ml. of the Li and Sr mixed solutions under anaerobic or aerobic conditions for 30 minutes. In this case, cation absorption under anaerobic and aerobic conditions represents exchange

adsorption and total absorption, respectively. Each concentration of LiCl and $SrCl_2$ in the mixed solution varied from 10^{-4} to 10^{-2} N. The ratio of Sr to Li in the solution was 1:1 on an equivalent scale. The roots were rinsed with distilled water immediately after the absorption period until the diffusible Cl^- disappeared from the last rinsing water. The rinses were usually carried out within two minutes with six 50 ml. portions of distilled water. The results are reported in Table 4.

Under the anaerobic condition, the sum of Li and Sr adsorption increased gradually with increasing concentrations in the external solution. Similar results were obtained by Briggs et al. (4) in beet disks where a gradual rise in the amount of Rb adsorbed in the DFS took place as the external Rb concentration rose from 1 to 20 meq./l. This gradual rise was explained as consistent with the Donnan anions having arisen from weak acids with a pK of about 3. The same explanation may be applied for the gradual rise in Li and Sr adsorption observed in this study.

The ratio of Sr to Li absorbed under the anaerobic condition increased markedly with decreasing concentrations in the external solution. These results indicate that Li and Sr absorption under anaerobic conditions are exclusively exchange adsorption and that the distribution of Sr and Li on the exchange sites follows the Donnan distribution.

Table 4. Li and Sr Adsorption by Excised Barley Roots as a Function of R*.

N-level in nutrient solution NH_4NO_3, mM.	Treatment		Amount of cation adsorbed (m mole/100g. dry roots)		
	O_2	R*	Li	Sr	Sr/Li
0	N_2	2x10-4	0.027	1.14	42.2
		2x10-3	0.10	1.33	13.3
		2x10-2	0.36	1.55	4.31
	Air	2x10-4	0.21	0.76	3.62
		2x10-3	0.36	1.05	2.91
		2x10-2	0.65	1.25	1.93
1	N_2	2x10-4	0.045	1.40	31.1
		2x10-3	0.16	1.63	10.2
		2x10-2	0.58	1.89	3.26
	Air	2x10-4	0.24	1.02	4.25
		2x10-3	0.39	1.40	3.08
		2x10-2	1.08	1.66	1.53

* $R = [Li^+]_o^2 / [Sr^{++}]_o$ in molar concentration.

The CEC of the roots which were grown in the nutrient solution with no nitrogen and with 1 m. mole/l. of NH_4NO_3 was found to be 7.31 and 9.44 meq./100 g. dry roots, respectively. If the volume of the DFS is the same for the roots grown under both conditions, the ratio of Sr to Li should be higher in the roots with higher root CEC measured. The results in Table 4, however, did not show the relationship expected. The alternative explanation for this result may be given in that a higher N level in the nutrient solution resulted in a higher amount of exchange sites per unit of dry weight and in much larger volume of the DFS. In short, the nitrogen pretreatment might result in the lower concentration of exchange sites in the DFS.

If the ratio of Sr to Li obtained represents the result of the Donnan distribution, it is possible to calculate Ec, the concentration of exchange sites in the DFS, from equation (6). The calculated value of Ec for the given roots, given in Table 5, do not deviate markedly in the different concentrations of the external solution. The mean concentration of exchange sites in the DFS was 761 and 440 meq./l. of the DFS for the roots grown under absence and presence of nitrogen in the nutrient solution, respectively. The estimated values of the concentration of the exchange sites in the DFS is similar to the value obtained by Briggs et al. (4) for beet discs. The method employed by them to estimate the concentration of the ex-

Table 5. Calculated Concentration of the Cation Exchange Sites in the Donnan Free Space.

N-level in nutrient solution NH_4NO_3, mM	R	Caluculated* Ec, meq./l.	Mean value of Ec, meq./l.
0	$2x10^{-4}$	721	761
	$2x10^{-3}$	736	
	$2x10^{-2}$	829	
1	$2x10^{-4}$	393	440
	$2x10^{-3}$	437	
	$2x10^{-2}$	490	

* The value of Ec was calculated from equation (6);

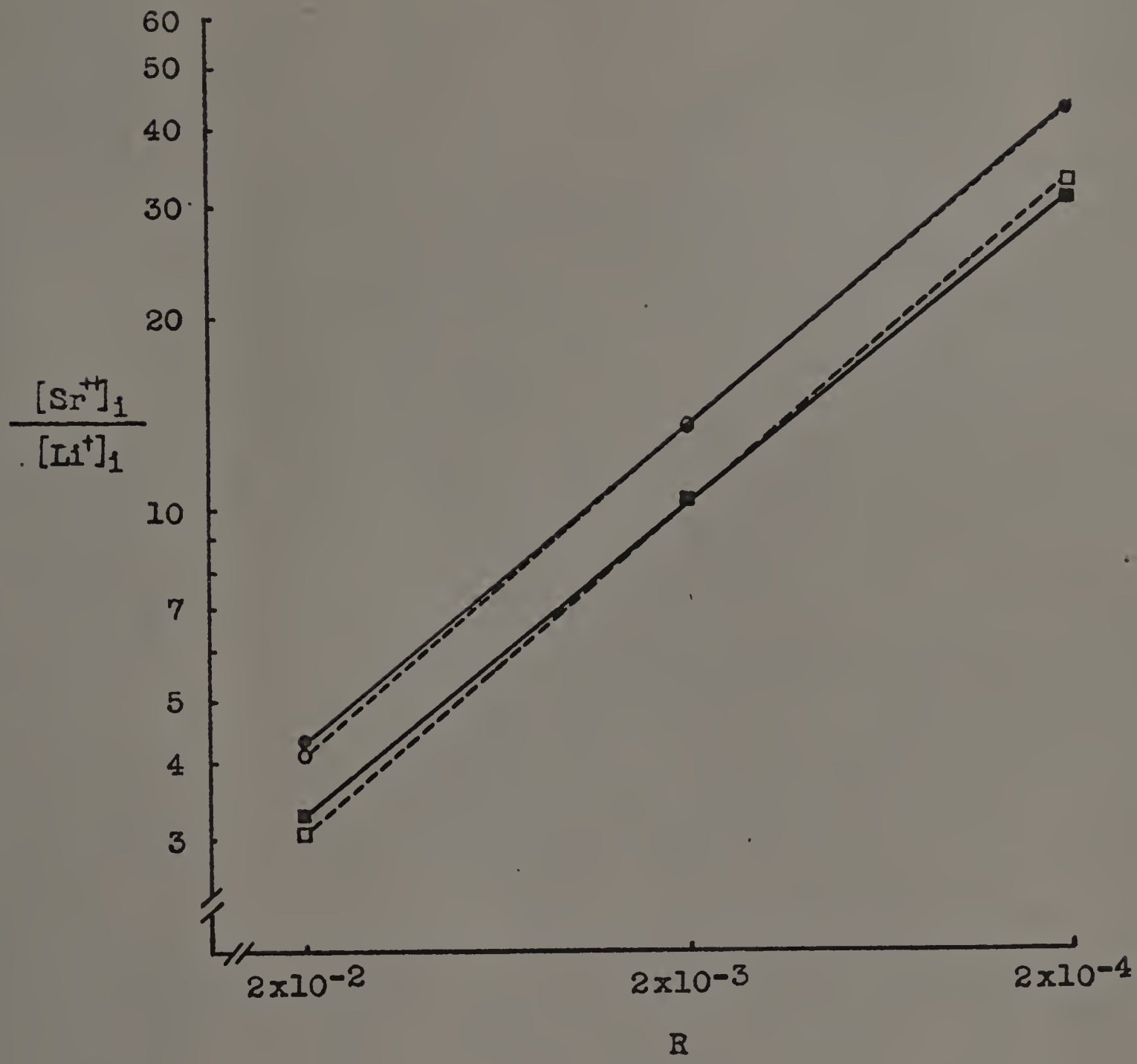
$$Ec = \frac{R}{8} \left\{ \left(\frac{[Li^+]_i \cdot 4}{[Sr^{++}]_i} + 1 \right)^z - 1 \right\}$$

change sites (non-diffusible anions in the DFS) is described as follows.

In disks pretreated with solutions of RbI to remove all other mobile ions from the free space, the amount of exchangeable I and Rb was measured by the uptake of I^{131} and Rb^{86} at various external concentrations. The excess of cations over anions (the extra exchangeable Rb') was used as an estimate of the amount of non-diffusible anions in the DFS. This was approximately constant at 10-13 meq./kg. of fresh weight. The volume of the DFS was estimated from the amount of extra exchangeable Rb in disks which had previously been treated so that the counterions in the DFS were exclusively Ca, and which were subsequently brought to equilibrium with various concentrations of RbBr. The mean volume from four experiments was 2.1 per cent, so that the concentration of exchange sites in the DFS was 560 meq./l.

If the concentration of the exchange sites in the DFS are assumed as 760 and 440 meq./l. of the DFS for the roots grown under absence and presence of nitrogen in the nutrient solution, respectively, it is also possible to calculate the ratio of Sr to Li adsorbed on the exchange sites in the DFS. The results, given in Figure 15, show that the experimental values almost exactly fit the theoretical values. Therefore, it is quite reasonable to conclude that exchange adsorption on the root exchange sites follows the Donnan

Fig. 15. Sr and Rb adsorption by excised barley roots as a function of R.



expectation.

The subsequent question is whether the Donnan distribution of cations on the root exchange sites results in a differential accumulation of divalent and monovalent cations inside of the roots. This question is equivalent to the question whether cation accumulation process occurs through the DFS of the roots. Epstein et al. (25) concluded that cation exchange adsorption is not essentially involved in the active accumulation process but that cations adsorbed on the exchange sites are absorbed very slowly by the metabolic processes in the absence of external reservoir of the ions in the solution. Lagerwerff and Peech (52) concluded that exchange adsorption and active accumulation of cations are two independent coexisting processes. The details of their experiment are reviewed on page 13 . However, it is obvious that they ignored the isotopic exchange of Ca between native Ca and Ca^{45} and the stimulative effect of $CaCl_2$ on Rb absorption which might cause erroneous values of Ca and Rb absorption. Therefore, their experiment is not helpful in the elucidation of the relationship between cation exchange adsorption and active accumulation.

The results given in Table 4 show that the ratio of Sr to Li absorbed under aerobic conditions increased with decreasing concentrations of the external solution. This result indicates that the valence effect is reflected in the total absorption. However, it does not necessarily

mean that exchange adsorption was involved in the active accumulation of cations because the exchange adsorption was the predominant process during the first 30-minute absorption period, even under aerobic conditions.

Figure 16 shows Rb and Sr absorption by the excised barley roots from 10^{-3} N single chloride or 10^{-3} N-RbCl and 10^{-3} N-$SrCl_2$ mixed solution under the aerobic condition. As discussed previously, the presence of Sr in the solution depressed Rb absorption by almost the same amount, in the presence of oxygen during the 60-150 minute absorption period. Hence the rate of Rb absorption after 60 minutes absorption period was almost the same in both the single and mixed solutions. The fact that the presence of divalent cations at 10^{-3} does not alter the monovalent cation after the first rapid absorption stage provided one of the strong bases for the viewpoint that exchange adsorption has no effect on active accumulation of cations (53). However, it will be dangerous to conclude so in this study from the limited information.

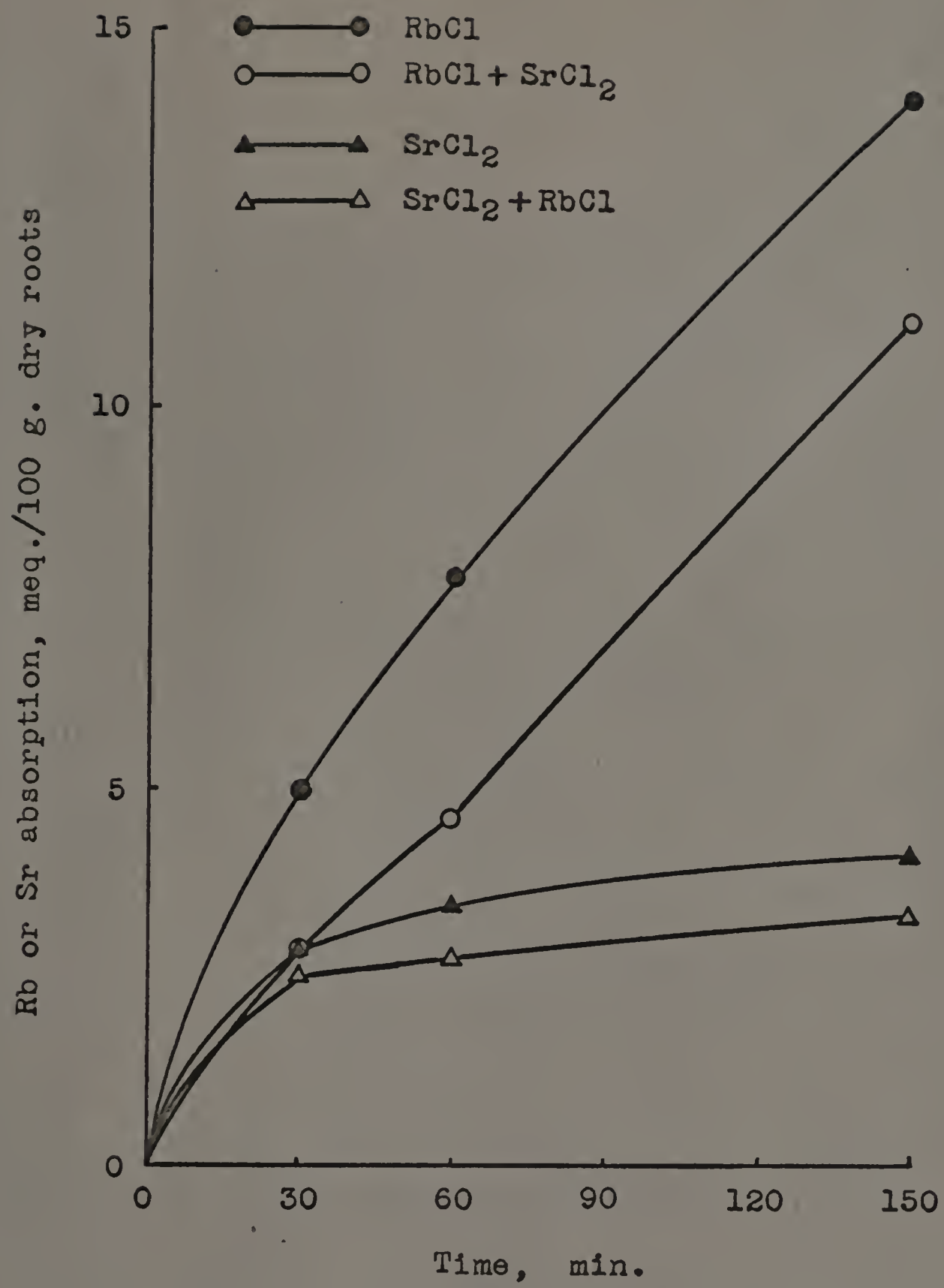


Fig. 16. Rb and Sr absorption by excised barley roots from 10^{-3}N single or mixed chloride solution.

VI. Summary and Conclusion

Cation absorption by excised barley roots was studied in relation to cation exchange capacity of the roots.

1). Studies on the methodology on the root CEC assay revealed that it is very difficult to determine accurately the root CEC by estimating the root exchange acidity. This is because it is difficult to saturate the roots with H^+ and to select an arbitrary end point of the titration. It was found that the Na-exchange method proposed by Bartlett (1) is not a quantitatively accurate method because of the contamination by the native and metabolically absorbed Na, and the short exchange period. As a result of these studies, it was proposed that the following conditions must be provided for the quantitative measurement of the root CEC by a direct exchange method.

   a). A proper exchange period.

   b). The use of exchange cations which are not normally contained in the roots.

   c). Elimination of metabolic absorption during the exchange period.

2). Characterization of the cation absorption under anaerobic conditions indicated that Li or Rb ab-

sorption in 30 minutes under anaerobic conditions was exclusively due to cation exchange adsorption. On the basis described above, a new method to determine the root CEC was developed. This method employs a technique using 0.1 N-LiCl as an exchange cation under anaerobic conditions during a 30-minute exchange period followed by distilled water rinses to remove the diffusible and occluded portion of Li from the roots.

3). Barley root CEC values determined by the Li exchange method increased with increasing nitrogen level up to 2.5 m. mole/l. of NH_4NO_3 in the nutrient solution being 10.2, 13.1 and 14.0 meq./100 g. dry roots for 0, 1 and 2.5 m. mole, respectively.

4). Calcium absorption from 10^{-3} N-$CaCl_2$ by excised roots under anaerobic conditions increased with time, reached a maximum within the first 60-minute absorption period and then gradually decreased with time. Potassium absorption was contrasted with Ca in that K absorption was greatly dependent on aerobic conditions. Excised barley roots under anaerobic conditions lost K in the solution regardless of the presence of 10^{-3} N-KCl in the solution.

5). Excised roots kept under anaerobic conditions for

60 minutes, when removed to aerated solution absorbed Rb at the same rate as roots not initially in anaerobic conditions. This indicated that a 60-minute period in the 0.2-0.3% O_2 as provided by bubbling Na gas had no adverse effect on Rb absorbability of the roots.

6). The relationship among the ratio of mono- to divalent cations adsorbed on the exchange sites in the Donnan Free Space (DFS), the concentration of the external solution and the concentration of exchange sites in the DFS was expressed as follows:

$$\frac{[M^{++}]_i}{[M^{+}]_i} \simeq \frac{1}{4}\left\{\left(1 + \frac{8\ Ec}{R}\right)^{\frac{1}{2}} - 1\right\} \qquad (A)$$

where $[M^{++}]i$ and $[M^{+}]i$ are molar concentrations of a divalent cation M^{++} and a monovalent cation M^{+} respectively, adsorbed on the exchange sites in the DFS, Ec is the concentration of exchange sites in the DFS in equiv./l. of the DFS, and R is expressed as $[M^{+}]_o^2 / [M^{++}]_o$, the ratio of the square of the molar concentration of monovalent cations to the molar concentration of divalent cations in the external solution.

7). The ratio of Sr to Li absorbed from a mixed solu-

tion of $SrCl_2$ and LiCl increased with decreasing R under anaerobic conditions, indicating that Sr and Li absorptions followed the Donnan distribution.

8). On the basis of the Donnan distribution, it is possible to calculate Ec, the concentration of the cation exchange sites in the DFS, from the ratio of Sr to Li absorbed (in 7). The calculated values of Ec for the barley roots which were grown in the nutrient solution without nitrogen and with 1 m mole/l. of NH_4NO_3 were 760 and 440 meq./l. of the DFS, respectively. These values did not deviate appreciably when 10^{-4}, 10^{-3}, and 10^{-2} N were the concentrations of the external solution.

9). The experimental values of the ratio of Sr to Li absorbed under anaerobic conditions exactly fitted to the theoretical values derived from the equation (A). This assumed that the concentration of exchange sites in DFS was as 760 and 440 meq./l. of the DFS of the roots which were pretreated by no nitrogen and 1 m mole/l. of NH_4NO_3 in the nutrient solution, respectively.

10). On the basis of the information obtained, the possible function of exchange adsorption in differential accumulation of mono- and divalent

cations by plants was discussed. It was concluded that at least cation absorption by exchange adsorption results in the differential absorption of mono- and divalent cations in accordance with the Donnan principle. Although the di/monovalent cation ratio increased in roots with decreasing concentration in the solution, this ratio did not increase with increased root CEC. Sr adsorption was increased by the N induced increase in root CEC, but Li uptake was also increased. Reasons for the latter were presented.

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