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THE EFFECT OF CALCIUM SALTS ON K
ABSORPTION BY EXCISED BARLEY ROOTS

by

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A Thesis

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I. INTRODUCTION

It has been recognized that salts of polyvalent cations often cause a striking increase in the absorption of K and Rb. Most investigators have attributed this "Viets effect" to the polyvalent cation (21,29,33,34,39,46,49,51, 68,69). On the other hand, Steward and Sutcliffe (61) and Briggs, Hope and Robertson (3) have suggested that this "Viets effect" is a result of stimulated K and Rb uptake by increased absorption of an accompanying anion.

Absorption mechanism consists of non-metabolic absorption and metabolic absorption. The former is believed to be due to diffusion and exchange adsorption (19,58) while the latter is believed to be mediated by the action of ion carriers or ion-binding compounds (8,9,12,14,20,29,31,32,33,41, 45,53). According to Epstein (8), the essential features of the functioning of carriers are the attachment of the ions to carrier molecules, the movement of the resulting carrier-ion complexes through some barrier intervening between an "outer" and an "inner" phase, and the subsequent discharge of the ions into the "inner" phase. Although there have been different hypotheses (49), the presence of such a substance in roots as the carrier or ion-binding compound seems to be a real feature of the metabolic absorption mechanism because of the movement of ions from external solution which has a lower concentration of the ions, into the cell.

In respect to the effect of accompanying anion on the

absorption of K, there have been several reports (13,22,25, 27,41,45,60), however, the effect has not been elucidated precisely. In this study, therefore, the effect of the accompanying anions as well as that of Ca on the K absorption, both in metabolic absorption based on the carrier concept and in non-metabolic absorption, will be discussed.

II. TERMINOLOGY

Some confusion has arisen in the past among investigators of salt absorption through differences in terminology. To avoid ambiguity, a number of words will first be defined based on Sutcliffe's idea (63).

Uptake and absorption are used synonymously, and do not imply any particular method of entry of salts. When these expressions are used, net movement is implied. Active absorption (or uptake) and metabolic absorption (or uptake) are used for absorption requiring metabolic energy. Passive absorption (or uptake) and non-metabolic absorption (or uptake) are used for exchange adsorption plus diffusion. Accumulation of ions is a result of metabolic absorption (or uptake), that is, this word implies entry of ions into the inner phase of root cells from the external solution.

III. REVIEW OF LITERATURE

It is generally accepted that absorption of ions by plant roots consists of non-metabolic absorption by diffusion and exchange adsorption and metabolic absorption effected by the action of carriers or ion-binding compounds (3,8,17,63).

The ions passively absorbed by diffusion and exchange adsorption are believed to exist in the "apparent free space" of the cell. Sutcliffe (63) defined the "apparent free space" as the apparent volume of a cell or tissue penetrated passively by salt in the external solution assuming that the concentration of the solution in the free space reached the same concentration as in the external solution. From measurements of passive salt uptake from salt solutions of different concentration, it is possible to deduce that the "apparent free space" consists of two components, a "water free space" which is a space into which salts diffuse to a concentration equalling that of the medium, and a "Donnan free space" containing immobile anions. Plant roots adsorb ions at exchange sites in the "Donnan free space" (3). According to Epstein (11), the "apparent free space" represents about 23 per cent of the volume of barley root tissue. While there is no competition among ions in the "water free space", there is competition among ions for exchange sites in the "Donnan free space".

Experiments by Sutcliffe (65) showed that the considerable initial uptake of alkali metal cations by red beet discs involves nonspecific ion exchange, unrelated to metabolic absorption. That is, Rb, Cs and Na all interfered with K absorption while Rb, Cs and K interfered with Na absorption. The relative rates of absorption of Na and K at 2° C (only exchange adsorption) were markedly different from the relative uptake of the two ions at 25° C (exchange adsorption plus metabolic absorption). Skelding and Rees (58) reported that the absorption of Mn ions by discs of beet-root tissue occurs in two phases, and suggested that the first rapid phase is physical in nature and the second slower phase is physiological in nature. Kylin and Hylmo (36) reported that the volume of apparent free spaces of intact and excised wheat root are 27.5 per cent and 18.0 per cent of total volume of the root respectively. In their experiment, the equilibration between apparent free space and nutrient solution was completed within 15 minutes. According to Lundegardh (44), the second slower phase of K and Cl absorption from 2 to 10 mM-KCl was attained after 30 minutes.

In 1936, Osterhaut (52) introduced a pioneering statement of carrier hypothesis in metabolic absorption mechanism of ions by algae. In his paper, he suggested that when NH_4^+ is absorbed from NH_4Cl solution, NH_4OH combines with a constituent HX of the protoplasm according to the equation $\text{NH}_4\text{OH} + \text{HX} = \text{NH}_4\text{X} + \text{H}_2\text{O}$. Then NH_4X diffuses, so that the

rate of entrance depends on the concentration of NH_4X in the protoplasm rather than on that of NH_4 in the external solution. Jacobson and Ovarstreet (31) advanced this idea as follows: ions react with metabolically produced carrier substances in the protoplasm to form complexes which subsequently move through the barrier and break down to release free ions. They expressed the reaction in the form of the chemical equations (31):



where R_1 and R_2 represent the metabolically produced cationic and anionic binding substances respectively. Epstein and Leggett (14) reported that radioactive Sr taken up by the respiration-dependent, active mechanism of barley roots was non-exchangeable with non-radioactive Sr in the external solution. They concluded that this result was caused by some barriers for movement of free ions. Sutcliffe (64) studied the exchangeability of monovalent ions in cells of red beet tissue. He also concluded that active absorption of these ions involved their passage, in combination with specific carriers, across a barrier highly impermeable to the free ions.

Lundegardh (45) introduced the anion respiration hypothesis in order to explain the mechanism of salt absorption. According to his hypothesis, an anion is absorbed through the cytochrome system and released from cytochrome b in the "in-

ner" phase to form acid which probably reacts rapidly with a cation carrier MR in the surroundings, thus forming the neutral salt M^+A^- . Consequently he suggested (41,45) that in plant roots it is the anions which are actively absorbed while cations move passively across the protoplasts along the electrical gradient so created. However, Epstein (10) found that absorption of cations as well as anions results in increased respiration, and questioned Lundegardh's anion respiration hypothesis. Leggett and Epstein (40) further reported that SO_4 absorption proceeds in three steps like cation absorption: 1) SO_4 ions reversibly combine with reactive sites of carriers, 2) the carrier- SO_4 complex traverses a membrane not permeable to free SO_4 ions, 3) SO_4 ions are released internally in a rate-limiting, essentially irreversible step. They found that SeO_4 competed with SO_4 but PO_4 , NO_3 , and Cl had no measurable affinity for the SO_4 - SeO_4 binding sites.

There is considerable controversy over the relationship between the amount of an ion passively absorbed and the rate of metabolic accumulation. Fried, Noggle, and Hagen (16) conducted absorption experiments with Sr (10^{-4} - 10^{-2} M, 30-180 min.), Rb (10^{-5} - 10^{-2} M, 5-40 min.), K (10^{-5} - 10^{-2} M, 5-60 min.), and Na (10^{-5} - 10^{-2} M, 5-35 min.), and reported that higher concentrations resulted in an increase in both rate of metabolic absorption and the amount of the ion passively absorbed and that the ratio of rate of accumulation to

the amount of the ion passively absorbed is approximately constant. They concluded that the amount of absorption not associated with ion-carrier complex MR is negligible. However, Lagerwerff and Peach (37) concluded that exchange adsorption and metabolic accumulation of cations are two independent co-existing processes after conducting experiments with combination of the activity ratio $(Rb^+)/\sqrt{(Ca^{++})}$ and the ionic strength. That is, in their experiment, excised barley roots were exposed to solutions containing Rb and Ca chloride in different combinations for 1 hour. In one set of exposure solutions, the activity ratio $(Rb^+)/\sqrt{(Ca^{++})}$, derived from millimolar concentrations, was varied from 0.02 to 1.28 while the ionic strength was maintained constant. In another set of exposure solutions, the ionic strength was varied from 4.40 to 118.80 millimoles per liter while the ratio $(Rb^+)/\sqrt{(Ca^{++})}$ was maintained constant. A comparison of the calculated percentage 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 percentage 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. Epstein and Leggett (14) reported that metabolic absorption of Sr from $SrCl_2$ is a linear function of time from the beginning of the absorption period. Thus, since

the adsorption-exchange reaction proceeds for at least half an hour, the accumulation rate is clearly independent of the saturation state of the adsorption sites. That the ions Ca, Sr, and Ba compete for identical binding sites was also reported by these investigators (14).

Collander (5) studied cation absorption by 21 different species of higher plants which were grown for approximately two months in complete nutrient solutions containing several cations in equivalent amounts. He found that all plants studied absorbed Rb and Cs with almost the same rapidity as K. There was a similar relationship between Ca and Sr. He also found that K and Rb mutually interfere with each other's uptake, somewhat as though they were identical ions or two isotopes of the same element. Sutcliffe (65) showed with red beet tissue that the alkali cations compete with one another for the same absorption mechanism, and that in this tissue, the ions are apparently preferred by the mechanism in the order $\text{Na}^+ > \text{K}^+ > \text{Rb}^+ > \text{Li}^+$. He further observed that when both K and Na are contained in an external solution, the red beet tissue absorbs much more Na and much less K than from single Na or K solution. He demonstrated that the uptake of a particular ion is not necessarily reduced in the presence of another when the two are competing for the same accumulation mechanism.

Viets (68) studied systematically the influence of salts of polyvalent cations (Ca, Mg, Sr, Ba, and Al) on K uptake

by excised barley roots. Each of the salts stimulated K absorption, Ca salt being the most effective. A ratio greater than 30 Ca ions to 1 K ion in the solution was necessary before K absorption could be depressed below the absorption from pure KBr. He suggested that polyvalent cations exerted their effect directly upon the protoplasmic membrane and that this surface effect, due either to the presence of the polyvalent cation or to its absorption in the surface, might conceivably be an effect on some phase of metabolism concerned with permeability, as dissociation, hydration of colloids, etc. Jacobson, Overstreet, and Handley (33) also studied the effect of Ca on the K absorption by barley roots for 3 hours and found that in a solution containing 0.01N-CaCl₂, as the KCl concentration decreased, the CaCl₂ had an increasing stimulating effect down to a concentration of 2×10^{-4} N-KCl. Below this concentration, the stimulating effect diminished and the CaCl₂ depressed the absorption of K below 5×10^{-5} N-KCl. They concluded that the stimulating effect of Ca on K absorption would best be explained either as an enhancement of the rate of formation of HR or an increased rate of utilization of KR, and that the depressing effect is caused by a competition between Ca and K for HR. That the effect is due to a non-metabolic Ca fraction rather than metabolically absorbed Ca is suggested by the observation that metabolic absorption of Ca occurs very slowly (34).

Moore, Jacobson, and Overstreet (50) found that the uptake of Ca by excised barley roots is largely non-metabolic, and that much of the Ca in the excised roots is associated with the cell surface region. Middleton and Russell (49) studied the entry of cations into carrot discs for periods ranging 5 minutes to 24 hours. They found that 1) in short periods of absorption, the competitive power of ions increases with atomic number and valence, thus the absorption of Rb is inhibited by the presence of Sr in short periods, 2) in experiments lasting 24 hours, the absorption of Rb is greatly stimulated in the presence of SrCl_2 . Based on these results, they explained the effect of polyvalent cation on the univalent cation absorption by the following concept: the characteristic of ions which favor retention in roots (i.e. high atomic weight or valence) are antithetic to those which favor their eventual accumulation (i.e. low atomic weight or valence). The characteristic of ions in plant tissue is the same as that of ions on cation-exchange columns. Thus they assumed that the stimulation of Rb absorption by SrCl_2 is caused by the extensive retention of Sr at the site of entry.

Although it is generally agreed that cations and anions are absorbed separately by plant tissues, several investigators have observed effects of anions on cation absorption. The effect of anions on the absorption of K was observed by Hoagland (22). He reported that accumulation of K from SO_4

solution was much smaller than from Cl, Br, or NO₃ solutions, as though the rate of accumulation of the cation or equilibrium value attained was markedly influenced by the mobility of the anion. Steward and Harrison (60) found in studies of Rb and Br absorption by potato discs that all the variables which affect Br absorption affect the uptake of Rb in a similar manner. Hurd and Sutcliffe (25) reported that increased K absorption by beet root discs at high pH could be attributed to an increased uptake of the associated anions, HCO₃⁻ and Cl⁻. According to Steward and Sutcliffe (61), absorption of anions and cations by barley roots is so closely related that an effect on one results in an effect on the other. Briggs, Hope, and Robertson (3) suggested that if the rate of accumulation of KCl is V and the resistance to movement of the cation of another chloride is so great that its rate of accumulation is negligible at the same concentration V, then the addition of this second chloride to the KCl should result in an increase in the rate of accumulation of K to a value greater than V while the second cation is not accumulated at all. Epstein, Rains, and Elzam (13) conducted an experiment on K absorption from solution ranging in concentration from 0.002mM-KCl to 50 mM with 0.5 mM-CaCl₂ throughout and found that over the 0.002 to 0.2 mM concentration range of K, a single Michaelis-Menton term described the relation between concentration and rate of absorption, but that over the high concentration range of 0.5 to 50 mM-K,

absorption rates consisted of two terms: the contribution from the low-range mechanism 1 and a contribution from a second mechanism with much lower affinity for K. And they observed that absorption of K or Rb by the high affinity mechanism 1 was autonomous cation transport independent of the rate of absorption of the anion, but that over the high concentration range, Rb absorption from SO_4 solution was greatly depressed, compared with absorption from Cl solution.

There have been several studies of the relationship between organic-acid metabolism and inorganic-ion absorption. It has been suggested that unequal amounts of metabolic absorption of cation and anion result in an increase or decrease in the organic-acid content of the cell. Ulrich (66) found that when excised roots absorbed cations in excess of anions from the salt in the culture solution, provided that an ample supply of sugar was available in the root cells, organic acids were formed as a response to the tendency toward an increase in pH of the root sap. Conversely, when anions were absorbed in excess of cations, the organic-acid anions tended to disappear leaving the base to balance the increase in inorganic anions. Hoagland and Broyer (23) also reported that when CaBr_2 was supplied to the roots as the culture medium, Br was rapidly absorbed (although to a considerably less extent than from a solution of KBr of equivalent strength), but little, if any, absorption of Ca occurred. This excess uptake of anion led to a marked decrease

of total organic acid in the sap.

Jacobson and Ordin (30) studied the relation between K-Br absorption and organic metabolism in barley roots and demonstrated that when excess cation absorption occurred, the production of malate was the most important means of ion compensation, and that on the other hand when excess anion absorption prevailed, much but not necessarily all of the compensation was due to a decrease in malate content. Furthermore, Jacobson (28) investigated the fixation of $C^{14}O_2$ by excised barley roots as influenced by equal and unequal cation and anion absorption. Under conditions of excess cation absorption (from KH_2PO_4), much more C^{14} was fixed than when cation and anion absorption were about equal (from KBr), and the reverse held when anion absorption exceeded that of cations (from $CaBr_2$). Jackson and Coleman (27) studied the K absorption from $KHCO_3$ solution and from KCl solution by bean roots and found the much greater absorption of K from $KHCO_3$ solution than from KCl solution. They suggested that the accumulation of the organic acids formed from HCO_3 enhanced the absorption of K.

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. (32,34). Fifty grams of seed were treated with 75 ml. of 10 per cent H_2O_2 for 20 minutes. The H_2O_2 solution was drained off and the seeds were soaked for 24 hours in 1500 ml. distilled water with continuous aeration. The seeds were then thoroughly washed several times with distilled water, spread out on cheese cloth supported by a nylon screen, and covered with another nylon screen and cheese cloth. The end of the cheese cloth dipped into the solution. The seedlings were grown in the dark using a dilute nutrient medium consisting of 0.1 m mole/l. each of KH_2PO_4 , $CaSO_4$, and $Mg(NO_3)_2$; the solution was gently aerated. Two days after transferring the seed to the nylon screen, the cheese cloth and the nylon screen covering the plants were removed. On the third day, the nutrient solution was replaced with fresh solution. At this time, the roots were rinsed twice in distilled water. On the fifth day, the nutrient solution was replaced with distilled water (51). The roots were used for an absorption experiment 24 hours later.

(2) Condition of Absorption Experiments.

The roots, grown for 6 days as described above, were

excised just below the seed, and thoroughly washed with ice-cold distilled water. A ratio of 1 gr. root material to 250 ml. of solution was employed in following absorption experiments, which were conducted at 25° C. The solutions were aerated continuously and pH was adjusted at 6.0 in all the experiments except one with 2,4-dinitrophenol (DNP). For the adjustment of pH, IRA 400 anion exchange resin in OH form was used (55,56) at 30 minute intervals.

(3) Analysis of the excised barley roots.

a) K and Ca.

After the absorption experiments, the excised roots were washed three times with cold distilled water, dried in an oven at 75° C, weighed to 0.1 mg., and ashed overnight in a muffle furnace at 450° C. Then K and Ca were determined using a Perkin-Elmer Model 214 Atomic Absorption Spectrophotometer.

b) Cl.

The excised roots were washed with cold-distilled water, dried in an oven and ground in a micro Wiley mill. It has been shown that boiling hot water can extract all of the absorbed Cl from the excised barley roots (6,7). A 200 mg. sample of the ground barley roots was weighed into a 25 ml. centrifuge tube, and 10 ml. of boiling de-mineralized water were added. The contents were stirred with a glass rod for 30 seconds and centrifuged at 3,000 r.p.m. for 5 minutes and the supernatant liquid was filtered through a Whatman No. 42

filter paper into a 25 ml. volumetric flask. The residue was washed three times with 5 ml. portions of boiling water, stirred each time for 30 seconds, centrifuged, and filtered. An aliquot of the filtrate was passed through an IR 120 cation exchange column into a 50 ml. volumetric flask. To insure complete passage of all anions, 5 ml. of distilled water was passed through the column. The pH of the effluent was neutralized with dilute nitric acid and ammonium hydroxide using phenolphthalein indicator. Then 2.5 ml. of N-nitric acid and 25 ml. of ethylene glycol monomethyl ether were added and the flask was filled to the mark with demineralized water. Approximately 100 mg. of mercuric chloranilate was added to the flask. The flask was allowed 15 minutes for color development, shaking at least once a minute. The contents were then filtered through Whatman No. 42 filter paper and the absorbance of the filtrate at $515\text{ m}\mu$ was read. (1).

c) Rb⁸⁶.

In short-term absorption, the amount of K absorption is so small that it can not be estimated accurately when non-radioactive K is used. Therefore the absorption rate of Rb was measured using Rb⁸⁶ as an isotopic tracer. Resemblance of Rb to K in its behavior in ion absorption by plant tissues has been found by many investigators (5, 12, 26, 48, 60). Manzel and Heald (48) found that there was a fixed relationship between the ratio of Rb to K in the solution and that of these elements absorbed by the millet, oats, buckwheat,

sweet clover, and sunflower: absorption of Rb was 0.85 times K. Jackson and Adams (26) obtained the same rates of K and Rb absorption from K^{42} -KCl and Rb^{86} -RbCl solutions. Based on these evidences, radioactive Rb^{86} -RbCl was used instead of K^{42} -KCl in the short-term experiments.

After an absorption experiment and washing, the roots were transferred to a 3.5 x 0.5 cm. planchet, dried over night in an oven at 75° C, weighed, and counted with a Geiger-Muller Autoscaler Model SC-51.

d) Cl^{36} .

Cl^{36} absorbed by the roots was measured using the method used for Rb^{86} .

e) Total non-volatile organic acids.

Total non-volatile organic acids were extracted by Falmer's water extraction method (54). After the absorption experiment, the roots were washed three times with cold-distilled water as quickly as possible. The roots were then heated at 95° C for 30 minutes in a ventilated oven and then transferred into another ventilated oven to dry at 75° C. The dried roots were ground in a micro Wiley mill. A 200mg. sample of the ground roots was weighed into a 25 ml. centrifuge tube, and 10 ml. of water was added. The tube was heated in a steam bath for 20 minutes, the contents being occasionally stirred with a glass rod. The rod was then rinsed off, the tube was centrifuged, and the supernatant liquid was filtered first through a Whatman No. 35 filter paper, then

No. 42 filter paper into a 25 ml. beaker. The residue was washed three times with 5 ml. portions of water, being heated and stirred each time for a few minutes, and centrifuged. The fluid was concentrated to 2 - 3 ml. at low temperature on a hot plate.

In order to determine the total non-volatile organic acids, Rexyn 201 anion exchange resin of 100-200 mesh size was prepared in formate form. The resin column was supported in glass tubing of 6 mm. inside diameter and approximately 15 cm. long. Height of the resin column was 4 cm. The root extract, concentrated to 2 - 3 ml., was placed on the column of formate resin. Elution was accomplished by passing two 6 ml. portions of 6 N-formic acid through the column at a rate of about 1 ml. per minute. Fractions of 6 ml. in volume were collected in 1.5 x 15 cm. test tubes and placed in a rectangular water bath at 46° to 48° C. A glass manifold constructed as described by Palmer (54), was placed on the row of tubes. The manifold tubes were of such a length that the accurately tooled capillary openings were approximately 2 cm. above the surface of the liquid. The test tubes were submerged in the warm water for about one-third of their length. Adjustment of the air flow to a point such that the surface of the liquid was considerably agitated but no splashing occurred brought about satisfactory volatilization rates. After evaporation of visible fluid, usually between 120 - 150 minutes, the test tube was removed from the water bath, and 2 ml. of

demineralized water were added to the tube. The solution was then titrated to the phenol red end point with CO_2 -free 0.002-NaOH.

V. RESULTS AND DISCUSSION

(1) Effect of Ca salts on the absorption of K by excised barley roots.

The effect of CaCl_2 and CaSO_4 , concentrations ranging from 0 to $2 \times 10^{-2}\text{N}$, on the absorption of K in 3 hours from $5 \times 10^{-3}\text{N-KCl}$ and K_2SO_4 solutions is shown in Fig. 1. K absorption from K_2SO_4 alone is only 70 per cent of the K absorption from KCl alone. A remarkable result in this experiment is that the stimulating effect of CaCl_2 on the absorption of K from both KCl and K_2SO_4 solutions is apparently much greater (36 per cent increase in KCl solution and 72 per cent in K_2SO_4 solution) than that of CaSO_4 (10 per cent in KCl solution and 8 per cent in K_2SO_4 solution).

That salts of polyvalent cations stimulate the absorption of K has been observed by several investigators (33,34,49,51,68). Viets (68) suggested that polyvalent cations exerted their effect directly upon the protoplasmic membrane and that this surface effect, due either to the presence of the polyvalent cation or to its absorption in the surface, might conceivably be an effect on some phase of metabolism concerned with permeability, as dissociation, hydration of colloids, etc. According to Jacobson et al. (33), the stimulating effect of Ca on the absorption of K would best be explained either as an enhancement of the rate of formation of HR or an increase rate of utilization of KR. Middleton

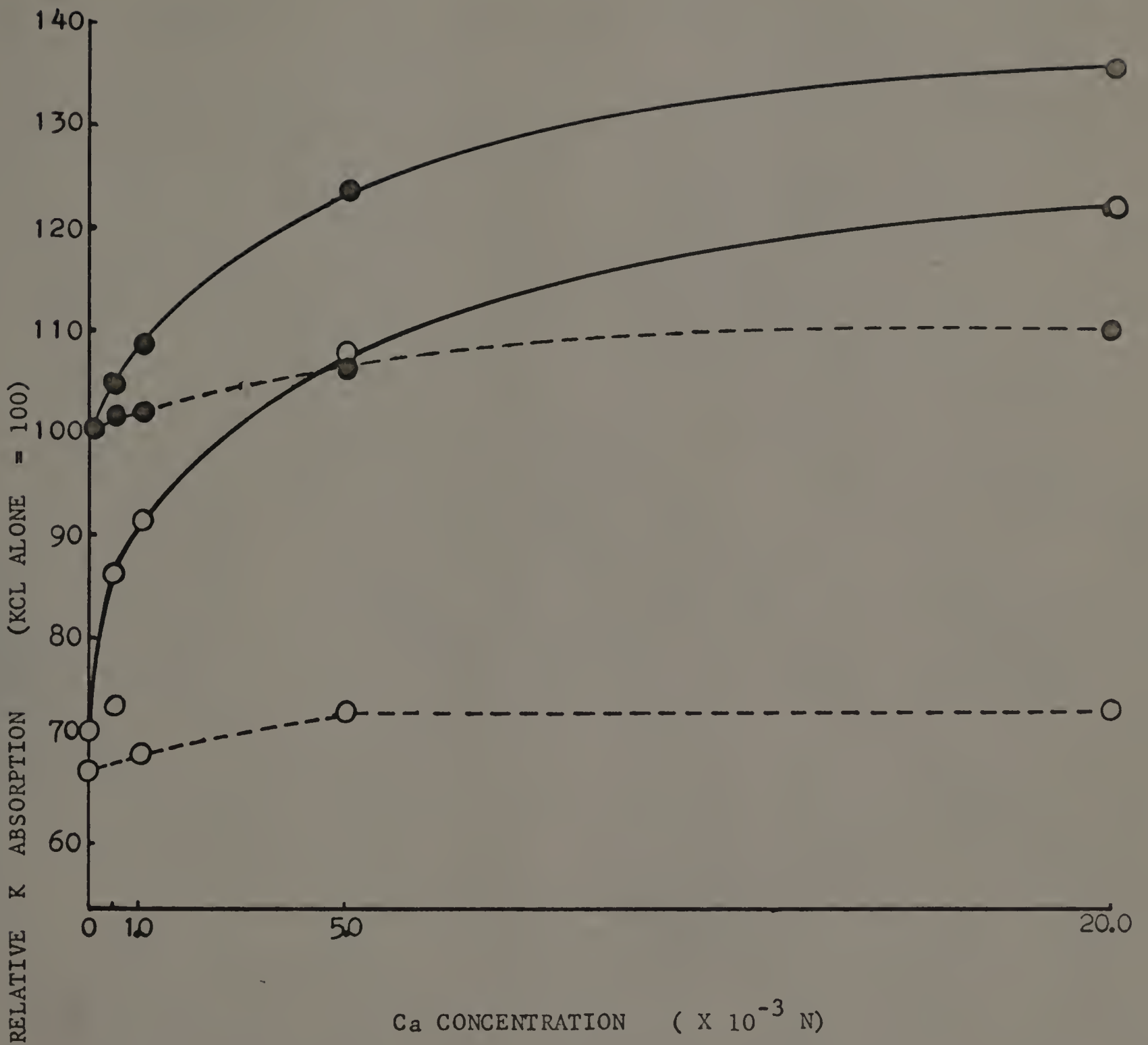


Fig. 1. Effect of CaCl₂ (—) and CaSO₄ (-----) on the absorption of K from KCl (●) and K₂SO₄ (○) for 3 hours. Concentration of KCl and K₂SO₄ is 5 x 10⁻³N.

and Russell (49) explained the stimulating effect of salts of polyvalent cations on univalent cation absorption by the following concept: the characteristic of ions which favor retention in roots (i.e. high atomic weight or valence) are antithetic to those which favor their eventual accumulation (i.e. low atomic weight or valence). The characteristic of ions in plant tissue is the same as that of ions on cation-exchange columns. Thus they assumed that the stimulation of Rb absorption by SrCl_2 is caused by the extensive retention of Sr at the site of entry. However, none of these concepts can explain the difference in stimulating effect of CaCl_2 and CaSO_4 on K absorption which is shown in Fig. 1.

In order to find out whether the stimulating effect of Ca salts on the K absorption is due to a non-metabolic ion fraction (Ca or accompanying anion) or a metabolically absorbed ion fraction (Ca or accompanying anion), the excised barley roots were first treated with 10^{-2} N- CaSO_4 , 10^{-2} N- CaCl_2 , or demineralized water for 3 hours. And then the roots were transferred to 5×10^{-3} N- K_2SO_4 solution for 1, 3, and 6 hours (Figs. 2 and 3). There is a marked increase in K-absorption by the roots pretreated with 10^{-2} N- CaCl_2 compared with water-pretreated or 10^{-2} N- CaSO_4 -pretreated roots (Fig. 2). Most of Ca ions which have been adsorbed and have diffused in apparent free spaces during pretreatment are removed by K in 1 hour (Fig. 3). Although it has been suggested by Jacobson et al. (33) that increased absorption of K is due

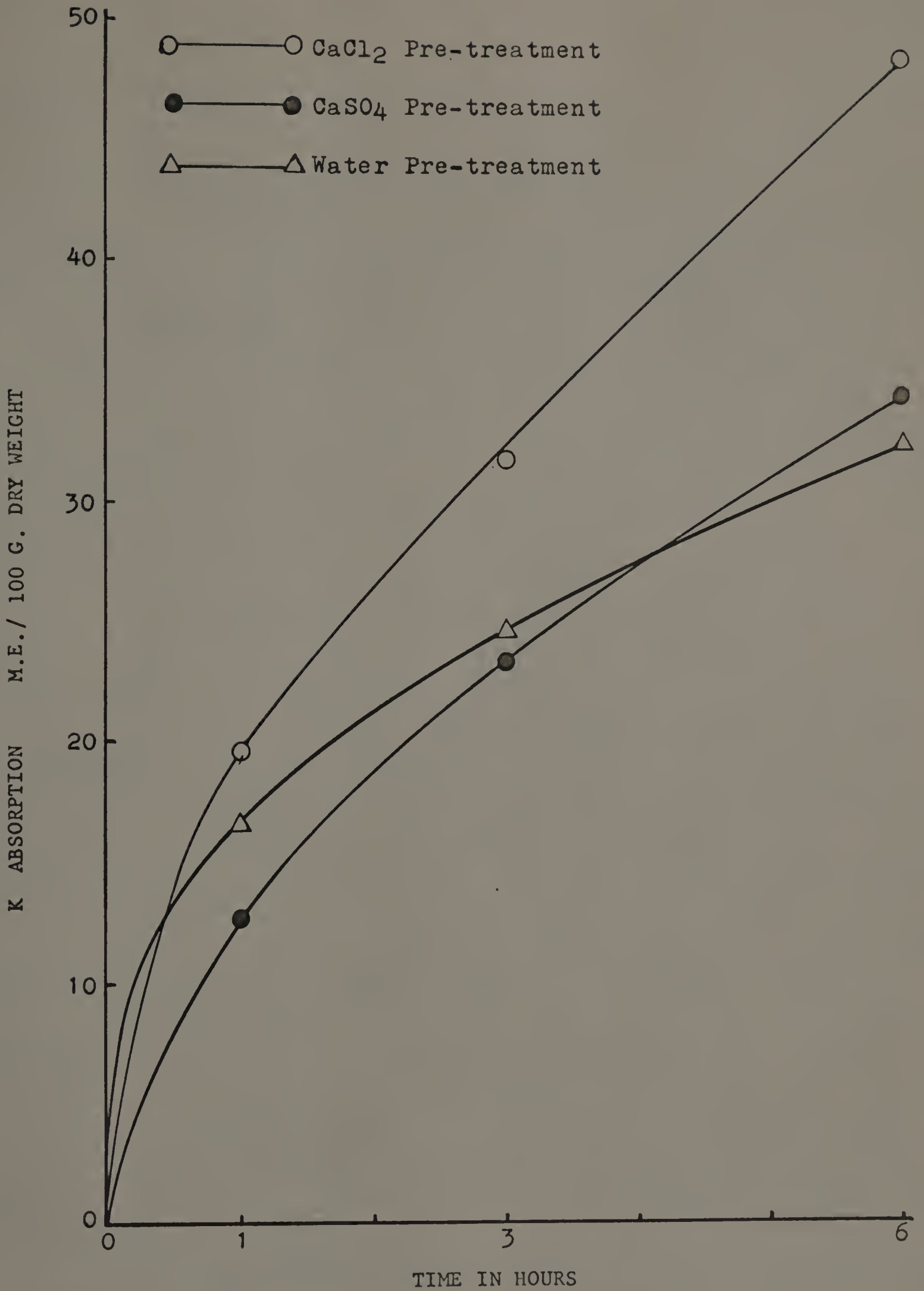


Fig. 2. Effect of pre-treatment for 3 hours with water, $10^{-2}N$ -CaSO₄, and $10^{-2}N$ -CaCl₂ on the absorption of K from $5 \times 10^{-3}N$ -K₂SO₄.

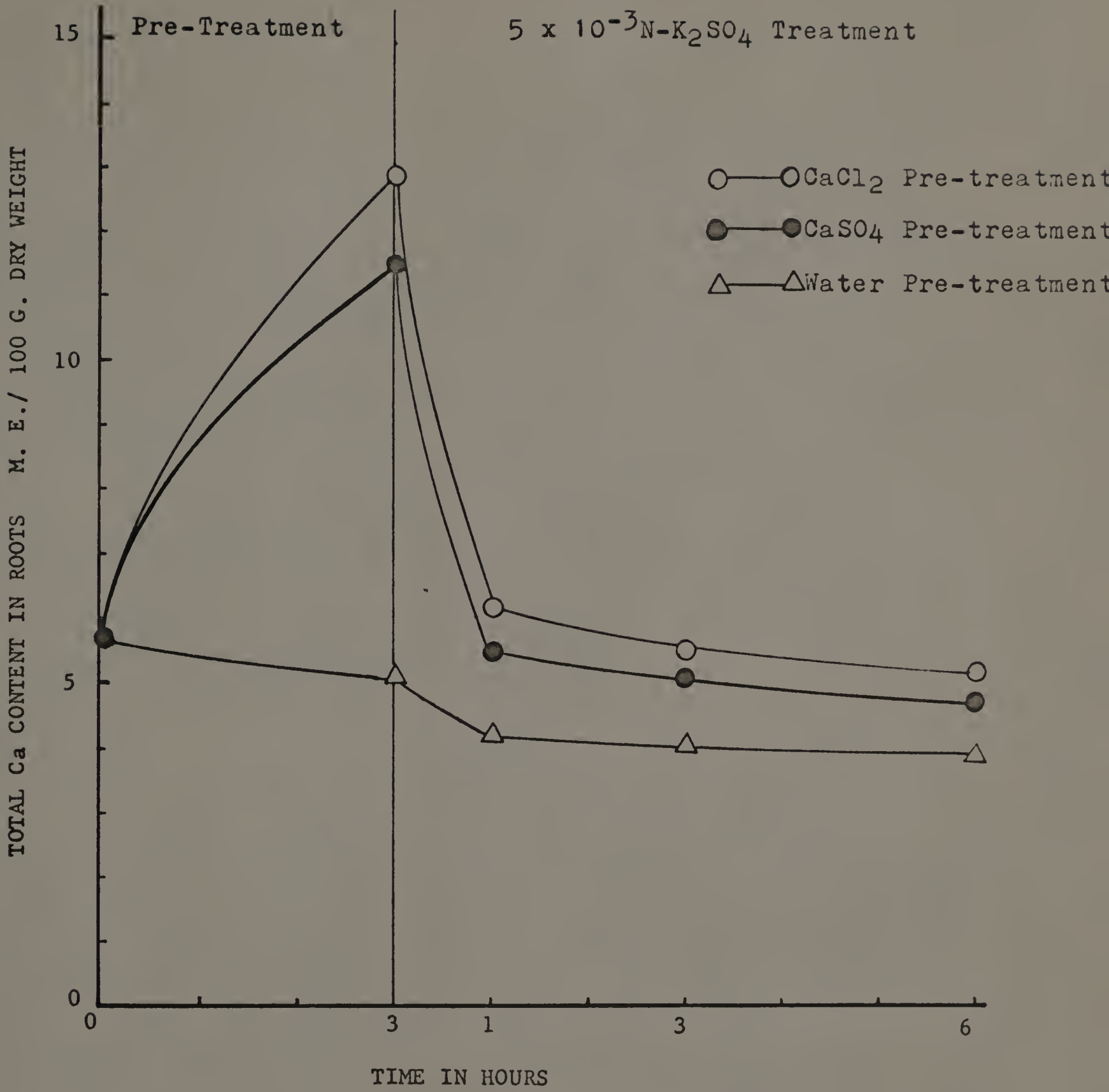


Fig. 3. Variation in Ca content in barley roots.

to the non-metabolically absorbed Ca, there is no stimulating effect of the non-metabolic ion fraction of Ca salts on the K absorption, because the absorption of K by the roots pretreated with 10^{-2}N-CaSO_4 is lower than that pretreated with water in 1 hour and 3 hours. If the non-metabolic Ca fraction has some effects, the absorption of K by the roots pretreated with 10^{-2}N-CaSO_4 should be higher than that by the roots pretreated with water. That the K absorption after 1 hour and 3 hours by the roots pretreated with 10^{-2}N-CaSO_4 is lower than that by water-pretreated roots, but slightly higher in 6 hours seems to indicate that first, there is a depressing effect of Ca on K adsorption and it is also possible that K adsorption at the carrier sites may be reduced to some extent by the CaSO_4 -pretreatment. Secondly, SO_4 accumulated previously has some stimulating effect on the metabolic absorption of K. According to Moore et al. (50), the uptake of Ca by excised barley roots is largely non-metabolic. They could not measure the metabolic absorption of Ca in 3 hours at pH 5.0. On the other hand, the metabolic absorption of SO_4 in 2 hours at pH 4.0 has been reported by Epstein (11). Therefore, it is probable that the amount of excess SO_4 absorbed metabolically may stimulate the metabolic absorption of K. The effect of the anion is much more obvious in the case of 10^{-2}N-CaCl_2 pretreatment (Fig. 2). In the same manner K exchanges for Ca at the exchange sites and SO_4 substitutes for Cl in water free space (Fig. 3). However, the

amount of Cl absorbed metabolically in 3 hours of pretreatment is much larger than the amount of Ca absorbed metabolically (25,28,30). Apparently, the stimulating effect due to excess Cl in the inner phase exceeds the depressing effect of Ca, observed in CaSO_4 -pretreatment, within 1 hour. Consequently, metabolic absorption of K by the roots pretreated with 10^{-2}N-CaCl_2 is greater than in the other treatments. The hypothesis that the stimulating effect of Ca salts on the absorption of K is not caused by Ca, but the accompanying anion is able to explain not only a result in Fig. 2, but also that in Fig. 1.

(2) Effect of Ca salts on K absorption in initial phase of absorption.

The initial phase of absorption has been assumed to consist of non-metabolic exchange at the exchange sites of cell membrane, diffusion into apparent free spaces, exchange at exchange sites of carrier or ion-binding compound, and entry of ions inside of barrier (38). In this phase, at first, non-metabolic exchange and diffusion are predominant, although, according to Epstein (14), exchange at carrier sites and entry of ions occurs from the beginning of the absorption period. However when an equilibrium between the apparent free spaces and external solution is attained, and non-metabolic exchange sites are at equilibrium with the external solution, the initial phase will come to an end. Since, presumably, exchange sites of carrier are also at

equilibrium with the external solution by this period, a slower and rather steady absorption will follow. It was previously suggested that there is a depressing effect of Ca on K absorption and this depressing effect may work on the exchange sites of the root cell. In order to find out the Ca effect at the non-metabolic exchange sites, one experiment with 2,4-dinitrophenol (DNP) was conducted (Fig. 4). In this experiment, excised roots were pretreated with 10^{-5} M-DNP at pH 5.0 for 30 minutes in order that there should be no lag-period in the inhibition of the accumulation of ions over the initial stage (68,78). Following this pretreatment, absorption of Rb^{86} from Rb^{86}Cl , $\text{Rb}^{86}\text{Cl} + 10^{-4}$ M-DNP, and $\text{Rb}^{86}\text{Cl} + \text{CaCl}_2 + 10^{-4}$ M-DNP at pH 5.0 was measured. The pretreatment solution was diluted to 10^{-5} M so that the pH of the solution was 5.0. Concentration of RbCl and CaCl_2 were 5×10^{-3} N. At this level of DNP and pH, it has been previously observed with barley roots (26) and wheat roots (42) that salt accumulation is arrested although there is a slight respiration (59). It has been suggested that DNP attacks the phosphorylating system in the cell and this attack may very well lead to a destruction of the normal spatial arrangement of some cell structures in the cell (59). In this experiment, metabolic absorption of Rb^{86} from solutions with DNP is almost ceased. This cessation can be seen from the Rb^{86} absorption rate from $\text{Rb}^{86}\text{Cl} + \text{CaCl}_2 + \text{DNP}$, that is, the Rb^{86} absorption rate during the second 30 minutes (30 minutes -

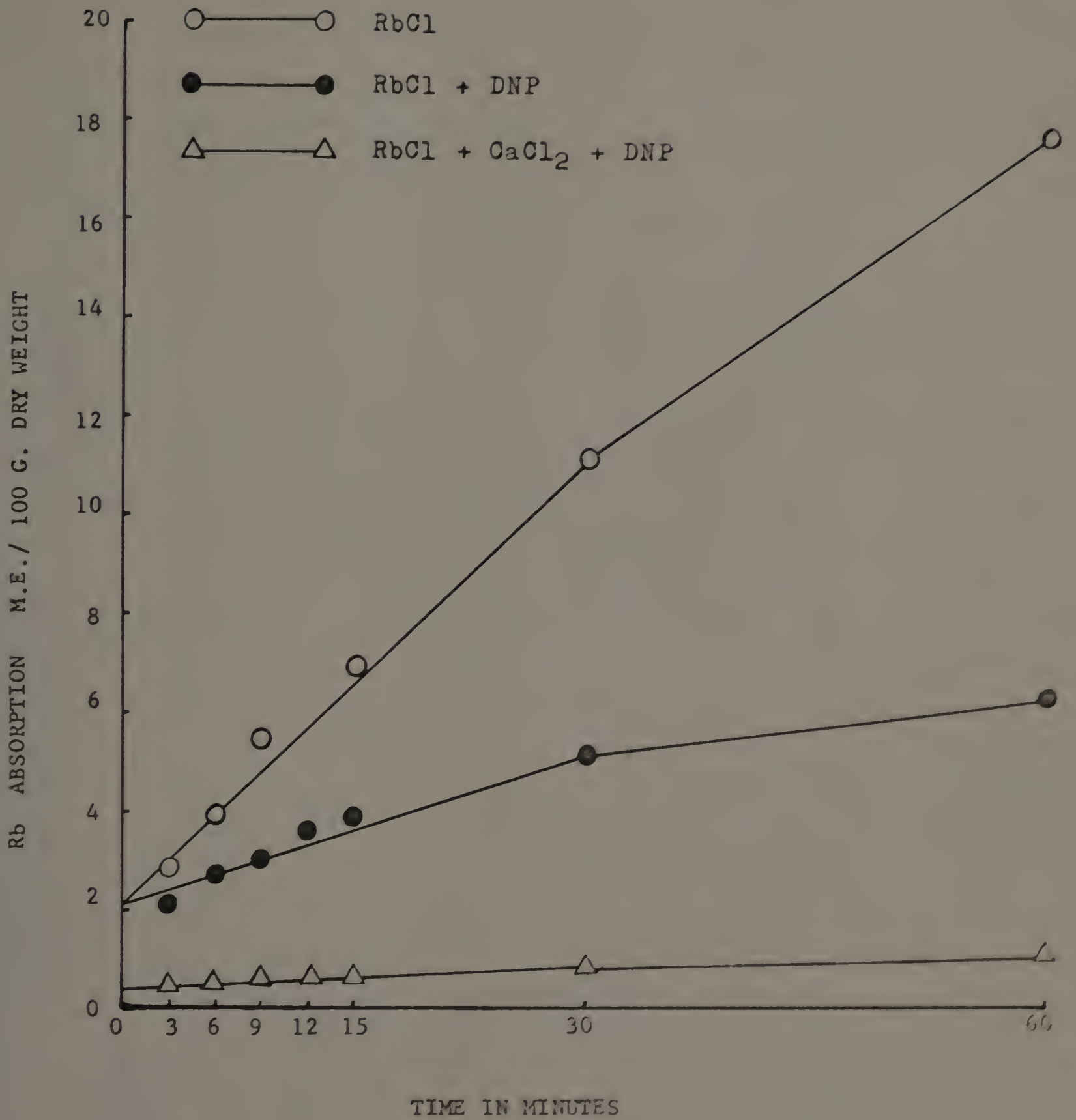


Fig. 4. Effect of DNP on Rb absorption.

60 minutes) is very slight. It is also obvious from the experimental data that, since Rb and K uptake is similar, the depressing effect of Ca on the absorption of K at the non-metabolic absorption sites is very large.

Although some investigators have tended to minimize the importance of cation uptake by exchange adsorption relative to diffusion (2,35,36), in this experiment much more Rb was absorbed passively by exchange than by diffusion. The amount of Rb^{86} absorbed from $\text{Rb}^{86}\text{Cl} + \text{CaCl}_2 + \text{DNP}$ is very low. This finding indicates that not only metabolic absorption is stopped by DNP, but also that the amount of Rb^{86} absorbed through the diffusion mechanism is very small. Even if the amount of Rb^{86} adsorbed at exchange sites is zero, although this is unlikely, Rb^{86} absorbed through diffusion mechanism is only 1.2 me./100 g. dry weight within 60 minutes (Fig. 4). Since some adsorption occurs, diffusion of Rb^{86} must be less than 1.2 me./100 g. dry weight. Also, the amount of Rb^{86} absorbed from $\text{Rb}^{86}\text{Cl} + \text{DNP}$ is about 5 times as much as that from $\text{Rb}^{86}\text{Cl} + \text{CaCl}_2 + \text{DNP}$. Since the concentrations of Rb^{86}Cl is the same in both solutions, the amount of Rb^{86} absorbed through diffusion should be the same. Thus most of the Rb^{86} ions absorbed non-metabolically from $\text{Rb}^{86}\text{Cl} + \text{DNP}$ are believed to be adsorbed by exchange. Consequently, since uptake of Rb and K are similar (5,48), the depressing effect of Ca on K absorption at the non-metabolic absorption sites is mostly through the exchange mechanism.

The effect of CaCl_2 and CaSO_4 on Rb^{86} absorption during the initial 15 minutes is shown in Fig. 5. The concentration of RbCl , CaCl_2 , and CaSO_4 is $5 \times 10^{-3} \text{N}$. When CaSO_4 or CaCl_2 is added to Rb^{86}Cl , Rb^{86} absorption is depressed equally. Within the initial 15 minutes, there is no difference between absorption rates of Rb^{86} from $\text{Rb}^{86}\text{Cl} + \text{CaCl}_2$ and $\text{Rb}^{86}\text{Cl} + \text{CaSO}_4$ (Fig. 5). Jackson et al. (26) reported that K absorption from 10^{-5} to 10^{-3}N-K with SO_4 , NO_3 , and PO_4 within 15 minutes showed no significant difference from Cl . The result shown in Fig. 5 agrees with their report. This result indicates that it takes at least fifteen minutes for the anion to affect the cation absorption and the depressing effect in initial phase is due to Ca at the exchange sites of root cell. Although the relationship between adsorption and absorption has been studied by many investigators (14,16,18,20,24,32,37,44,47,57,58,65), significance of the adsorption process has been obscured because on the one hand the observable exchange reaction has been considered the first step in ion accumulation (16,18,20,24,32,44,57) while on the other hand it has been shown that the demonstratable exchange reaction is quite extraneous to the accumulation process (14,37). There may be exchange sites on cells in addition to those associated with carrier molecules. And exchange adsorption at the "non-metabolic" exchange sites and accumulation of cations would be expected to be two independent processes. However, adsorption at the carrier sites and accum-

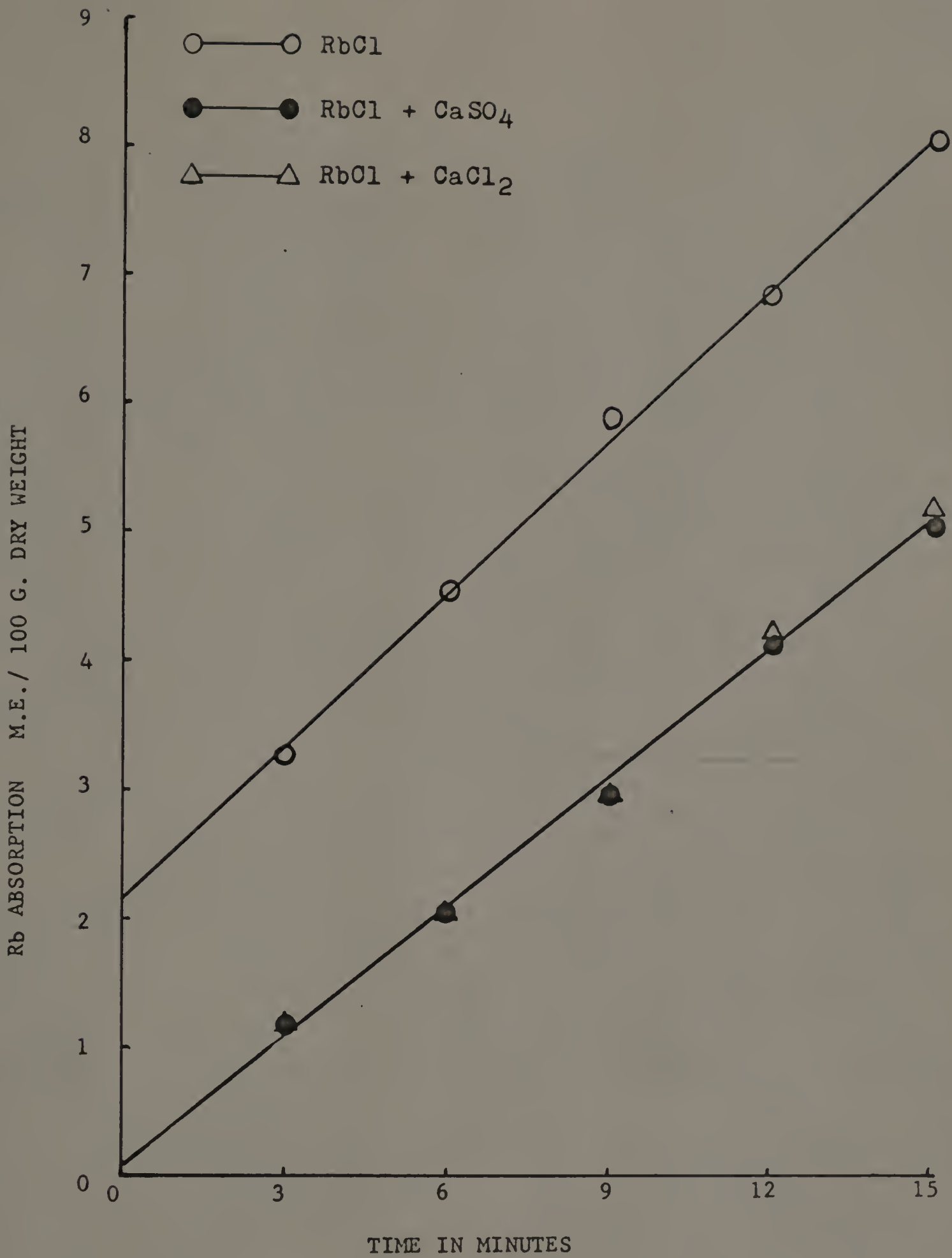


Fig. 5. Effect of Ca salts on the absorption of Rb.

ulation of cations may still be related.

Although Fried et al. (16) reported that the amount of Sr adsorption not associated with ion-carrier complex MR is negligible, most of Sr absorbed within 180 minutes from 10^{-4} - 10^{-2} M-SrCl₂ is non-metabolic (14). Therefore their conclusion is misleading. If the rate of Rb accumulation is related to the amount of Rb-carrier complex, the data presented in Fig. 5 indicate that most of the Rb is adsorbed at exchange sites not associated with carrier molecules.

However, whether the competitive effect of Ca on K and Rb absorption is conducted at the exchange sites of carrier or ion-binding compound is not clear. Franklin (15) reported that there are two kinds of exchange sites on root cell and that ions are removed easily from one site but with difficulty from the other site. He found that the exchangeable ions removed easily are not correlated to uptake while those removed with difficulty are correlated to uptake; R values range from 0.879 to 0.979. His results (15) and Fig. 4 and 5 suggest that the exchange sites of carrier or ion-binding compound may favor K specifically or the mode of exchange at the carrier sites may not be the same as that at the "non-metabolic" exchange sites which is the same as an exchange resin (14). That is, even if the exchange sites of the carrier can adsorb Ca, the adsorbability of Ca may be much lower than that of K.

(3) Effect of Cl on the absorption of K from KCl + CaCl₂.

The time course of Rb^{86} and Cl^{36} absorption from Rb^{86}Cl , RbCl^{36} , $\text{Rb}^{86}\text{Cl} + \text{CaCl}_2$, and $\text{RbCl}^{36} + \text{CaCl}_2^{36}$ in short time intervals is shown in Fig. 6. Concentration of each solution is $5 \times 10^{-3}\text{N}$. From this figure, it is apparent that the amount of Cl^{36} absorption from $\text{RbCl}^{36} + \text{CaCl}_2^{36}$ is considerably higher than from RbCl^{36} alone over the whole range of the time course study. On the other hand, as shown in Fig. 5, the rate of Rb^{86} accumulation is practically the same in the presence and absence of CaCl_2 during the first 15 minutes. As shown in Fig. 6, the rate of Rb^{86} uptake from RbCl alone decreases sharply after about 40 minutes, but in the presence of CaCl_2 the rate decreases slightly after about 50 minutes of the experiment.

Hoagland (22) reported that accumulation of K from SO_4 solution was much smaller than from Cl, Br, or NO_3 solution, as though the rate of accumulation of the cation, or the equilibrium value attained, was markedly influenced by the mobility of the anion. Steward and Sutcliffe (61) reported that the absorption of anions and cations by barley roots is so closely related that an effect on one results in an effect on the other. Briggs et al. (3), based on Lundegardh's anion respiration hypothesis (43), suggested that if the anion is actively transported, the rate of the transport will tend to increase with the anion concentration, and hence the addition of further anion, whether accompanied by the same cation or a different one, will tend to increase the rate of accumulation of both cations and anions.

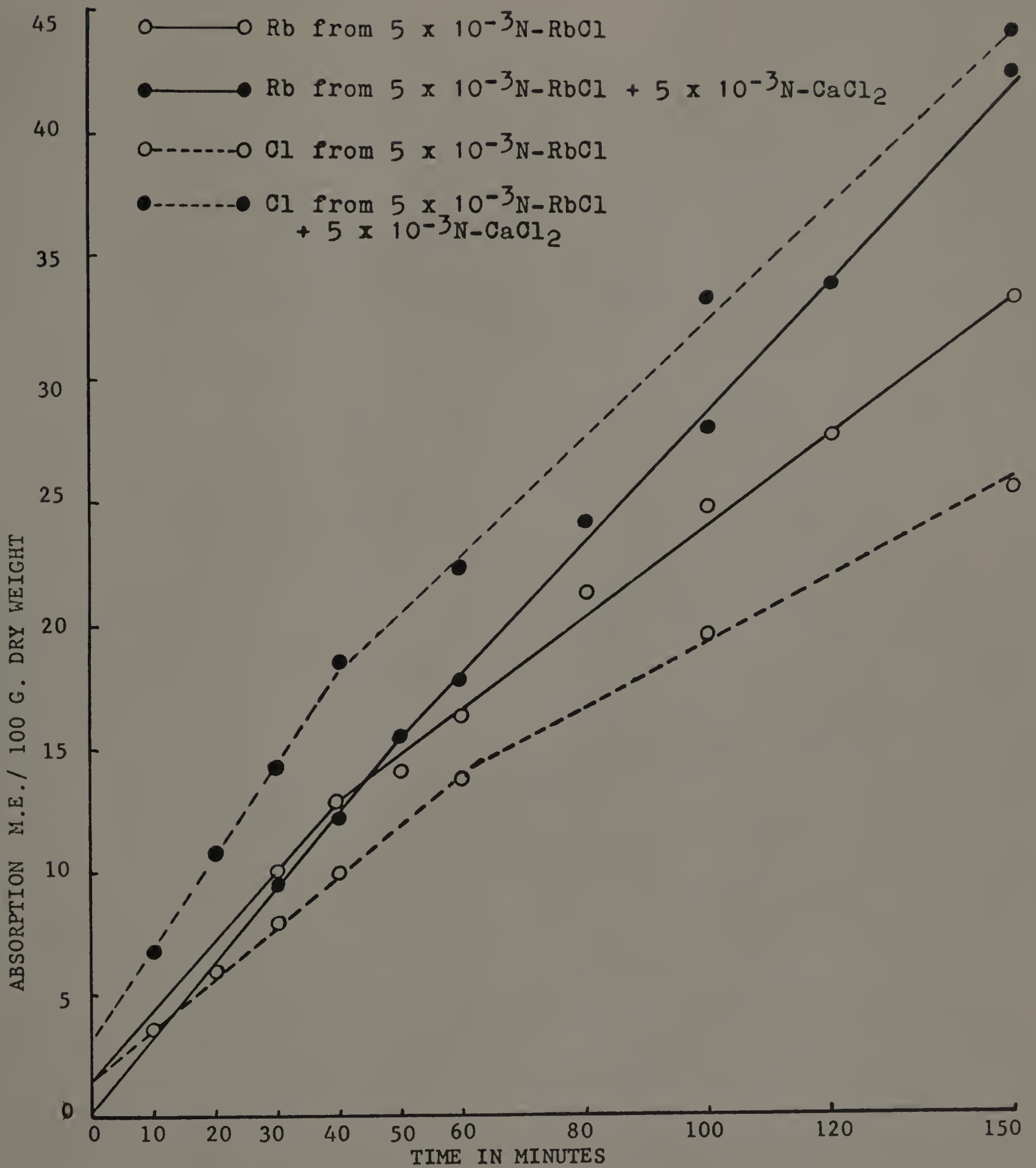


Fig. 6. Time courses of Rb and Cl absorption during 150 minutes.

Since it has been proved that effect of Ca is not a stimulating but a depressing one, it is believed that the increase in Cl absorption from $\text{RbCl}^{36} + \text{CaCl}_2^{36}$ compared with that from RbCl^{36} alone results in the increased absorption of Rb from $\text{Rb}^{86}\text{Cl} + \text{CaCl}_2$ compared with Rb^{86}Cl alone.

The effect of Cl on the absorption of K was further investigated in an experiment which was conducted over 9 hours (Figs. 7, 8, and 9). In this experiment, absorption of K and Cl from KCl alone, $\text{KCl} + 5 \times 10^{-3}\text{N-CaCl}_2$, and $\text{KCl} + 2.5 \times 10^{-2}\text{N-CaCl}_2$ at 1, 2, 3, 6, and 9 hours were determined. Concentration of KCl was always maintained at $5 \times 10^{-3}\text{N}$. As Cl concentration in external solution increases, Cl absorption increases and K absorption also increases but to a lesser extent than the increase in Cl absorption. It appears that the K absorption is almost parallel to the Cl absorption after 2 hours from $5 \times 10^{-3}\text{N-KCl}$, after a certain time between 2 hours and 3 hours from $5 \times 10^{-3}\text{N-KCl} + 5 \times 10^{-3}\text{N-CaCl}_2$, and after 3 hours from $5 \times 10^{-3}\text{N-KCl} + 2.5 \times 10^{-2}\text{N-CaCl}_2$. Thus it is indicated that the increase in Cl absorption from $5 \times 10^{-3}\text{N-KCl} + 5 \times 10^{-3}\text{N-CaCl}_2$ or $5 \times 10^{-3}\text{N-KCl} + 2.5 \times 10^{-2}\text{N-CaCl}_2$ compared with that from $5 \times 10^{-3}\text{N-KCl}$ results in the increased absorption of K.

Data which have been presented in Figs. 5, 6, 7, and 8 are brought together in Fig. 10, a time course study of K and Cl absorption from $5 \times 10^{-3}\text{N}$ solution, and Fig. 11, absorption from $5 \times 10^{-3}\text{N-KCl} + 5 \times 10^{-3}\text{N-CaCl}_2$. Experimental

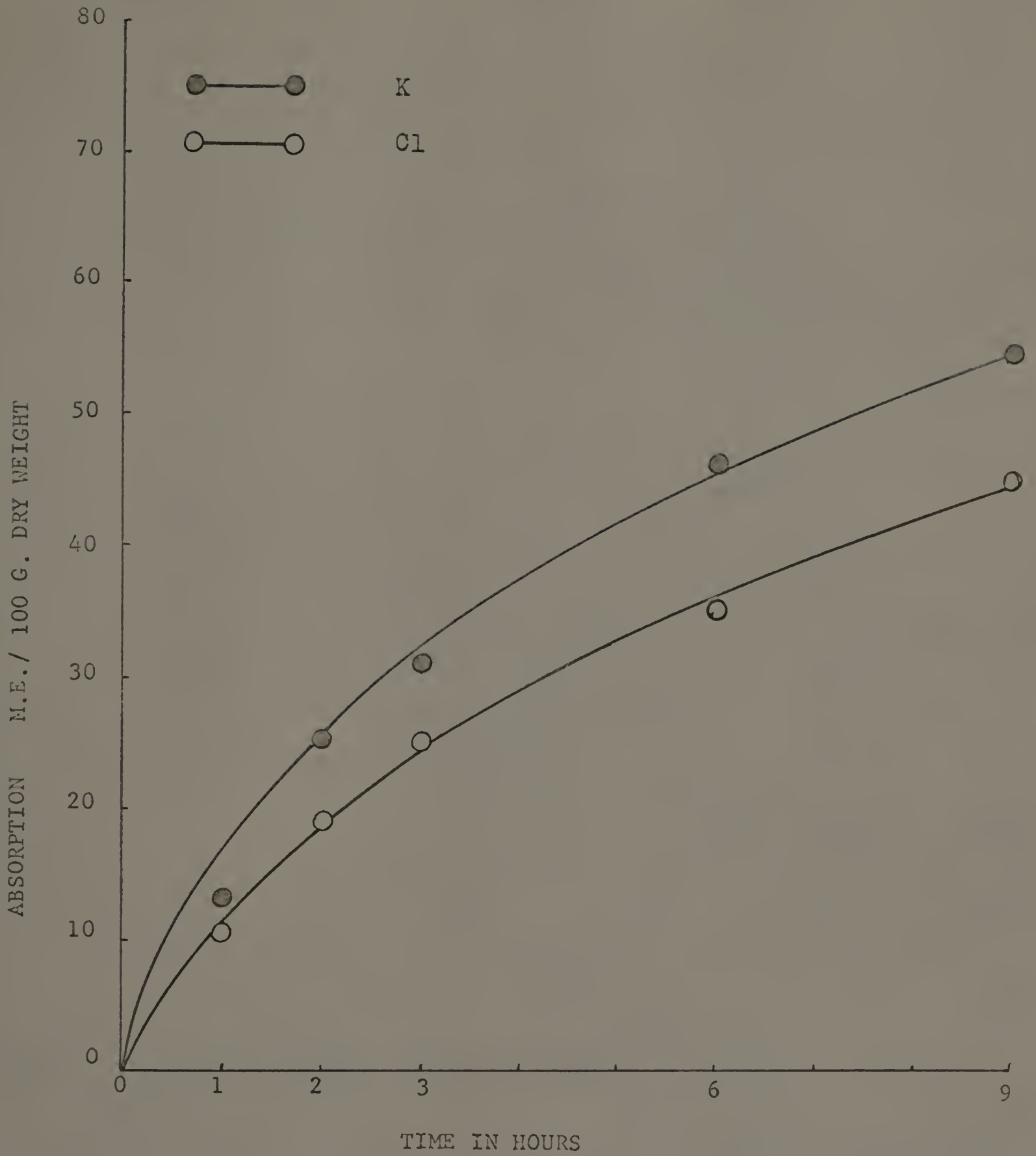


Fig. 7. Time courses of K and Cl absorption from $5 \times 10^{-3}N$ -KCl during 9 hours.

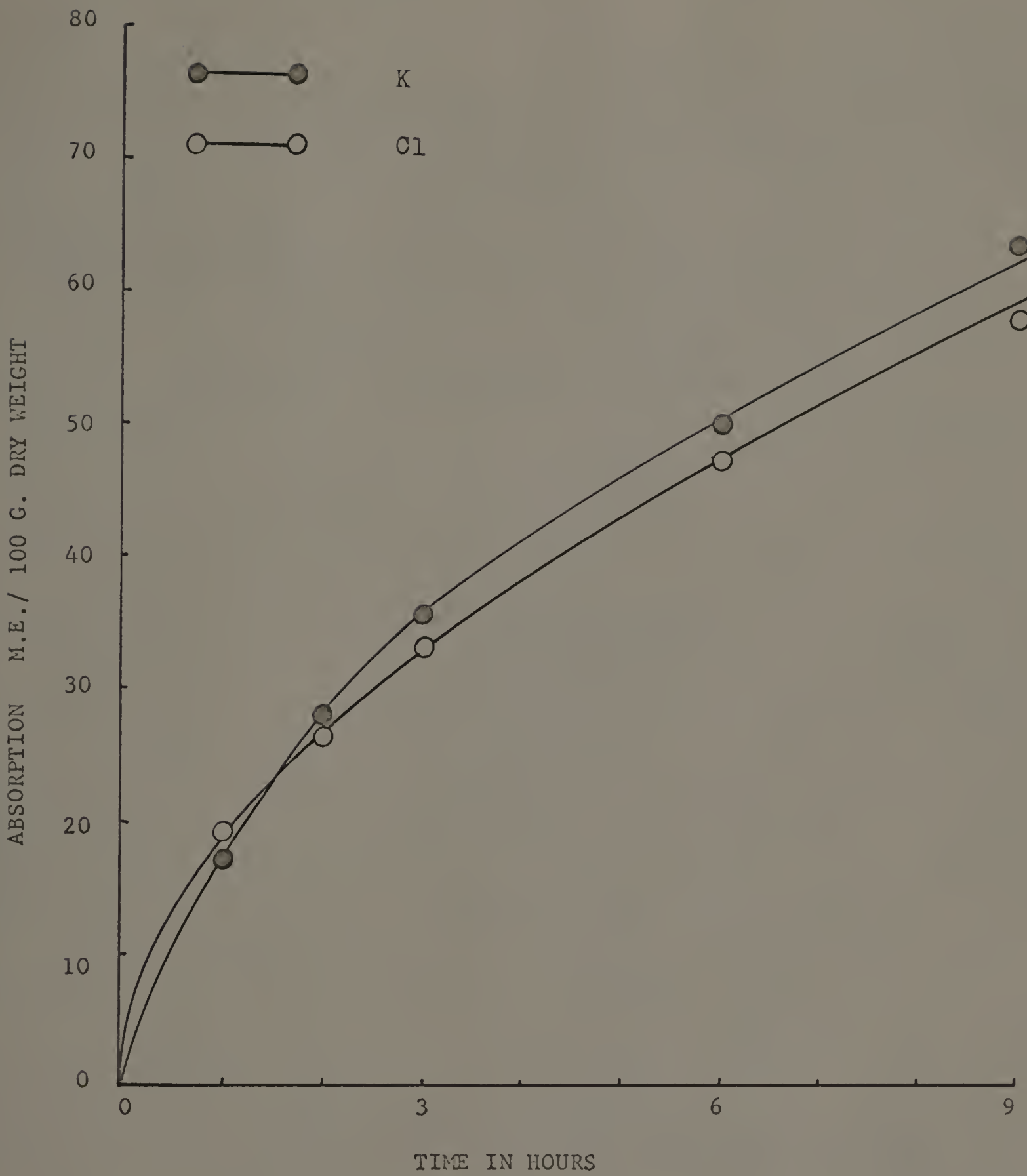


Fig. 8. Time courses of K and Cl absorption from $5 \times 10^{-3} \text{N-KCl} + 5 \times 10^{-3} \text{N-CaCl}_2$ during 9 hours.

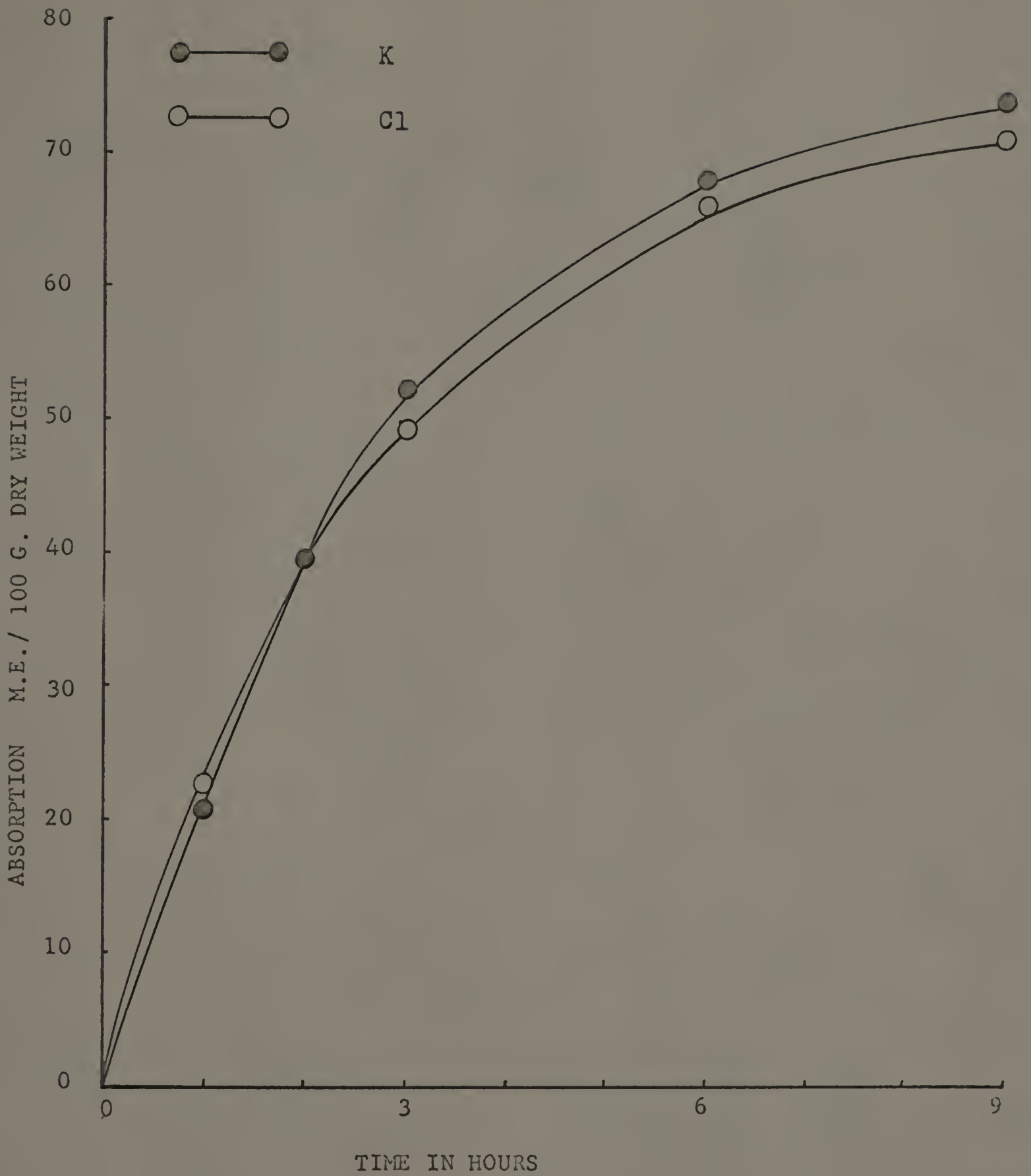


Fig. 9. Time courses of K and Cl absorption from $5 \times 10^{-3}\text{N-KCl} + 2.5 \times 10^{-2}\text{N-CaCl}_2$ during 9 hours.

results obtained from radioisotopic and non-radioisotopic experiments agree closely except for a few cases. It has been reported by many investigators (2, 4, 11, 14, 16, 24, 36, 37, 40, 44, 58) that there are two phases in the time course of absorption of ions, one being a relatively brief interval of rapid uptake, the other being a slower but more prolonged period of steady-state absorption. As discussed previously, sites of non-metabolic absorption will be almost filled up by the end of the initial phase. However, it is suggested from Figs. 9, 10, and 11 that there are two phases in the latter phase, one being a transition phase in which K absorption is not parallel to Cl absorption, the other being a final phase in which K absorption rate is parallel to Cl absorption rate. From Figs. 6 and 10, it is recognized that the initial rapid phase of absorption comes to an end after about 30 - 40 minutes and the final phase is attained at about 120 minutes in $5 \times 10^{-3}\text{N-KCl}$ solution. In $5 \times 10^{-3}\text{N-KCl} + 5 \times 10^{-3}\text{N-CaCl}_2$ solution, the initial phase comes to an end at about 50 - 60 minutes (Figs. 6 and 11) although exchange sites of the root cell must have been saturated by K and Ca by some time before 40 minutes. This prolongation in the initial phase would be caused by the increase in Cl absorption. The final phase in this solution is attained at a certain time between 120 and 180 minutes. In $5 \times 10^{-3}\text{N-KCl} + 2.5 \times 10^{-2}\text{N-CaCl}_2$ solution, the initial phase continues up to at least 120 minutes as for the same

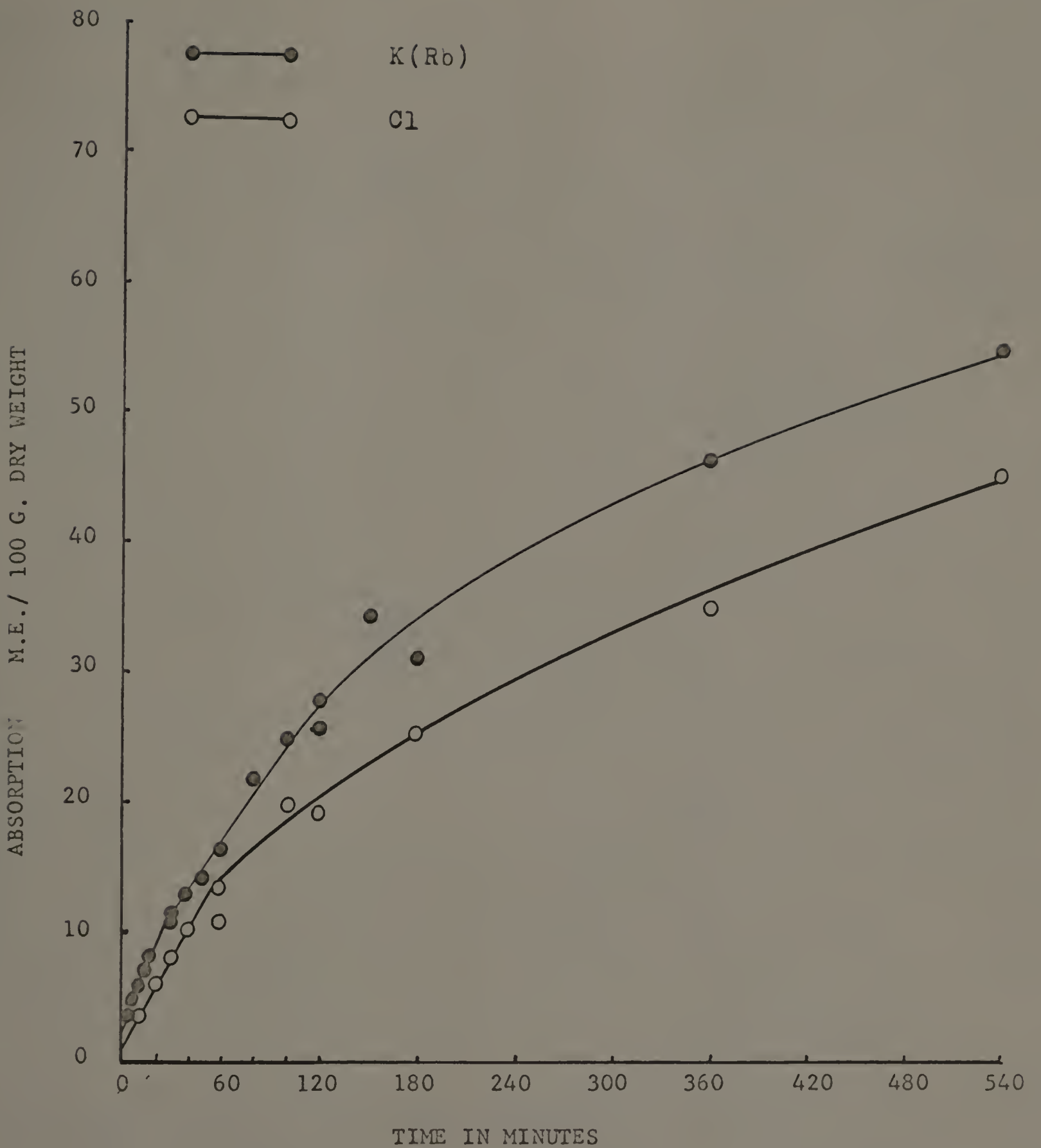


Fig. 10. Time courses of K(Rb) and Cl absorption from $5 \times 10^{-3} \text{N-KCl(RbCl)}$ during 9 hours. This figure is derived from Figs. 5, 6, and 7.

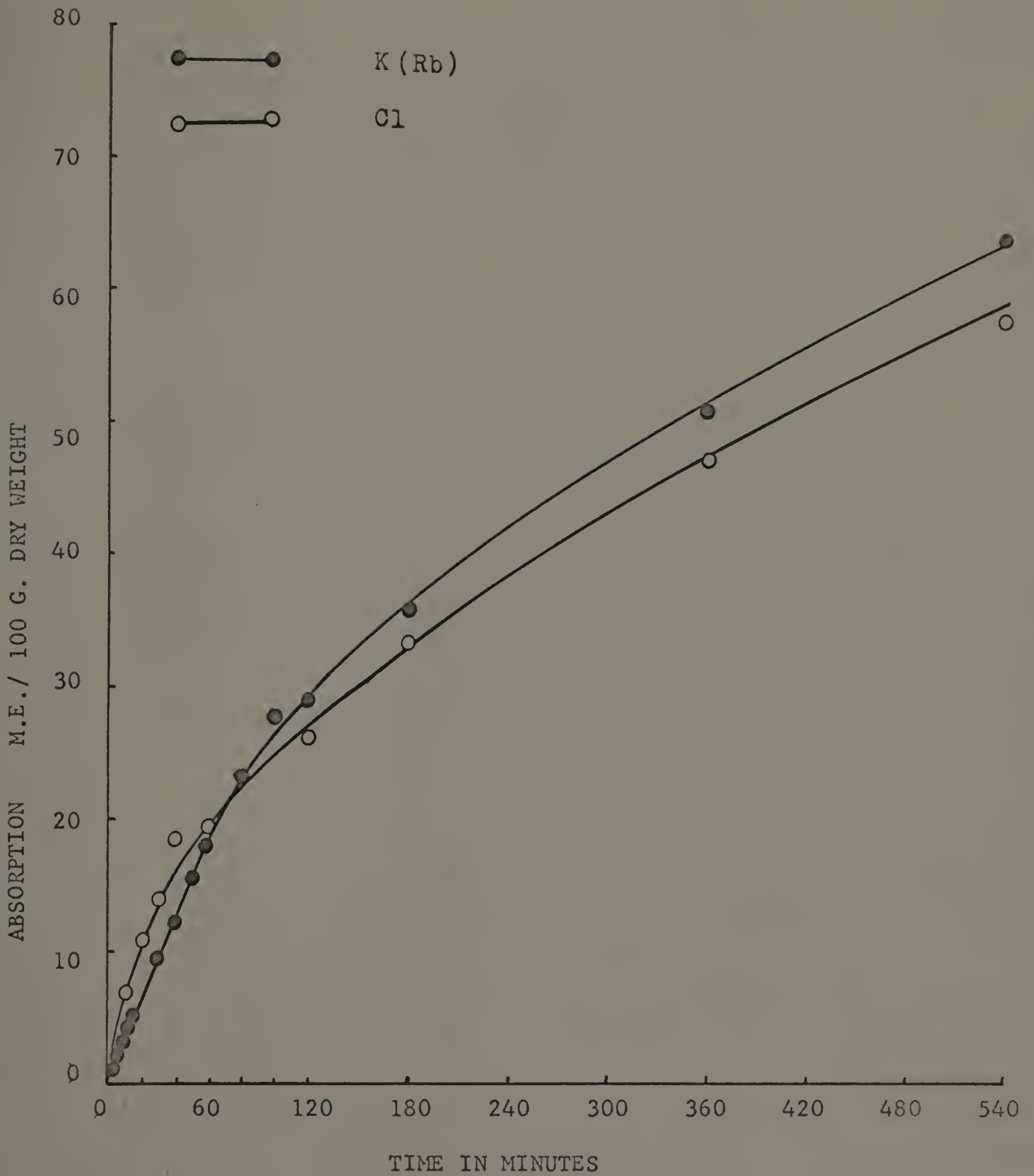


Fig. 11. Time courses of K(Rb) and Cl absorption from $5 \times 10^{-3}\text{N-KCl(RbCl)} + 5 \times 10^{-3}\text{N-CaCl}_2$ during 9 hours. This figure is derived from Figs. 5, 6, and 8.

reason previously stated. And the final phase is attained at about 180 minutes (Fig. 9).

It is instructive to compare the rates of Rb absorption in the different experiments. It is apparent from Fig. 5 that after the first 3 minutes the rates of absorption of Rb are constant and not greatly different in the presence and absence of Ca salts. The rate of Rb absorption during this time is $400 \mu\text{moles}/\text{min.}/100 \text{ g. dry weight}$ in the absence of Ca salt and $334 \mu\text{moles}/\text{min.}/100 \text{ g. dry weight}$ in the presence of $5 \times 10^{-3} \text{N-CaCl}_2$ or CaSO_4 . In the presence of DNP (Fig. 4), there is a rapid uptake of Rb during the first 3 minutes followed by a slow but continuous uptake, which is much more rapid in the absence than in the presence of CaCl_2 . As discussed previously, the continuous uptake of Rb in the presence of DNP is largely the result of exchange adsorption of Rb at the more inaccessible exchange sites. If Rb uptake in the presence of DNP is assumed to be completely physical, then the rate of metabolic accumulation of Rb can be calculated by subtracting the rate of uptake in the presence of DNP from the rate in the absence of DNP. If this calculation is done, the rate of metabolic accumulation of Rb is found to be about $320 \mu\text{moles}/\text{min.}/100 \text{ g. dry weight}$ in the presence and absence of Ca salts during the initial phase of absorption. However, it is shown in Fig. 12, that the rate of Rb uptake in the absence of CaCl_2 does not continue at this rate, but decreases after about 30

minutes. On the other hand, in the presence of CaCl_2 , the rate, about $320 \mu\text{ moles/min./100 g.}$ lasts for a longer time, about 50 - 60 minutes when the CaCl_2 concentration is $5 \times 10^{-3}\text{N}$ (Figs. 6, 11, and 12) and 120 minutes when the CaCl_2 concentration is $2.5 \times 10^{-2}\text{N}$ (Figs. 9 and 12). Thus the addition of CaCl_2 and consequent increase in Cl uptake apparently make it possible for the roots to maintain the first rate of Rb or K accumulation for a longer time.

(4) Relation of organic acid content in the root to the effect of Cl on the absorption of K.

It has been clearly indicated above that the stimulating effect of CaCl_2 on the absorption of K is caused by Cl, however, the mechanism of the stimulation of K absorption due to Cl is still unknown. The relationship between concentration of organic acid anion and that of inorganic ions in the plant cell has been studied (23,25,27,28,30,66,67). Ulrich (67) showed that when roots of barley were placed in a solution of K_2SO_4 , considerable K entered the cells, but the external concentration of the anion did not decrease appreciably. The K ions entering the cells were balanced by an increase in the organic acid anions so that the pH of the sap extracted did not change appreciably. When a salt with a divalent cation (CaBr_2) was provided in the external solution, the inorganic anion was absorbed in excess of the cation and the organic acid anion in the cells decreased with simultaneous increase in the respiratory quotient.

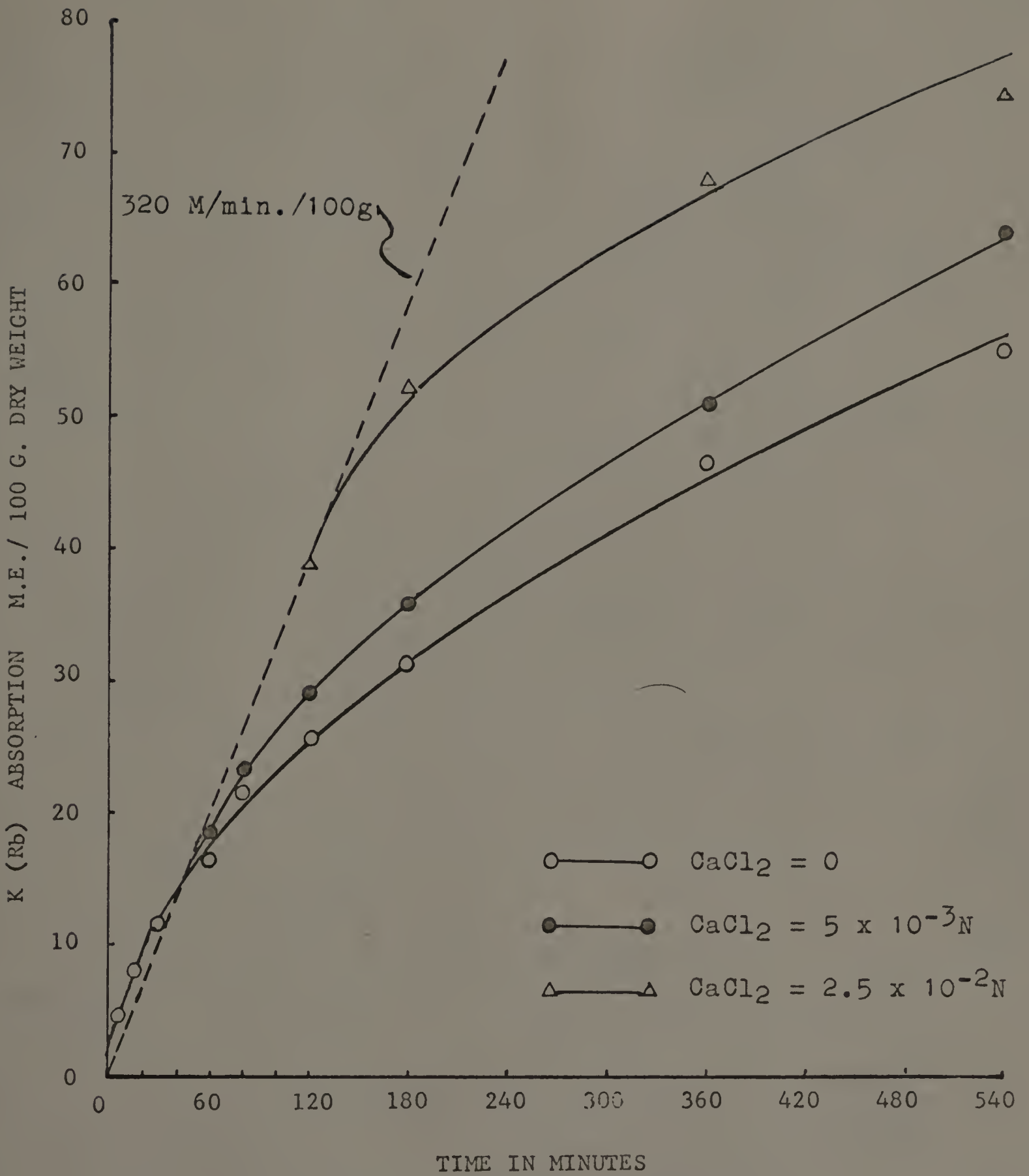


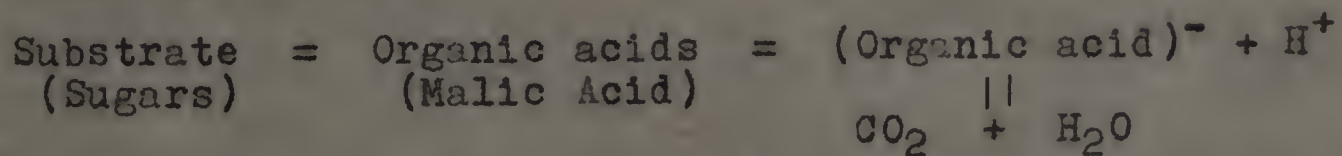
Fig. 12. Time courses of K(Rb) absorption from 5 x 10⁻³N-KCl(RbCl). This figure is derived from Figs. 5, 6, 7, 8, and 9.

Jacobson et al. (30) have confirmed this effect and reported that the production or decomposition of malate was the most important means of ion compensation. Hurd and Sutcliffe (25) suggested that the organic acid is lost in exchange for the excess Br absorbed from CaBr_2 , that is, exchange occurs between internal bicarbonate and external Br. The carboxylating reaction by which HCO_3 is converted to organic acids is likely to be reversible, and will have a definite equilibrium constant. If external anions exchange with HCO_3 present in the cell, organic acids will break down to renew the equilibrium. However, the concept of Briggs et al. (3) is more reasonable. They described the mechanism as follows. Just as the accumulation mechanism works on ions in the external solution which diffuse into the cell surface, so too the mechanism will be expected to accumulate, in the vacuole, ions produced in the cytoplasm by metabolic activity. This accumulation would keep the concentration of such ions from building up in the cytoplasm and prevent diffusion of the ions from the cytoplasm to the external solution. If the ion accumulation mechanism works in this way on anions, then the anions supplied in the external solution may be regarded as competing with the endogenous anions of metabolism. Since there is a reversible equilibrium between substrate (sugars) and organic acids in the formation of organic acids, the absorption of excess cations available from the external solution may increase the formation of

organic acids, and the presence of excess anions may decrease the amount of organic-acid anions.

As shown in Figs. 7, 8, and 9, when $5 \times 10^{-3} \text{N-CaCl}_2$ or $2.5 \times 10^{-2} \text{N-CaCl}_2$ is added to $5 \times 10^{-3} \text{N-KCl}$, the concentration of Cl in the external solution is increased in twice with $5 \times 10^{-3} \text{N-CaCl}_2$ or 6 times with $2.5 \times 10^{-2} \text{N-CaCl}_2$, as a result the Cl absorption increases. However the amount of the Cl absorbed from each solution does not increase in proportion to the increase in solution concentration because the accumulatory mechanism is limited in its capacity (3). As shown in Fig. 13, in $5 \times 10^{-3} \text{N-KCl}$ solution, organic acid content increases 7.5 m.e./100 g. dry weight compared with original roots during 3 hours. As the concentration of CaCl_2 added to the KCl increases, however, the rate of increase of the organic acid content in the root cell becomes smaller, 0.5 m.e./100 g. dry weight in $5 \times 10^{-3} \text{N-KCl} + 5 \times 10^{-3} \text{N-CaCl}_2$ during 3 hours.

It has been reported that there is a reversible equilibrium between substrate of organic acid formation and organic acids and between organic acids and CO_2 in the cell (3, 25, 27, 28, 30, 62).



Since the pH in the cell changes whenever the amounts of cation and anion absorbed metabolically are different, if the amount of cation absorbed metabolically is larger than

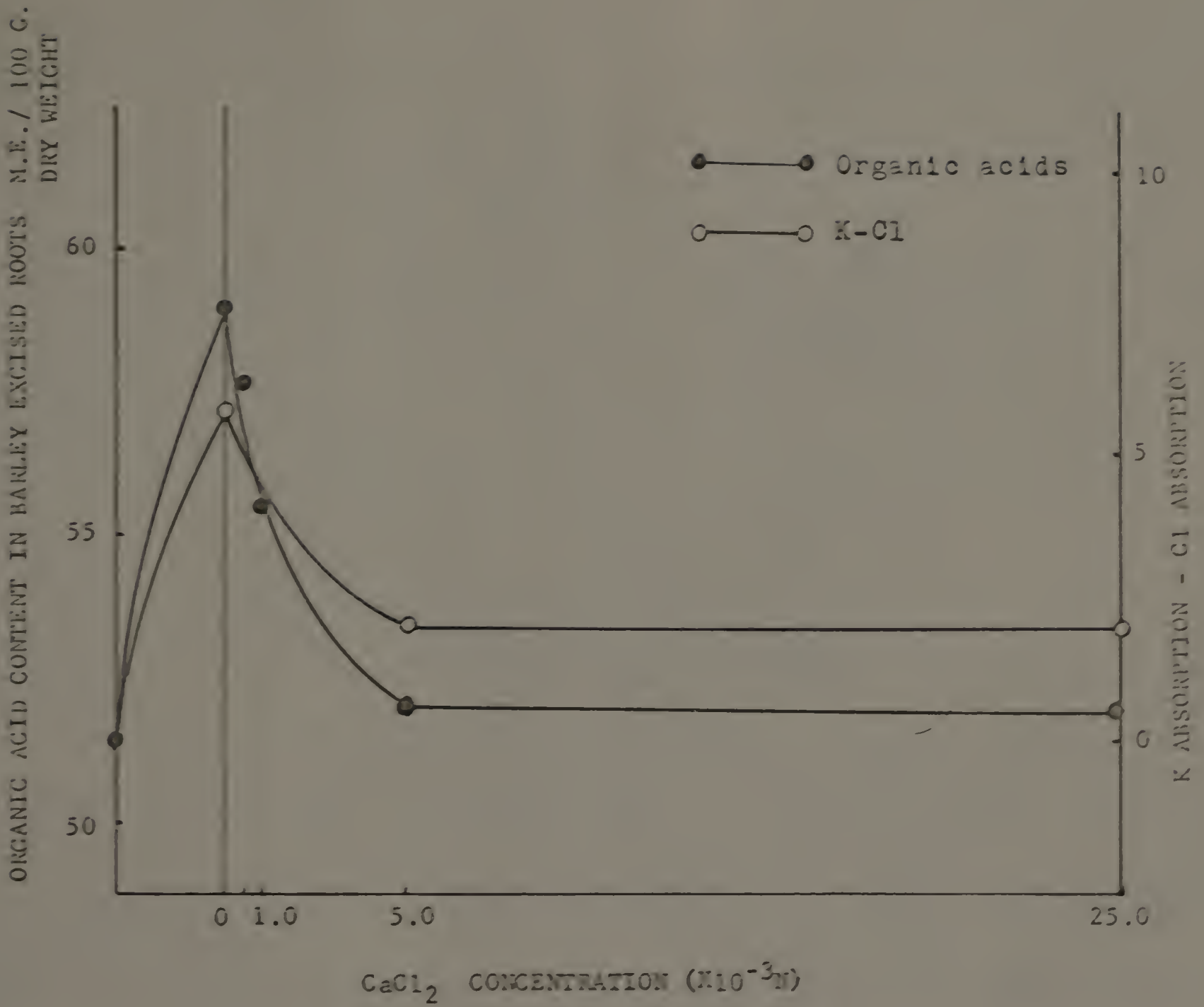


Fig. 13. Effect of CaCl_2 on the organic-acid content of excised barley roots after 3 hours in $5 \times 10^{-3}\text{N-KCl}$ solution.

the anion, organic acid formation will occur thus preventing the pH in the cell from rising. This is assumed to be the reason why organic acid content in the root increased 7.5 m.e./100 g. dry weight in $5 \times 10^{-3}\text{N-KCl}$ solution while it increases only 0.5 m.e./100 g. dry weight in $5 \times 10^{-3}\text{N-KCl} + 5 \times 10^{-3}\text{N-CaCl}_2$ solution during 3 hours.

Since the method (54) employed for determination of organic acid contents in the roots in this experiment is not sufficiently accurate, the increased amount of organic acids in the cell is not exactly equal to the difference between amounts of K and Cl absorption (Fig. 13).

Although it has been assumed that changes in organic acid content in the root cell is a result of difference between amounts of inorganic cation and anion in the cell, it is also assumed that organic acid in the root cell will have some regulatory effect on metabolic absorption of ions. Since the metabolic absorption of ions by roots in the initial phase is a rather rapid phenomenon, while changes in organic acid content is a much slower process (30), it is probable that when much more Cl is absorbed metabolically than K (Fig. 8, 9, 11), the organic acid content in the root will tend to decrease at a slower rate, consequently the initial rapid Cl absorption will be slowed down while the initial rate of K absorption continues for a longer time. Thus amount of K absorption exceeds that of Cl absorption after about 70 minutes in $5 \times 10^{-3}\text{N-KCl} + 5 \times 10^{-3}\text{N-CaCl}_2$ (Fig 11)

and after about 120 minutes in $5 \times 10^{-3} \text{N-KCl} + 2.5 \times 10^{-2} \text{N-CaCl}_2$ (Fig. 9). It is assumed that the amount of K-carrier complex (KR) limits the initial rapid but constant rate K absorption. When metabolic absorption of K exceeds that of Cl, organic acid formation in the root cell occurs. In the case of $5 \times 10^{-3} \text{N-KCl}$ alone (Fig. 7, and 10), metabolic absorption of K is larger than Cl from the initial time, therefore an increase in both Cl and organic acids occurs. Thus it is suggested that parallel absorption of K and Cl occurs when an equilibrium among substrate, organic acids, and CO_2 in the cell is attained.

VI. SUMMARY AND CONCLUSION

Effect of Ca salts on K absorption by excised roots of 6 day-old barley was investigated.

It was found that:

1. The stimulation effect of CaCl_2 on the absorption of K for 3 hours from K_2SO_4 and KCl was much greater than that of CaSO_4 (Fig. 1).
2. The stimulating effect of Ca salts on the K absorption was caused by a metabolically absorbed ion fraction (Figs. 2 and 3).
3. The depressing effect of Ca on the non-metabolic absorption of K was very large. This was mostly through the cation competition in the exchange mechanism (Fig. 4).
4. Most of the non-metabolic absorption was completed within 30 to 60 minutes (Fig. 4).
5. When CaSO_4 or CaCl_2 is added to Rb^{86}Cl , Rb^{86} absorption within 15 minutes is depressed identically. This result indicates that the accompanying anion does not affect the cation absorption within at least 15 minutes and the depressing effect in the initial phase is due to Ca at the exchange sites of the root cell (Fig. 5).
6. The amount of Cl absorption from $5 \times 10^{-3}\text{N-RbCl} + 5 \times 10^{-3}\text{N-CaCl}_2$ was considerably higher than from $5 \times 10^{-3}\text{N-RbCl}$ alone while the amount of Rb^{86} absorption from $5 \times 10^{-3}\text{N-RbCl} + 5 \times 10^{-3}\text{N-CaCl}_2$ became larger than from $5 \times$

10^{-3}N-RbCl alone after 45 minutes (Fig. 6).

7. The increase in Cl absorption from $5 \times 10^{-3}\text{N-KCl} + 5 \times 10^{-3}\text{N-CaCl}_2$ or $5 \times 10^{-3}\text{N-KCl} + 2.5 \times 10^{-2}\text{N-CaCl}_2$ compared with that from $5 \times 10^{-3}\text{N-KCl}$ alone resulted in prolongation of the initial rapid but constant rate of K absorption (Figs. 7, 8, and 12).
8. An absorption phase in which the absorption rate of K was equivalent to that of Cl was attained at about 120, 120-180, or 180 minutes in $5 \times 10^{-3}\text{N-KCl}$ (Figs. 6 and 10), $5 \times 10^{-3}\text{N-KCl} + 5 \times 10^{-3}\text{N-CaCl}_2$ (Figs. 6 and 11), or $5 \times 10^{-3}\text{N-KCl} + 2.5 \times 10^{-2}\text{N-CaCl}_2$ (Fig. 9) respectively.
9. In $5 \times 10^{-3}\text{N-KCl}$ solution, organic-acid content increased 7.5 m.e./100 g. dry weight compared with original roots during 3 hours. And as the concentration of CaCl_2 added to the KCl increased, the increase in the organic-acid content in the roots became smaller (Fig. 13).

As a result of these experimental results, it is concluded that effect of Ca salts on K absorption consists of two different effects, one being a depressing, competitive effect caused by Ca, the other being a stimulating effect, which is actually a prolongating effect of the initial rapid but constant rate of K absorption, due to accompanying anion.

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