

Salinity and the Pattern of Selective Ion Transport in Plants

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FOREWORD

This is the one hundred and sixty-first of a series of reports designed to present accounts of progress in saline water conversion with the expectation that the exchange of such data will contribute to the long-range development of economical processes applicable to large-scale, low-cost demineralization of sea or other saline water.

Except for minor editing, the data herein are as contained in the reports submitted by the University of California, under Grant No. 14-01-0001-264, covering research carried out through June 30, 1965. The data and conclusions given in this report are essentially those of the Contractor and are not necessarily endorsed by the Department of the Interior.

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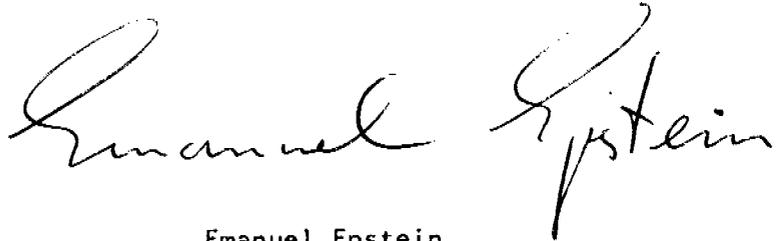
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The following persons participated with the author in this research:

Dr. R.L. Jefferies, Dr. W.E. Schmid, Dr. R.C. Smith, Mr. O.E. Elzam, and Mr. D.W. Rains. Mr. Elzam and Mr. Rains used results of this research in their graduate dissertations in partial fulfillment of the requirements for the degree of Doctor of Philosophy. This investigation was supported in part by the National Science Foundation. The University of California Lawrence Radiation Laboratory, Livermore, California, cooperated by making available potassium-42, through the courtesy of the Director, Dr. John S. Foster, Jr.

A handwritten signature in cursive script that reads "Emanuel Epstein". The signature is written in black ink and is positioned above the printed name and title.

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I. SUMMARY

Biological membranes and the mechanisms of ion transport across such membranes differ in fundamental respects from non-living membranes and their transport processes. In particular, biological membranes possess carrier mechanisms which transport ions against chemical and electropotential gradients, through the expenditure of metabolic energy, and do so with a high degree of selectivity. An investigation is reported on transport of potassium, sodium, and chloride by plant cell membranes.

When the rate of absorption of potassium by excised roots of barley is measured as a function of the external concentration of KCl, over the range 0.002 - 50 mM, a plot of the relation results in a complex isotherm as follows. Over the range 0.002 to 0.20 mM, the rate of absorption is a function of the concentration of potassium in strict conformance with Michaelis-Menten kinetics. The Michaelis constant (the concentration resulting in half the theoretical maximal rate) is on the order of 0.02 mM, and at 0.20 mM, the observed rate of absorption is about 90% of the theoretical maximal rate. The mechanism of absorption operating over this range of concentrations (membrane mechanism 1) is indifferent to the identity and rate of absorption of the anion; it is a mechanism of autonomous cation transport. The mechanism is highly selective, being insensitive to even large excess concentrations of sodium.

At concentrations of about 1 mM and above, a second mechanism of potassium transport comes into play. Its affinity for potassium is lower by two orders of magnitude or more than that of mechanism 1. It is severely inhibited when sulfate instead of chloride is the anion. Mechanism 2 is not highly selective for potassium but transports sodium as well. The isotherm depicting the rate of absorption of potassium and sodium over the range of concentrations where mechanism 2 comes into play (1-50 mM) shows several inflections which are interpreted as evidence that mechanism 2 possesses a number of transport sites differing in their affinities for any given cation. Absorption of chloride shows a similar dual pattern of transport.

Evidence is presented which indicates the generality of this complex pattern of ion transport in diverse plant tissues. Dual ion transport systems as traced in this investigation are briefly discussed in regard to their significance in regulating the salt economy of plants.

II. INTRODUCTION

As the need for usable water becomes more acute with the growth of the population and increased per capita consumption of water, attention will focus increasingly on irrigation agriculture which uses more water than any other single economic activity of man. Salinity represents the most pressing challenge in connection with irrigation in precisely those regions where irrigation is most important - the arid and semi-arid regions of the world. The research reported here is based on the premise that gains in our knowledge of the cellular mechanisms governing the salt economy of plants can contribute very significantly to the solution of problems posed by our need for gaining greater usable supplies of water.

The boundaries between the metabolic centers of plant cells and the solutions bathing the cells are the cell membranes. These membranes represent the structural and functional entities which confront the extra-cellular environment and regulate the flux of ions into and out of the cells. The mechanisms of ion transport residing in these membranes are by far the most sophisticated ion transport devices of which we have any knowledge. Basic research on these mechanisms is expected to make a dual contribution toward the solution of problems posed by saline water.

First, the biological membranes which effect ion transport and selective ion separations operate in a manner which is fundamentally different from the operation of man-made membranes. Synthetic membranes permit or restrict the passage of ions when existing chemical or electropotential gradients tend to induce a flux of ions across the membranes. Biological membranes, on the other hand, possess ion transport "carriers" or "pumps" which in many situations cause both cations and anions to move across the membrane against prevailing chemical or electropotential gradients. This means that these membranes are devices whereby metabolic energy is expended to do osmotic work. In doing this, the membranes often are highly selective or discriminating even between closely related ions such as potassium and sodium. Knowledge of these mechanisms may lead to new design principles to be incorporated into industrial analogs.

Second, research on biological ion transport may lead to more immediate and direct benefits involving the use of living plants. Most crop species demand water of high purity, with a salt concentration of not more than a few hundred parts per million. Yet there are many species of wild plants and microorganisms that cope amazingly well with concentrations of salt up to and exceeding the salt concentration of sea water. Gains in our knowledge of biological salt transport and toleration mechanisms may lead to techniques for rendering existing crop species more tolerant to salt, and supply background information for a genetic approach to breeding plants better able to cope with saline waters than existing plant species and

varieties. Use of salt-tolerant plants would permit the use of salinized water for irrigation, making non-saline water (either naturally existing or secured by desalination) available for those urban and industrial uses absolutely requiring water of high purity.

III. THEORETICAL

I. Metabolically "Active" Ion Transport

Non-living membranes permeable to ions permit the flux of ions according to the prevailing concentration and electropotential gradients. Given time, an equilibrium is established in which the activities of the ions on both sides of the membrane are equal.

Biological membranes, on the other hand, are capable of transporting ions against activity and electropotential gradients, the energy necessary for this "uphill" transport being furnished by metabolism. Ion transport across such membranes may continue for long times at steady rates, resulting in intracellular concentrations of the ions greater by several orders of magnitude than their concentrations in the solution bathing the cells.

Classical concepts of permeability are inadequate to account for these and other features of biological ion transport. Indeed measurements of permeability proper show many biological membranes, especially those of plant cells, to be rather impermeable to many polar solutes including inorganic ions.

The "carrier" concept has been invoked as a mechanism of selective ion transport across a membrane impermeable to free ions (7). According to this concept, the ion combines at the external side of the membrane with a carrier molecule which resides in and is part of the membrane, considered largely impermeable to the free ion (ion not combined with a carrier). Following this initial step, the carrier-ion complex migrates across the membrane, rotates within the membrane, or undergoes some other spatial rearrangement within the membrane as a result of which the ion is brought to the far side of the membrane. Through a change in the carrier molecule the ion is then released to an inner (transmembrane) compartment or space. Once there, the ion is not free to migrate back because of the impermeability of the membrane and the lack of affinity for the ion of the carrier when in the configuration it assumes at the inner surface of the membrane. That is, the carrier operates as a transport enzyme, shifting the ion from one side of the membrane to the other.

Specificity or selectivity in ion transport, according to this model, is the result of the existence of ion-specific or group-specific active sites of the carriers to which the ions are bound during their transport.

The kinetics of such carrier-mediated transport have been shown to correspond to classical enzyme kinetics (7). The enzyme-kinetic model of carrier-mediated, metabolically active transport, developed in connection with studies on ion transport in barley roots (8), forms the theoretical basis of the kinetic studies presented in this report.

2. Metabolic Transport vs. Exchange Adsorption

The structural cell wall of plant cells, as distinct from the functional cell membrane, represents a cation exchange material on which cations exchange reversibly and non-metabolically, as on any other cation exchanger. Adsorption of cations on the (mainly carboxylic) cation exchange sites of plant cell walls does not constitute absorption or transport in the sense discussed in the preceding paragraphs. Experimentally, it is necessary to take into account this non-metabolic exchange adsorption and to subtract this contribution from total ion uptake, so that only membrane transport proper will be measured (9, 13, 14, 20).

3. Cellular vs. Long-distance Transport

Membrane transport of ions at the cellular level is not the only transport phenomenon of interest in multicellular organisms, and specifically in higher plants. Ions having initially been absorbed by the cortical cells of the root are eventually delivered into the tubular, non-living conducting elements of the xylem tissue and translocated upward to the shoot. Once delivered into the extracellular space of leaves they are then absorbed by the leaf cells. In effect this means that ions absorbed by leaf cells have undergone at least two cellular transport processes: transport into cortical tissue of the root, and transport into a leaf cell. There is evidence that in many species, salinity exerts its most damaging effects on the metabolism of leaf tissue. In the absence of suitable technique, no previous work has been done on the kinetics and selectivity of ion transport in leaf tissue of terrestrial plants. These phenomena are among the problems included in the present studies.

4. Genetic Control of Selective Ion Transport

In both microorganisms and higher plants, selective ion transport has been shown to be under genetic control (6, 10). For example, there are mutants of the bacterium, Escherichia coli, specifically deficient in the potassium accumulating mechanism, and there is a strain of celery deficient in the capacity to absorb magnesium from media in which the concentration of magnesium in available form is low. In each of these cases, a single gene controls the specific ion transport mechanism, and there is no evidence

to the effect that the transport of any other ions is impaired. These findings confirm the conclusions from kinetic experiments in regard to ion transporting mechanisms which are specific for certain ions. The genetic control of selective ion transport demonstrated in several studies affords a powerful tool for the study of selective transport mechanisms, and for their manipulation in the interest of desired ion transport performances.

IV. MATERIALS AND METHODS

The experiments reported here were done mainly with roots of barley, Hordeum vulgare, and some other grasses. For experiments with barley roots, seeds of the variety Arivat are germinated in aerated water for 24 hours after which the seeds are planted over a 2×10^{-4} M solution of CaSO_4 which is aerated (4). Seedlings are grown in the dark for five days. At this point, the roots are about 12 to 15 cm long (fig. 1). The roots are then excised and 1.00-gm (fresh weight) samples are weighed out and enclosed in a "tea bag" made of cheesecloth with a very open weave. Before the absorption period proper, the samples are suspended in an aerated solution of 0.50 mM CaCl_2 or CaSO_4 maintained at the same temperature at which the roots will be kept during the absorption period (30°C).

The roots are then transferred to the aerated experimental solution containing the ion whose absorption is under study (the substrate ion) in radioactively labeled form. Potassium is labeled with K^{42} except in the experiment shown in fig. 8, labeled with Rb^{86} . Sodium is labeled with Na^{22} , and chloride with Cl^{36} . In addition to the salt of the substrate ion, the experimental solution contains 0.50 mM Ca, except when the effect of omitting it is being studied. The pH (unbuffered) is approximately 5.7. Figure 2 shows a root sample in the aerated experimental solution, and fig. 3 shows an array of experimental flasks in the temperature controlled water bath.

At the end of the absorption period, the tissue is lifted from the experimental solution and transferred to a cold solution, at about 8°C, containing the substrate ion at a concentration of 5 mM, unlabeled, and 0.50 mM Ca. Transfer into this solution instantly stops the absorption, by isotopic dilution, and serves to remove diffusible and readily exchangeable labeled substrate ions from the outer (extracellular) space of the tissue. This leaves for final radioassay only those labeled substrate ions that have been transported by metabolically active processes into the inner, i.e., trans-membrane spaces or compartments of the cells. Once there, the ions are not subject to ready isotopic exchange with exogenous ions (1, 2, 9, 13, 14, 16, 17). Radioassay is by counting with a thin-window, gas flow counter. The details of the experimental methods have been described (4, 14, 20).

V. RESULTS AND DISCUSSION

1. Absorption from Solutions of Low Salt Concentrations

When the rate of potassium absorption by barley roots is examined as a function of the external concentration of KCl or K₂SO₄, at concentrations up to 0.20 mM, the resulting absorption isotherm strictly follows Michaelis-Menten kinetics (fig. 4). The line is a plot of the Michaelis-Menten equation,

$$v = \frac{V_{\max} \cdot (S)}{K_m + (S)}$$

where v is the observed rate of absorption of the substrate ion present at concentration (S) , V_{\max} is the theoretical maximal rate of absorption at non-limiting external concentrations of the substrate ion, and K_m is the Michaelis constant - that concentration of the substrate ion resulting in a rate of absorption equal to one half the theoretical maximal rate. This conformance of absorption kinetics with classical enzyme kinetics is consistent with the hypothesis that transport entails the formation of a transient intermediate complex between the ion and a carrier or transport enzyme, as outlined in the Introduction.

Attention is drawn to the following two points. At an external concentration of 0.20 mM, the observed rate of absorption of potassium approaches to within about 10% of the theoretical maximal rate of absorption, and the rates of absorption of potassium are not appreciably influenced by the identity of the anion - the rates are essentially the same, whether the counterion is chloride or sulfate.

Figure 5 shows the results of a similar experiment on absorption of chloride. The actual rates and the calculated maximal theoretical rate are lower than these values for potassium absorption, but the Michaelis constant is similar and the kinetics are the same. As for potassium absorption, the theoretical maximal rate is closely approached, to within about 10%, at an external concentration of 0.20 mM. It is immaterial whether chloride is furnished as the potassium or the calcium salt.

The close correspondence between the kinetics of potassium absorption and those of chloride absorption naturally leads to the idea that the rate of absorption of the cation might be determined by the rate of absorption of the anion, or vice versa: anion absorption might be governed by cation absorption. However, the results do not support the conclusion that such tight coupling exists between cation and anion transport. In fig. 4, the absorption of potassium is shown to be indifferent to the identity of the anion, despite the fact that the rate of absorption of chloride is about

half the rate of absorption of potassium while the rate of sulfate absorption is extremely low, on the order of 1-2% of the rate of chloride absorption (16). Potassium absorption by the transport mechanism under consideration is therefore not mediated by the anion. Neither is the absorption of chloride governed by concomitant cation transport. In fig. 5, chloride absorption is shown to be independent of the cation, although potassium absorption proceeds at a rate about twice that of chloride absorption while the rate of calcium absorption is almost nil (5).

The evidence presented is to the effect that the kinetics of potassium and of chloride absorption are very similar. The Michaelis constants are similar, and both ions approach the theoretical maximal rate of absorption at an external concentration of 0.20 mM. Nevertheless, there is no close mutual dependence between potassium and chloride in regard to their absorption. Potassium shows these kinetics regardless of the anion, and chloride shows them regardless of the cation. In other words, we are dealing with autonomous potassium and chloride transport mechanisms.

That this is indeed so is brought out by yet another aspect of these transport mechanisms. Conformance with classical enzyme kinetics is not the only feature they share with enzymic processes. Another is specificity or selectivity. Figure 6 shows the results of an experiment on potassium absorption similar to that shown in fig. 4, except that it was done both in the absence and presence of sodium at 0.50 mM. Sodium is almost without effect on potassium absorption, even at the lowest potassium concentration where the sodium concentration exceeds that of potassium by a factor of 100. Even at much higher concentrations of sodium, potassium absorption by this mechanism is highly indifferent to sodium (4, 17).

These results are most readily accounted for by the hypothesis that just as enzymes have active sites to which the substrate is bound, so the carriers have active sites to which the ions are bound in the process of transport across the membrane, and that these sites are specific for certain ions or groups of closely related ions. For example, the potassium carrying sites of this transport mechanism make hardly any distinction between potassium and rubidium, but have very little affinity for sodium (4, 12). There are similar findings for chloride transport. In experiments resembling that shown in fig. 5, bromide has been found to compete with chloride, but the chloride-bromide transporting sites have very low affinity for fluoride and iodide as evidenced by the failure of these two ions to compete with chloride in the process of chloride absorption (2).

The properties of the mechanisms of potassium and chloride absorption discussed so far may be summed up as follows. Transport by these mechanisms strictly follows Michaelis-Menten kinetics, with Michaelis constants on the order of 0.02 mM. As expected for mechanisms with this Michaelis constant, the rate of absorption at 0.20 mM is about 90% of the theoretical

maximal rate calculated for non-limiting external concentrations. Both potassium and chloride are accumulated from solutions of very low concentrations; in other words, we are dealing with metabolically active transport. The mechanisms are highly specific or selective, and there is no close mutual dependence between the absorption of the cation and the anion of a salt. The mechanisms thus characterized shall be referred to as mechanisms 1 of potassium and chloride transport, respectively.

2. Absorption from Solutions of High Salt Concentrations

Membrane mechanism 1 was explored in experiments in which the salt concentrations did not exceed 0.20 mM. This concentration corresponds to 8 ppm potassium and 7 ppm chloride. These concentrations are entirely realistic for non-saline conditions, but much higher concentrations must be included in experiments in which the responses of biological transport mechanisms exposed to saline media are to be explored.

Figure 7 shows the results of an experiment on absorption of potassium by barley roots in which the concentration of KCl ranged from 0.002 mM to 50 mM. The left side of the figure covers the range of low concentrations, from 0.002 to 0.20 mM KCl. The solid line, continued by the dashed line, is a plot of the Michaelis-Menten equation, with the constants given. This part of the figure represents the operation of mechanism 1 discussed above (cf. fig. 4, 6).

The abscissa is broken between 0.20 and 0.50 mM, and the right half of the figure portrays the findings for the 0.50 to 50 mM range or concentrations. The dashed line represents the rate of absorption calculated by the Michaelis-Menten equation on the basis of the constants which apply to mechanism 1. At these high concentrations, the calculated line simply represents the maximal velocity for the low-concentration mechanism 1.

However, the observed rates of absorption at the high concentrations rise to values far above the maximal rate calculated on the basis of mechanism 1, indicated by the dashed line. This is considered evidence that at these high concentrations, a second mechanism of absorption comes into play. Mechanism 2 has been examined at concentrations up to 50 mM which is equivalent to 2000 ppm K, 1150 ppm Na, and 1775 ppm Cl. Mechanism 2 differs in important respects from mechanism 1.

The first and most obvious difference relates to the matter of affinity. Whereas membrane mechanism 1 of potassium transport operates at half-maximal rate at an external concentration of about 0.02 mM, and at about 90% of its maximal rate at 0.20 mM, mechanism 2 makes no appreciable contribution to potassium transport at 0.20 mM, and indeed makes very little of a contribution below 1.0 mM. At concentrations above 1.0 mM, the fraction of the

total observed rate of absorption assignable to mechanism 2 becomes appreciable, and at a potassium concentration of 50 mM, both mechanisms contribute about equally to the measured rate.

A second difference between the two mechanisms concerns the role of the anion. Substitution of sulfate for chloride as the anion makes no difference to potassium transport via mechanism 1 (fig. 4), but potassium transport by mechanism 2 is severely inhibited when sulfate instead of chloride is the counterion (fig. 8).

A third difference relates to selectivity. Potassium absorption mediated by mechanism 1 is unaffected or nearly so by sodium (fig. 6 and ref. 4, 12), but sodium severely competes with potassium in transport by mechanism 2 (12, 17). For example, at a potassium concentration of 50 mM, the rate of absorption in the absence of sodium was 26.5 μ mole per gram per hour, which in the presence of sodium at 50 mM was reduced to 8.0 μ mole per gram per hour - slightly below the maximal rate of absorption due to mechanism 1. In other words, sodium eliminates potassium transport by mechanism 2 but even at 50 mM it makes only a small inroad into potassium transport by mechanism 1 (cf. ref. 1, 4, 17, 21).

The findings concerning competition by sodium with potassium transport in mechanism 2 suggest that mechanism 2 may be a mechanism for sodium transport. Experiments in which the absorption of sodium was studied over a wide range of external sodium concentrations have confirmed this hypothesis (11, 17, 18). Figure 9 shows the results of an experiment in which the sodium concentration was varied from 0.005 to 50 mM, in the presence of 1.0 mM KCl as well as 0.50 mM CaCl_2 . At low concentrations of sodium (less than 0.20 mM), sodium absorption under these conditions is all but eliminated. Absorption at these low concentrations would have to be via mechanism 1, but inclusion of potassium at 1.0 mM concentration causes this mechanism to be preempted by potassium. In other words, mechanism 1 transports potassium at maximal rate, and is therefore unavailable for sodium transport: potassium acts as a selective inhibitor of sodium transport by mechanism 1.

At higher concentrations, on the other hand (above 0.20 mM), sodium is absorbed. At these concentrations, mechanism 2 comes into play, and this mechanism, unlike mechanism 1, has affinity for sodium as well as potassium. That is, in mechanism 2 sodium competes with potassium and is itself absorbed (11, 17, 18).

It has been found that mechanism 2 is heterogeneous (11, 17). The evidence is for a number of transport sites to which the ions are bound with slightly different affinities. Figure 10 shows the rate of potassium absorption as a function of the potassium concentration over the 1-50 mM range of concentrations. The lowest concentration is 1 mM at which

mechanism 1 operates at its maximal velocity, indicated by the dashed line. Over the high concentration range where mechanism 2 operates, the rate of potassium absorption does not rise as a smooth function of the potassium concentration but stepwise. The inflections in this isotherm are taken as evidence for a number of transport sites which are saturated one by one at progressively higher concentrations of potassium until, at 50 mM, a total of 4 sites appears to be involved in potassium transport by mechanism 2. Since mechanism 2 transports sodium as well as potassium it was predicted that sodium transport by this mechanism would show a similar heterogeneity, and this was found to be so (fig. 11).

Another way of showing this heterogeneity is to examine the rate of absorption of one of the alkali cations, present at a given, high concentration, as a function of increasing concentrations of the other alkali cation. Figure 12 shows that the diminution of the rate of absorption of sodium, present at 50 mM, is a complex function of increasing potassium concentrations. Conversely, when potassium is present at 50 mM and the concentration of sodium is progressively increased, the rate of potassium absorption decreases not as a smooth function of increasing sodium concentrations but stepwise (17). The findings are consistent with the interpretation suggested above, to the effect that transport via mechanism 2 involves the operation of a number of sites differing in their affinity for any given cation.

When absorption of chloride is examined over the extended range of concentrations, from 0.005 to 50 mM, the kinetics of this process show a similar complex pattern (fig. 13). The insert of the figure shows the low concentration range, up to 0.20 mM (cf. fig. 5). At these concentrations, mechanism 1 of chloride transport operates which has been characterized above in regard to kinetics and selectivity. At higher concentrations a second mechanism of chloride transport comes into play, and like mechanism 2 of alkali cation transport, it is heterogeneous (3).

3. Comparative Aspects

The results discussed so far were obtained in experiments with just one experimental object: roots of barley, Hordeum vulgare var. Arivat. It was desired to establish the pattern of selective transport of those ions of prime interest in the context of salinity, and to characterize these transport processes in their responses to salt concentrations ranging from very low levels to values so high as to fall in the category of saline conditions. Once the over-all pattern of kinetics and selectivity was established, work was initiated to extend this approach in a comparative manner in two directions.

First, experiments were done with leaf tissue. A technique was devised to by-pass the leaf epidermis with its impervious cuticle so that leaf mesophyll cells could be exposed directly to the experimental solutions (20). Second, experiments were done with a number of species and with different varieties of the same species to gain information on the comparative physiology and genetic basis of selective transport (10). These investigations, which will be continued, have already led to a number of conclusions in regard to the generality of the pattern of selective ion transport which has been traced in this investigation.

First, mechanism 1 of potassium transport is remarkably uniform in systems as widely disparate as root tissue of barley and leaf tissue of corn, Zea mays. The results of an experiment on potassium absorption by corn leaf tissue, over the concentration range 0.002 to 0.20 mM, are presented in fig. 14. They closely resemble those of figs. 4, 6, and 7 (left part) portraying potassium absorption by barley roots over the same range of concentrations - the concentrations at which mechanism 1 operates. In respect to selectivity, also, mechanism 1 of potassium transport in corn leaf tissue resembles that of barley roots. Specifically, sodium competes very ineffectively in potassium transport by this mechanism (21). The far-reaching correspondence between the characteristics of alkali cation transport in root tissue of barley and leaf tissue of corn leads to the conclusion that the biochemical entities effecting the transport (carriers), and their mode of operation, are identical or nearly so in the cellular membranes of these species (21).

Second, sodium transport in barley leaf tissue resembles sodium transport in barley roots, over the entire range of concentrations explored (fig. 15 and cf. fig. 9).

Third, comparisons of Michaelis constants for mechanism 1 of alkali cation transport by root tissue of different varieties of barley show reproducible differences which may be related to the relative salt tolerance or sensitivity of the varieties (15).

Fourth, two species of wheatgrass, Agropyron, which differ greatly in salt tolerance, show marked differences in respect to ion transport (1). Roots of A. elongatum, tall wheatgrass, which is quite salt tolerant, accumulate sodium from 0.20 mM NaCl at a rate several times greater than that for A. intermedium, intermediate wheatgrass, a less tolerant species. These and other findings suggest a link between salt transport, on the one hand, and salt toleration, on the other. Such a link is expected on theoretical grounds, since plant cells exposed to saline media must maintain intracellular osmotic pressures higher than those of the ambient solutions if they are not to be dehydrated by osmotic withdrawal of water.

Fifth, in species other than barley there is evidence for a membrane mechanism 2, with a number of transport sites possessing relatively low affinity for the ions and a low degree of selectivity (1, 15).

VI. CONCLUDING COMMENTS

This report traces a dual pattern of selective ion transport by plant tissue. Initial evidence for this duality was obtained in 1952 (8), but a definitive delineation of this pattern in all its complexity awaited the demonstration of the essentiality of calcium for selective ion transport (4, 19) and the application of improved technique for precise measurement of absorption rates both from extremely dilute and from relatively concentrated salt solutions, unobscured by non-metabolic, non-selective, reversible exchange adsorption unrelated to transport proper (14).

The pattern which emerges is a dual one, reflecting the operation of ion transport mechanisms with greatly different responses to salt solutions of low and high concentrations, respectively. At potassium concentrations of 0.20 mM or less, a potassium absorption mechanism operates which displays strict adherence to Michaelis-Menten kinetics. It has a high affinity for potassium, with a Michaelis constant of about 0.02 mM. Its affinity for sodium is very low, and it is independent of the identity and rate of absorption of the anion.

At much higher concentrations (1-50 mM), a second and very different absorption mechanism comes into play. Its affinity for alkali cations is lower than that of mechanism 1 by two orders of magnitude or more, it is not selective for potassium but carries sodium as well, is considerably influenced by the anion, and is itself heterogeneous. A similar dual pattern emerges from kinetic experiments on chloride absorption. This complex pattern has been observed in diverse plant tissues.

Evidence for these dual transport systems has come from short-term experiments with excised tissues. The question therefore arises how this evidence squares with what is known about the absorption of ions by growing plants, especially plants in saline media.

Such plants generally absorb potassium (the essential alkali metal) in preference to sodium, despite the wide ratio of the concentration of sodium to that of potassium prevailing under conditions of salinity. This evidence for preferential potassium transport and discrimination against sodium by growing plants parallels the observed properties of membrane mechanism 1 with its high, and highly selective, affinity for potassium vis-a-vis sodium ions. On the other hand, many general and indiscriminate mutual interactions among ions have been recorded in the literature. Such "antagonisms" would result from the operation of transport mechanisms with a low degree of selectivity, like mechanism 2 described in this report. Similar considerations apply to chloride transport. It is thus likely that dual ion transport mechanisms as traced in this investigation are generally involved in the regulation of the salt economy of plants.

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VIII. FIGURES



Fig. 1. Barley seedlings after growing for five days in 2×10^{-4} M CaSO_4 solution, in the dark. The roots are excised and used in short-term experiments at this stage.



Fig. 2. A 1.00 - gm (fresh weight) sample of excised roots enclosed in its "tea bag".

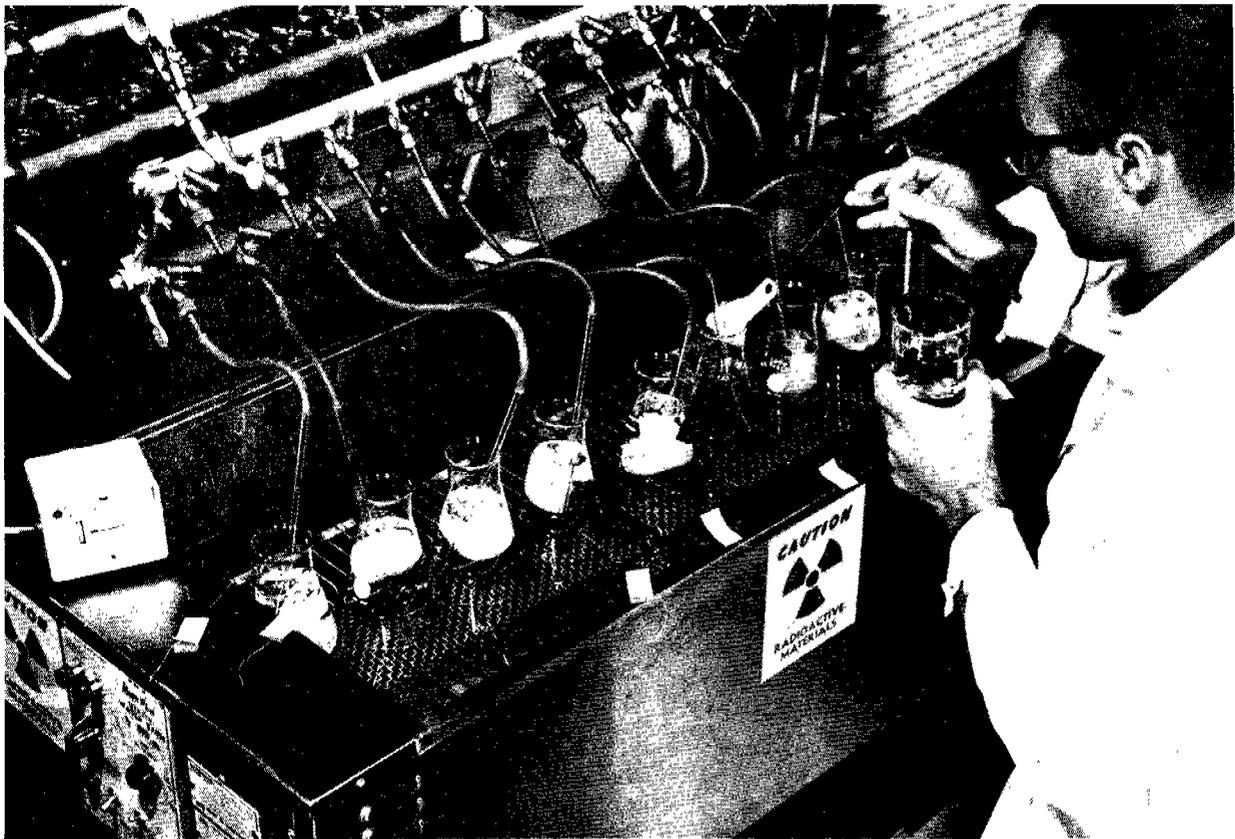


Fig. 3. An array of flasks with root samples, in the temperature-controlled water bath.

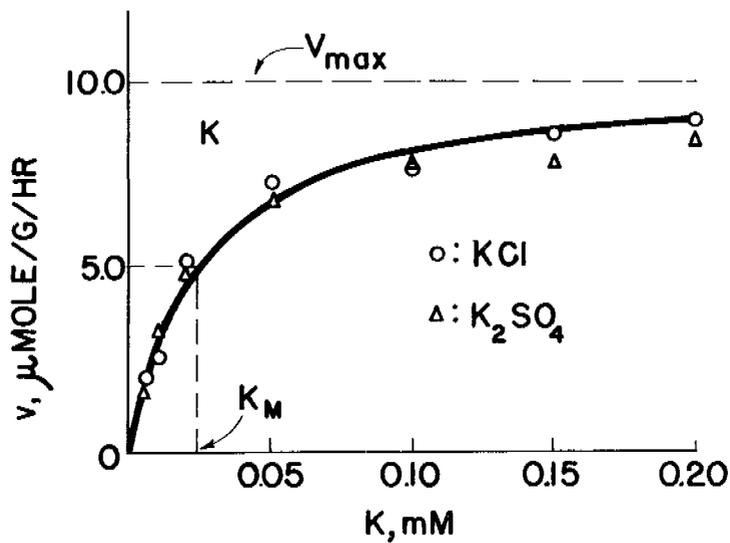


Fig. 4. Rate of absorption of K as a function of the concentration of KCl (○) and K₂SO₄ (△). CaCl₂ and CaSO₄, respectively: 0.50 mM. The line is a plot of the Michaelis-Menten equation, using the means of the KCl and K₂SO₄ values. K_m: 0.023 mM; V_{max}: 10.0 μmole/gm/hr.

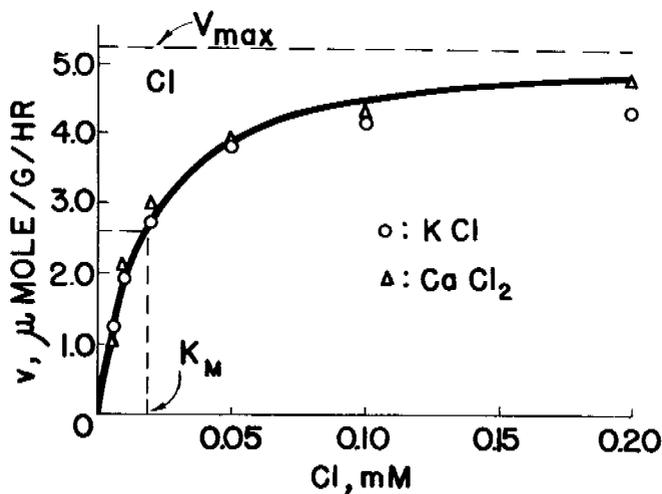


Fig. 5. Rate of absorption of Cl as a function of the concentration of KCl (○) or CaCl₂ (△). CaSO₄: 0.50 mM. The line is a plot of the Michaelis-Menten equation, using the means of the KCl and CaCl₂ values. K_m: 0.017 mM; V_{max}: 5.25 μmole/gm/hr.

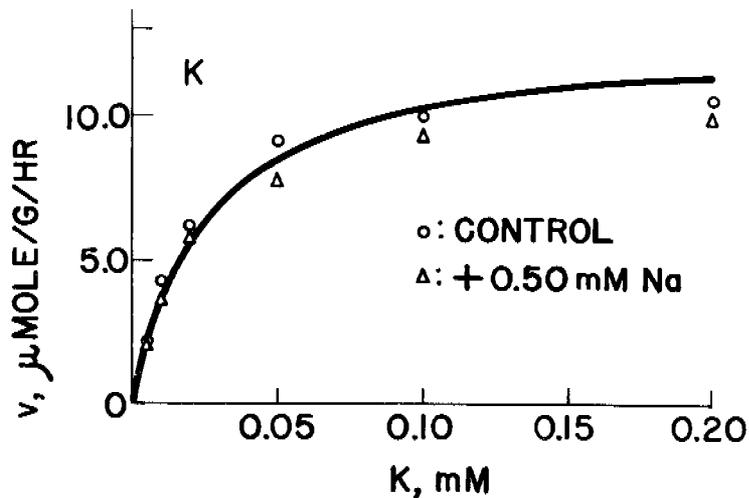


Fig. 6. Rate of absorption of K as a function of the concentration of KCl with and without NaCl at 0.50 mM. CaCl_2 : 0.50 mM. The line is a plot of the Michaelis-Menten equation, using the means of the control and plus sodium values.

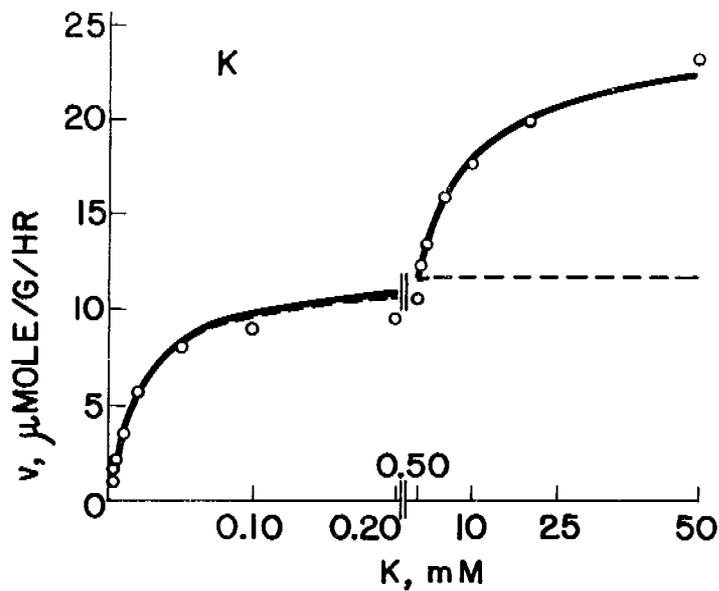


Fig. 7. Rate of absorption of K as a function of the concentration of KCl. CaCl_2 : 0.50 mM. The horizontal (concentration) scale is broken between 0.20 and 0.50 mM. The solid line at the low concentrations, continued by the dashed line, is a plot of the Michaelis-Menten equation. K_m : 0.021 mM; V_{max} : 11.9 μmole/gm/hr.

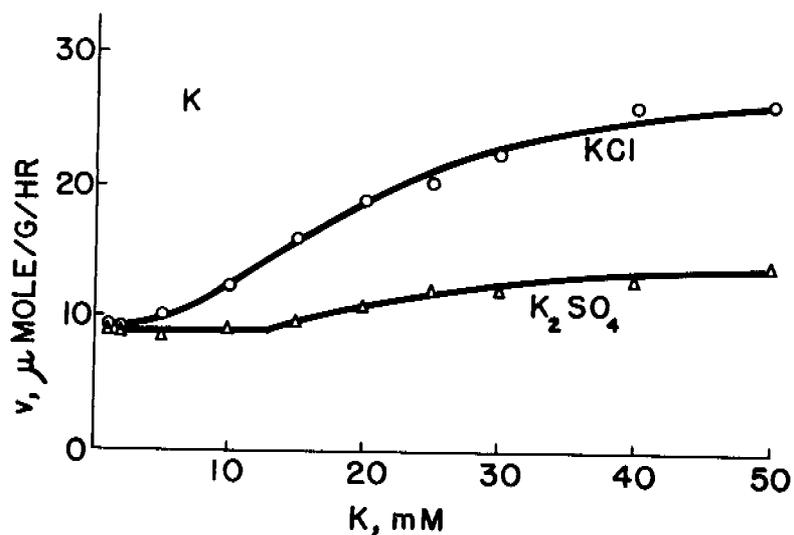


Fig. 8. Rate of absorption of K as a function of the concentration of KCl (o) and K₂SO₄ (Δ). CaCl₂ and CaSO₄, respectively: 0.50 mM.

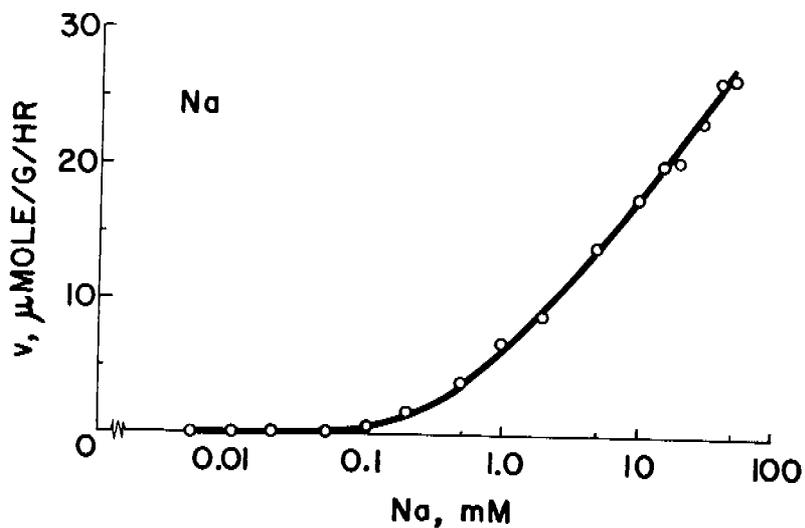


Fig. 9. Rate of absorption of Na as a function of the concentration of NaCl, plotted logarithmically over the range 0.005 to 50 mM. CaCl₂: 0.50 mM; KCl: 1.00 mM.

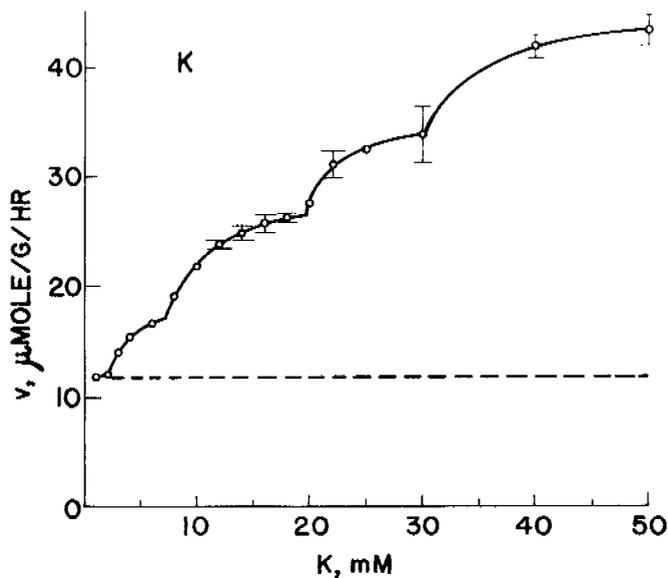


Fig. 10. Rate of absorption of K as a function of the concentration of KCl. CaSO_4 : 0.50 mM. All treatments replicated; circles represent the means of two individual values indicated by the short horizontal lines. Horizontal lines are not drawn where the distance between them would have been equal to or less than the diameter of the circle. Dashed line represents the maximal rate of absorption, V_{max} , by mechanism 1 (cf. fig. 7).

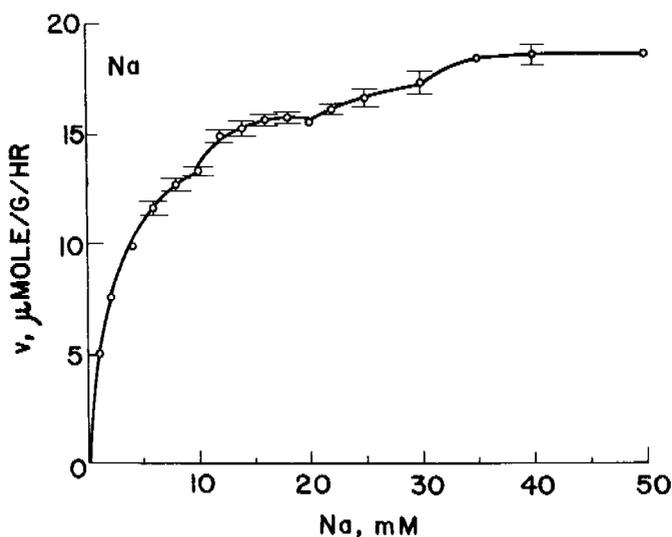


Fig. 11. Rate of absorption of Na as a function of the concentration of NaCl. CaCl_2 : 0.50 mM, KCl: 1.00 mM. Other conditions and conventions as for fig. 10.

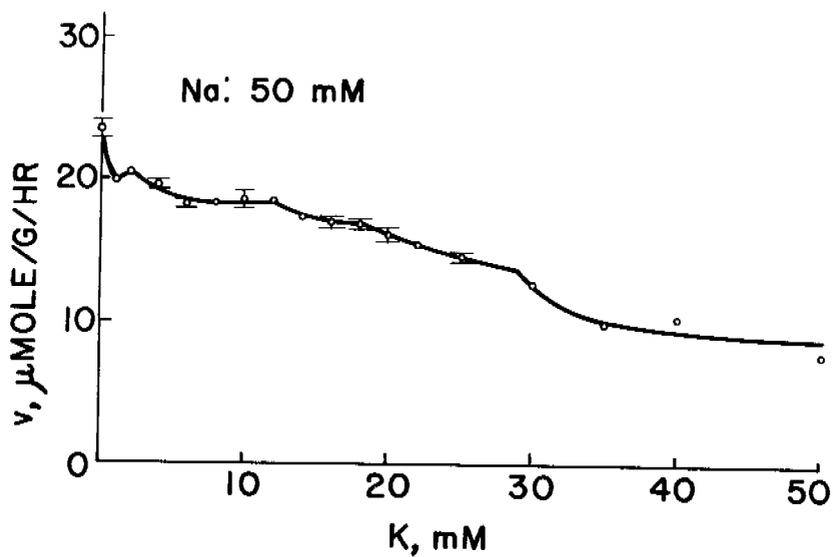


Fig. 12. Rate of absorption of Na as a function of the concentration of KCl. NaCl: 50 mM; CaCl₂: 0.50 mM.

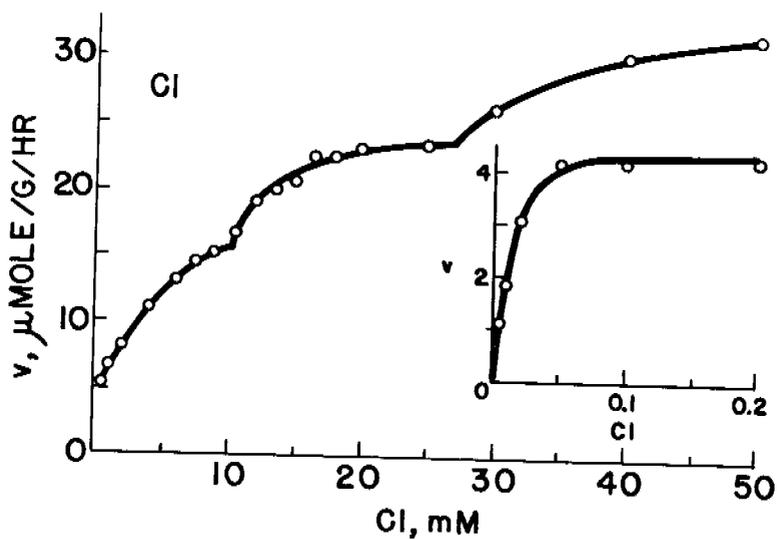


Fig. 13. Rate of absorption of Cl as a function of the concentration of KCl. CaSO₄: 0.50 mM. Results for the 0.005 to 0.20 mM range of concentrations shown in the insert.

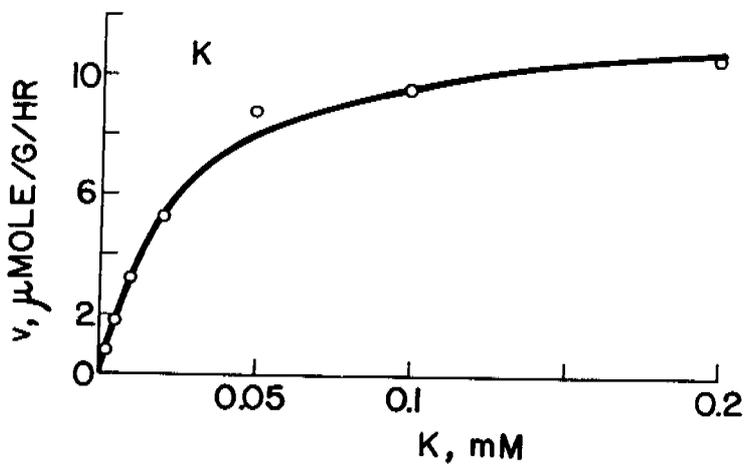


Fig. 14. Rate of absorption of K by corn leaf tissue as a function of the concentration of KCl. CaCl_2 : 0.50 mM. The line is a plot of the Michaelis-Menten equation. K_m : 0.027 mM; V_{\max} : 12.1 $\mu\text{mole/gm/hr}$.

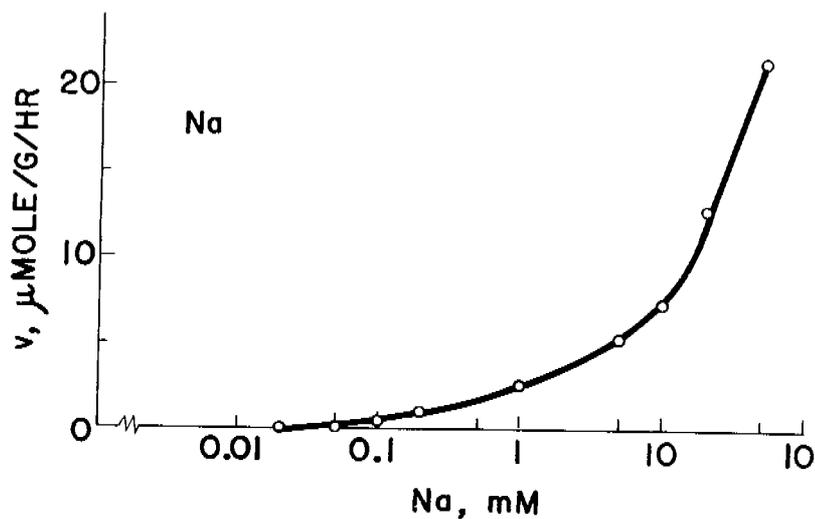


Fig. 15. Rate of absorption of Na by barley leaf tissue as a function of the concentration of NaCl, plotted logarithmically over the range 0.02 to 50 mM. CaCl_2 : 0.50 mM.