Plant Physiol. (1975) 56, 377–380

The Regulation of Potassium Absorption in Barley Roots

Received for publication January 14, 1975 and in revised form April 23, 1975

ANTHONY GLASS
Department of Botany and Zoology, Massey University, Palmerston North, New Zealand

ABSTRACT

The dynamics of changes in K^+ influx across the plasmalemma and of internal K^+ concentrations $[K^+]_i$ of intact barley (*Hordeum vulgare*) roots were examined as the roots were converted from 'high-salt' to 'low-salt' roots. Following the transfer of plants grown in 0.5 mM $CaSO_4$ solutions plus various concentrations of KCl to 0.5 mM $CaSO_4$ solutions, influx rates increased and internal K^+ concentrations declined as a function of time and the initial K^+ status of the tissue. The relationship between plasmalemma influx and $[K^+]_i$ was examined over a wide range of $[K^+]_i$ values by growing intact plants in various concentrations of KCl. Plasmalemma influx was inversely correlated with the square of $[K^+]_i$. A model for the regulation of plasmalemma influx by $[K^+]_i$ is considered.

Ever since the pioneering studies on the mineral nutrition of higher plants by Hoagland and Broyer (10), and later by Epstein (8), and co-workers, experiments on the uptake of mineral ions by plant roots have routinely been performed with roots which have been starved of inorganic ions for varying periods before the actual uptake experiment. Such "low-salt" roots absorb ions at rates many times greater than roots (so-called "high-salt" roots) which have been grown in solutions rich in mineral ions. Until recently the implications of the above observations have received relatively little attention. Workers have, in the main, required high, and consequently easily-measurable rates of influx, and therefore have routinely used these low-salt roots. However, the relationship between ion status and uptake outlined above clearly indicates the operation of some form of regulatory mechanism whereby the uptake of a given ion is controlled by the nutrient status of the plant. Following the absorption of significant quantities of ions, low-salt roots gradually lose their high capacity for ion uptake, becoming, in effect, high-salt roots. It seems to be well established that in barley the basis for the reduced uptake rates of high-salt roots is reduced influx rather than increased efflux (3, 5, 13). Several workers have shown that influx values may be negatively correlated with the internal concentration of a particular ion (3, 15). Cram (3) has established that Cl^- influx in barley root and carrot tissue is negatively correlated with log of $(Cl^- + NO_3^-)$ concentration of the vacuole. In *Lemna* (15), K^+ influx has been shown to be inversely proportional to the square of the internal K^+ concentration. By analogy with biochemical pathways, it has been suggested that ion uptake in plants may be regulated by a form of feedback control in which the absorbed ion acts as the regulator of further uptake through its effects upon influx (14).

The series of events whereby the regulator signal, presumably the ion itself, is perceived and translated to give control over influx remains far from being understood.

Studies with microbial systems (2, 6, 7, 9) indicate that the regulation of active transport systems may be achieved by the induction (*e.g.*, β-galactoside permease) or repression (*e.g.*, K^+ and sulphate absorption) of carrier synthesis and by the operation of a system of feedback inhibition (*e.g.*, sulphate absorption). There are good indications (15) that similar systems might operate in higher plants although, for various reasons, progress in elucidating the details of these systems has been slow. Control of influx by means of regulation of carrier synthesis might be effected at any one of several levels from transcription through translation. In addition a direct effect upon influx could be achieved by feedback inhibition (analogous to enzymic allosteric interaction). These ideas are expressed diagramatically in Figure 1. Many aspects of this model have been expressed or implied previously in relation to higher plants (3, 14, 15).

To date, there appears to be little in the way of experimental evidence to support any specific mechanism for the regulation of ion uptake in higher plants. Several studies (11, 12) have examined the changes of influx which follow the transfer of low-salt roots to high-salt conditions. Such conditions may lead to a dramatic decline in rates of influx. It was considered that the reverse situation, the transfer of high-salt roots to low-salt conditions, might provide a system more suitable for the examination of the mechanism of control since these conditions lead to the development rather than the disappearance of the influx system. The experiments described below examine the dynamics of increases in K^+ influx following transfer of high-salt barley roots to low-salt conditions, and the relationship between influx and internal (vacuolar) K^+ concentrations.

MATERIALS AND METHODS

Growth of Plants. Seeds of *Hordeum vulgare* cv. Carlsberg were treated for 10 min in a solution of 1% sodium hypochlorite. They were then washed several times in distilled H_2O before their aeration at 26 ± 1 C for 24 hr. After this treatment, seeds were placed upon layers of cheesecloth supported on stainless steel wire frames which fit over the tops of 3-liter capacity plastic trays. The trays contained 2.5 liters of 0.5 mM $CaSO_4$ which was supplemented by various concentrations of KCl according to the experiment. In all the growth and pretreatment methods described henceforward, 0.5 mM $CaSO_4$ solution was always present alone or in addition to various concentrations of KCl. Solutions in the trays were changed at intervals of time as described in specific experiments. After the 24 hr imbibition, plants were grown for 5 more days in the dark at 26 ± 1 C. Solutions in the trays were aerated continuously.

Pretreatment of Roots. After 5 days' growth in various KCl

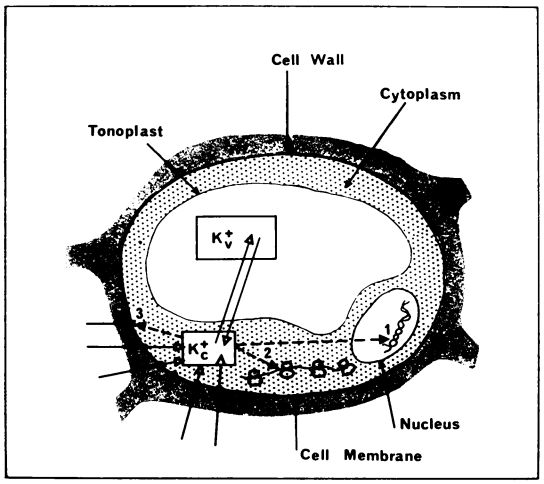

FIG. 1. Diagram showing a schematized cortical root cell and the likely sites for regulation of potassium uptake. 1: Repression by cytoplasmic K^+ (K_c^+) of transcription of DNA coding for carrier protein; 2: inhibition by cytoplasmic K^+ of ribosomal synthesis of carrier protein; 3: allosteric inhibition of carrier mediated uptake of K^+. K_v^+ represents the vacuolar pool of potassium ions. Carrier molecules are represented by dark circles (●).

solutions, plants were normally removed from their trays, still supported upon the wire frames, rinsed briefly in 0.5 mM $CaSO_4$ solution to remove any KCl solution, and then transferred to fresh trays containing $CaSO_4$ solution at 26 C. At various intervals of time, root samples were removed from the trays and allowed to equilibrate for 15 min in aerated $CaSO_4$ solution at 30 C. After equilibration, excess water was removed from the roots and 0.5 or 1 g samples were weighed out.

Determination of K^+ Influx Values. The uptake media for influx determinations was buffered at pH 7.1 (tris-HCl) and contained 0.5 mM $CaCl_2$, 0.05 mM KCl, and approximately 0.05 μCi of ^{86}Rb. This solution was contained in 150-ml beakers during influx measurements and maintained at 30 C in a thermostatically controlled water bath. Solutions were aerated continuously during the uptake periods, which were usually of 30-min duration. After this uptake period roots were rinsed for 1 min in ice-cold isotope-free uptake medium and then were allowed to stand at room temperature for 30 min in fresh isotope-free uptake medium. At the end of this desorption period, the roots were ashed and the radioactivity of the ash was determined by means of a Nuclear Chicago Geiger Counter. Influx measurements based upon such long desorption periods have been claimed to represent influx rates across the tonoplast (4). However, at low external ion concentrations influx across the tonoplast does not differ from that across the plasmalemma. The author confirmed that this was the case in preliminary experiments and established that influx rates based upon a 30-min uptake period followed by a 30-min desorption period, and those based upon a 10-min uptake period followed by a 10-min wash did not differ at all. Influx values quoted throughout this paper may therefore be taken to represent ϕoc[1]. Wherever presented, influx rates are given in μmoles of K^+ g fresh weight^{-1}. Values shown in the various kinetic experiments are the means of four or five replicates.

Measurement of K^+ Content of Tissue $[K^+]_t$. At the same time that root samples were weighed out for influx determinations, 0.5- or 1-g samples of roots were weighed out for K^+ determination. These roots were repeatedly extracted in boiling distilled H_2O, and the aqueous extracts were filtered and pooled. The K^+ concentrations of these solutions were determined by means of an EEL flame photometer. Each datum point shown in the figures is the average of two determinations.

[1] Abbreviation ϕoc: plasmalemma influx.

RESULTS AND DISCUSSION

Time Course of Development of Increased Influx Values. Figures 2, 3, and 4 demonstrate the dynamics of increases in K^+ influx (ϕoc) after the transfer of roots from KCl solutions to 0.5 mM $CaSO_4$ solutions. The rates of increase of influx values were found to vary according to the initial concentration of KCl ($[K^+]_t$) of the roots before their transfer to $CaSO_4$ solutions. Figure 2 shows data for roots grown in 5 mM KCl solutions. In this experiment, solutions in the tray were changed daily so that a high internal concentration of K^+ (49.3 μmoles g fresh weight^{-1}, was maintained. During the course of the experiment, ϕoc increased from 0.13 μmoles g fresh weight^{-1} hr^{-1} at the time of transfer to 0.53 μmoles g fresh weight^{-1} hr^{-1}, 24 hr later. This is equivalent to a rate of increase of ϕoc of 0.02 μmoles g fresh weight^{-1} hr^{-2}. Figure 2 also shows the changes of $[K^+]_t$, which involved a decline from 49.3 to 32 μmoles g fresh weight^{-1} for the same period. This is equivalent to a 35% dilution of K^+ concentration. The data shown in Figure 3 are for roots grown in 5 mM KCl solution without replacement with fresh KCl solution during the growth period. During this period the barley roots reduced the external KCl concentration from 5 mM at day 1 to 1.69 mM at day 5. The internal K^+ concentration of these roots was much lower than when KCl solutions were changed daily. Initial influx rates were higher and the rate of increase of influx rates following transfer to $CaSO_4$ solution was higher. During the 24-hr period after transfer $[K^+]_t$ declined from 26.3 to 18.3 μmoles g fresh weight^{-1} (31% dilution), while ϕoc increased from 0.66 to 1.96 μmoles g fresh weight^{-1} hr^{-1}. Expressed as a rate of increase of influx, this amounts to 0.06 μmoles g fresh weight^{-1} hr^{-2}. Figure 4 shows the increase of ϕoc and decrease of K^+ concentration after transfer of roots

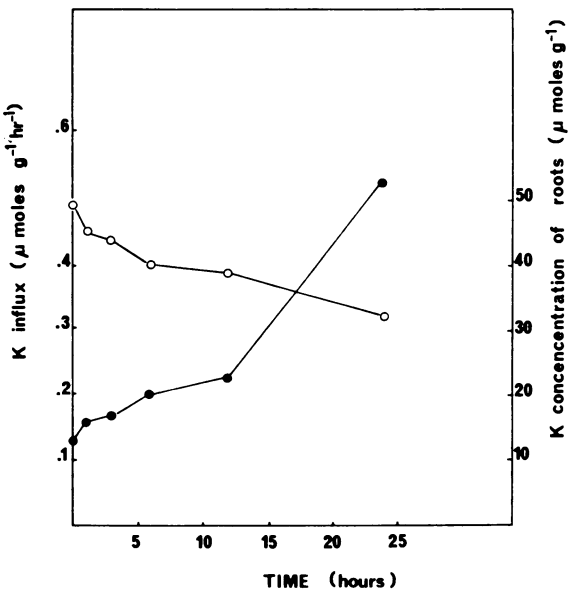

FIG. 2. Changes in K^+ influx values and K^+ concentration of high salt barley roots grown in 5 mM KCl plus 0.5 mM $CaSO_4$ (changed daily) after transfer to 0.5 mM $CaSO_4$ (—●—: K^+ influx; —○—: K^+ concentration of roots). Uptake media contained 0.5 mM $CaCl_2$, 0.05 mM KCl plus approximately 0.05 μCi of ^{86}Rb.

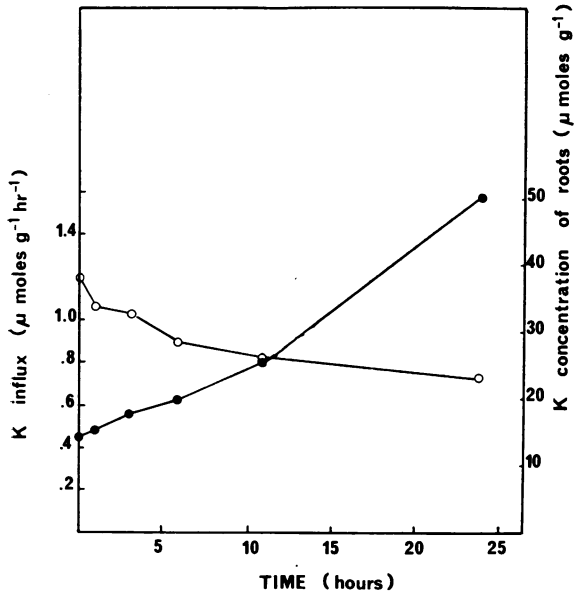

FIG. 3. Changes in K^+ influx values and K^+ concentration of high-salt barley roots grown in 5 mM KCl plus 0.5 mM $CaSO_4$ (without change of medium) after transfer to 0.5 mM $CaSO_4$ (—●—: K^+ influx; —○—: K^+ concentration of roots). Uptake media contained 0.5 mM $CaCl_2$, 0.05 mM KCl, plus approximately 0.05 μCi of ^{86}Rb.

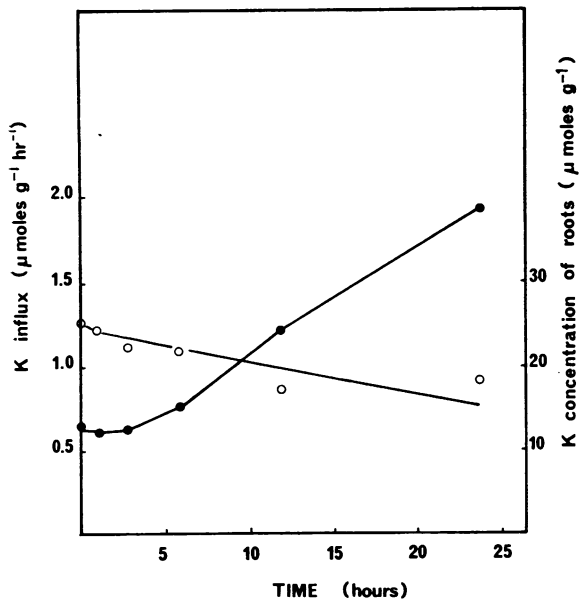

FIG. 4. Changes in K^+ influx values and K^+ concentration of 'high-salt' barley roots grown in 1 mM KCl plus 0.5 mM $CaSO_4$ (changed daily) after transfer to 0.5 mM $CaSO_4$. (—●—: K^+ influx; —○—: K^+ concentration of roots). Uptake media contained 0.5 mM CaCl, 0.05 mM KCl, plus approximately 0.05 μCi of ^{86}Rb.

grown in 1 mM KCl (changed daily) to $CaSO_4$ solution. $[K^+]_t$ declined from 38 to 24 μmoles g fresh weight^{-1} (37% dilution) and ϕoc increased from 0.4 to 1.7 μmoles g fresh weight^{-1} hr^{-1}, an increase rate of 0.05 μmoles g fresh weight^{-1} hr^{-2}.

These experiments establish that the initial K^+ influx rates in barley may vary considerably according to the previous nutrient conditions under which roots have been grown. After transfer to K^+-free solutions, influx rates may increase slowly or quite dramatically depending upon the nutrient status of the roots in relation to K^+. The dilution of $[K^+]_t$ in the various tissues shown in Figures 2, 3, and 4 (31–37%) is remarkably similar despite wide differences in initial K^+ concentrations. This dilution could be achieved by root growth, by translocation to the shoot and/or by efflux. Growth measurements over this period showed that roots increased their fresh weight at the rate of 0.2 day^{-1}. The lowest dilution (31%) for the data of Figure 3 would require a growth rate of 0.4 day^{-1} to account for this dilution solely on the basis of root growth. It is, therefore, likely that translocation to the shoot or efflux or both make some contribution to the observed decline of K^+ concentration.

In order to be certain that the increased influx values observed after transfer to $CaSO_4$ solution was not attributable to some endogenous changes, unrelated to the transfer, ϕoc was measured at intervals over an 18-hr period before transfer. Roots were grown in 5 mM KCl solution for 4 days, without replacement, and at the time of transfer, one group of plants was replaced in fresh 5 mM KCl solution while the other group was placed in $CaSO_4$ solution as in the other experiments. The results as shown in Figure 5 demonstrate that influx varied only slightly over the 18 hr before transfer. Influx values in the $CaSO_4$-treated roots demonstrated a rapid rise whereas rates in the KCl-treated roots declined slightly.

In order to examine the relationship between $[K^+]_t$ and ϕoc over a much wider range of K^+ concentration, roots were grown in KCl solutions, ranging in concentration from .05 mM to 10 mM, which were replaced daily. The results of these experiments as well as the data from the kinetic experiments are shown in Figure 6. The relationship between ϕoc and $[K^+]_t$ seems to be an exponential one similar to that observed by Cram (3) for Cl^- influx and vacuolar ($Cl^- + NO_3^-$) concentration. The regression of ϕoc on $[K^+]_t$ gave a correlation coefficient of −0.83. This correlation was increased to −0.93 for ϕoc against log $[K^+]_t$ and to +0.98 for ϕoc against

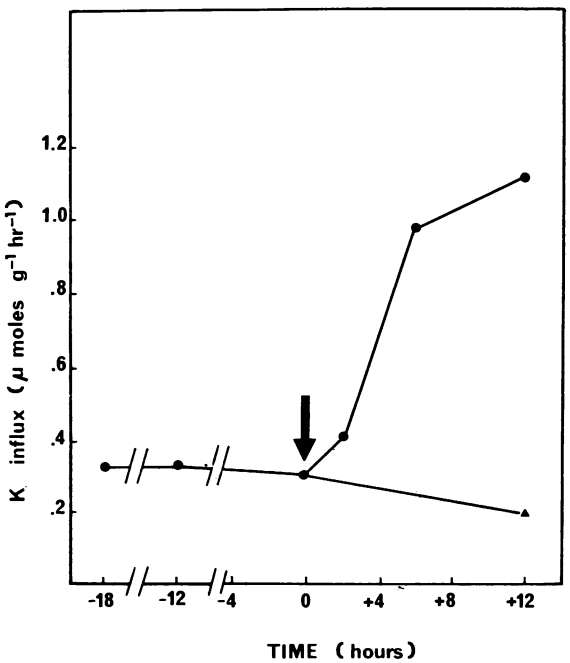

FIG. 5. Changes in K^+ influx values over a period of 18 hr before and 12 after transfer of high-salt roots to 0.5 mM $CaSO_4$ (—○—) or fresh 5 mM KCl (—▲—). The arrow indicates the time of transfer. Uptake media contained 0.5 mM CaCl, 0.05 mM KCl, plus approximately 0.05 μCi of ^{86}Rb.

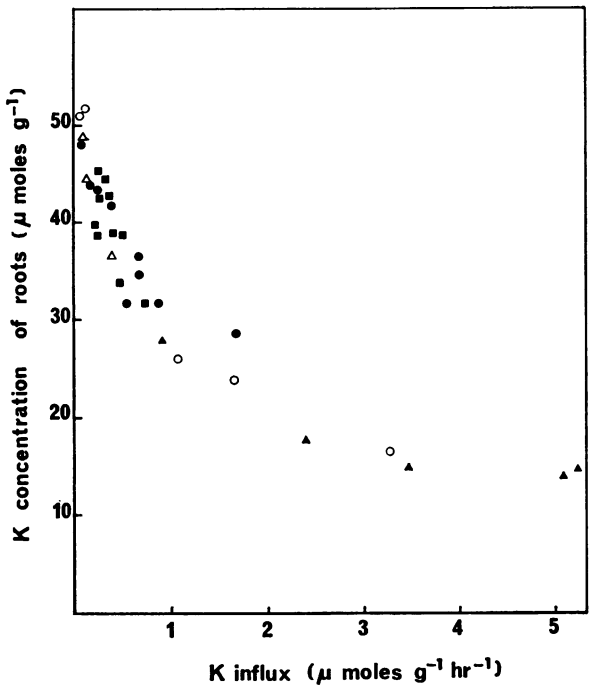

FIG. 6. Plot of K^+ influx values of roots grown in various concentrations of KCl against the internal concentration of K^+. The various symbols employed to represent points on the plot refer to separate experiments.

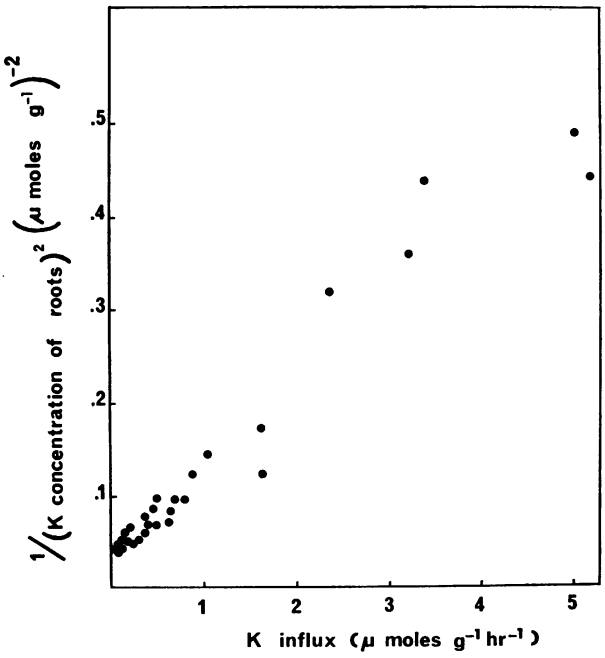

FIG. 7. Plot of K^+ influx (data of Fig. 6) against the reciprocal of internal K^+ concentration squared ($1/[K^+]_i^2$).

$1/[K^+]_i^2$. Figure 7 shows the plot of ϕoc against $1/[K^+]_i^2$. Similar observations have been recorded for K^+ uptake and internal K^+ concentrations in *Lemna* (14).

In the introduction I suggested that the absorption of ions in higher plants might be regulated by a process of induction or repression of carrier synthesis in addition to a form of allosteric inhibition of carrier activity. The present studies establish that K^+ influx is strongly correlated with internal K^+ concentration. The plot of ϕoc against $[K^+]_i$ (Fig. 6) strongly resembles the sigmoid relationship which exists between concentration of allosteric inhibitor and enzyme activity in known cases of allosteric inhibition, *e.g.*, the relationship between the concentration of isoleucine and the activity of threonine deaminase of *E. coli* (1). Furthermore the rapidity with which ϕoc increases after transfer to $CaSO_4$ (Figs. 2 and 4) is strongly suggestive of an immediate allosteric effect associated with the dilution of $[K^+]_i$. Effects via derepression of carrier synthesis would be anticipated to require much longer to become evident.

Nevertheless, after sufficient time has elapsed for significant changes in $[K^+]_i$ to bring about derepression of carrier synthesis, *de novo* carrier synthesis could well contribute to the observed increased influx values. If this were the case, as $[K^+]_i$ declined one would predict an initially slow rate of increase of ϕoc, because of relief of the allosteric inhibition, followed by a much more rapid rate of increase of ϕoc as carrier synthesis became activated. In the experiments described in Figures 2, 3, and 4, there is an exponential increase in ϕoc with time. Further support for this interpretation of these data comes from preliminary studies on the sensitivity of high-salt barley roots to inhibitors of protein synthesis (cycloheximide and Actinomycin D) during the period after transfer to $CaSO_4$ solutions. These observations indicate that even in the absence of protein synthesis there is initially a significant increase of ϕoc.

In studies of the regulation of K^+ uptake by *Lemna*, Young *et al.*, (15) claimed that allosteric effects of internal K^+ are significant in the determination of K^+ influx rates. Clearly regulation of ion uptake by induction or derepression of carrier synthesis like the regulation of biosynthetic pathways by enzyme induction or repression is effective but relatively slow. Rapid and fine control can be achieved by the addition of an allosteric system of negative feedback.

LITERATURE CITED

1. COHEN, G. N. 1968. In: The Regulation of Cell Metabolism. Holt, Rinehart, and Winston, New York.
2. COHEN, G. N. AND J. MONOD. 1957. Bacterial permeases. Bacteriol. Rev. 21: 169–194.
3. CRAM, W. J. 1973. Internal factors regulating nitrate and chloride influx in plant cells. J. Exp. Bot. 24: 328–341.
4. CRAM, W. J. AND G. G. LATIES. 1971. Use of short-term and quasi-steady state influx in estimating plasmalemma and tonoplast influx in barley root cells at various external and internal chloride concentrations. Aust. J. Biol. Sci. 24: 633–646.
5. CRAM, W. J. AND G. G. LATIES. 1973. Chloride fluxes in cells of the isolated root cortex of *Zea mays*. Aust. J. Biol. Sci. 26: 757–779.
6. DREYFUSS, J. 1964. Characterization of a sulphate- and a thiosulphate-transporting system in *Salmonella typhimurium*. J. Biol. Chem. 239: 2292–2297.
7. DREYFUSS, J. AND A. B. PARDEE. 1966. Regulation of sulphate transport in *Salmonella typhimurium*. J. Bacteriol. 91: 2275–2280.
8. EPSTEIN, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. John Wiley and Sons, New York.
9. GOLDMAN, D. G., S. G. SCHULTZ, AND W. EPSTEIN. 1966. Repressive control of potassium transport in *Escherichia coli*. Biochim. Biophys. Acta 130: 546–548.
10. HOAGLAND, D. R. AND T. C. BROYER. 1936. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. 11: 471–507.
11. JOHANSEN, C., D. G. EDWARDS, AND J. F. LONERAGAN. 1970. Potassium fluxes during potassium absorption by intact barley plants of increasing potassium content. Plant Physiol. 45: 601–603.
12. LEIGH, R. A. AND R. G. WYN JONES. 1973. The effect of increased internal ion concentration upon the ion uptake isotherm of excised maize root segments. J. Exp. Bot. 24: 787–795.
13. PITMAN, M. G. 1963. The determination of the salt relations of the cytoplasmic phase in beetroot tissue. Aust. J. Biol. Sci. 16: 647–668.
14. PITMAN, M. G. AND W. J. CRAM. 1973. Regulation of inorganic ion transport in plants. In: W. P. Anderson, ed., Proc. Workshop "Ion transport in Plants". Academic Press, London pp. 465–481.
15. YOUNG, M., R. L. JEFFERIES, AND A. P. SIMS. 1970. The regulation of potassium uptake in *Lemna minor* L. Abh. Dtsch. Akad. Wiss. Berl. b, 67–82.