Plant Physiol. (1972) 49, 16–19

The Release of Potassium and Sodium from Young Excised Roots of *Zea mays* under Various Efflux Conditions

Received for publication April 14, 1971

KONRAD MENGEL AND REINHARD PFLÜGER
Agricultural Research Station Büntehof, 3 Hannover, Germany

ABSTRACT

The release of potassium and sodium from excised roots of *Zea mays* having similar contents of potassium and sodium was studied. At low temperature (2 C) the efflux rates of both cations were very similar, but at higher temperature (20 C) the potassium release was reduced considerably, whereas the sodium release was hardly affected. Also, under anaerobic conditions the potassium efflux rate was nearly as high as the sodium efflux rate, but with normal O_2 supply the potassium release was reduced to about one-fifth. Since a changing efflux medium compared with a constant efflux medium had no great influence upon the sodium release but influenced the potassium release very much, it is assumed that the low potassium release under normal metabolic conditions is due to a reabsorption of effluxed potassium from free space. For sodium this reabsorption is of minor significance, as the uptake potential of maize roots for sodium is very poor. It is concluded that the release of potassium and sodium is a diffusion process and that the cell membranes have rather similar diffusivities for these two cations.

The release of ions by higher plants to the external medium has been described in numerous papers. For the most part, the authors have examined the efflux of individual ions, such as K^+ and Na^+ (12, 14–16). The efflux is regarded as a passive process, not directly influenced by metabolism, and it is presumed that diffusion or exchange diffusion is responsible for the release of ions. Another group of researchers, on the basis of results obtained in investigations on electrochemical potential differences, postulate an active release process (4, 5, 7, 18, 19). This applies particularly to the Na^+ efflux (Na^+ pump) because the distribution of K^+ often corresponds to the electrochemical equilibrium. The existence of such a Na^+ pump in cells of different animal tissues could be established in various experiments (9, 20). It is supposed that also lower plants living in sea water possess such a Na^+ pump (1, 2, 8). For higher plants, its existence has been postulated by several authors (4, 5, 7), but it is still unproved. If the release of Na^+ is mainly due to a Na^+ pump and the release of K^+ to an outward diffusion, there should exist different efflux patterns for Na^+ and K^+. In order to test this question, the K^+ and Na^+ release by young roots of *Zea mays* L. was studied under various efflux conditions.

MATERIAL AND METHODS

The experiments were carried out with secondary roots of *Zea mays* L. (cv. KC 3), 12 to 15 cm in length, grown in solution culture (3 mM KNO_3, 3 mM $MgSO_4$, 1 mM NH_4NO_3, 0.5 mM $Ca(H_2PO_4)_2$, 0.5 mM $CaCl_2$, 14 μM $MnSO_4$, 1.4 μM $CuSO_4$, 1.4 μM $ZnSO_4$, 10 μM H_3BO_3, 0.3 μM $(NH_4)_6Mo_7O_{24}$, 10 μM Fe^{3+} as chelate).

In all cases, several (up to 5) generations of secondary roots could be obtained from each plant series grown. In order to get roots with similar contents of K^+ and Na^+, for each experimental set 60 excised roots having a dry weight of about 0.9 g were incubated for 20 hr in 500 ml 30 mM NaCl. The roots were then rinsed three times with H_2O, and two times with 0.5 mM $CaSO_4$, each rinse lasting 5 min, and then washed again with H_2O. By this procedure most of the Na^+ in the free space of the roots is removed. For the efflux investigations 10 roots having a total dry weight of about 0.15 g were selected for each sample. In order to prevent ions exuded from the cut basal end of the roots moving into the external medium, the excised roots, having a length of about 14 cm, were placed into test tubes in such a way that the basal end of the roots (about 1.5 cm) did not dip into the external medium (efflux medium). This basal end was wrapped in thin strips of plastic foam, in order to fasten the roots to the test tubes (14). The efflux medium for each sample was 25 ml of 0.5 mM $CaSO_4$. The Ca^{2+} in the outer solution prevents abnormally high ion releases, caused by a lack of Ca^{2+} at the cell boundaries (3, 10, 16, 21). The efflux solution for the different experimental series was replaced 6 to 12 times. The effluxed amounts of K^+ and Na^+ were determined by atomic absorption spectrophotometry. Chloride determinations were carried out potentiometrically with the Aminco-Cotlove Titrator. Labeled K^+ (^{42}K) was analyzed by a well scintillation counter (Friesecke u. Höpfner, Model FHT 20 D).

In one experimental series, the efflux medium (25 ml of 0.5 mM $CaSO_4$) was replaced 20 times at intervals of 10 min. This procedure was compared with a efflux medium of 500 ml of 0.5 mM $CaSO_4$ that was not replaced during the whole efflux period of 200 min. All data given in the figures and tables are mean values of three to four samples for each treatment.

RESULTS

Roots having similar contents of K^+ and Na^+ released Na^+ at a higher rate than K^+ at room temperature (Fig. 1). The release rate for K^+ was rather constant during the various replacements of the efflux medium, whereas the Na^+ release rate declined. But still at the sixth replacement, the efflux rate of Na^+ was about five times higher than the K^+ efflux rate. The roots released into the sixth efflux medium about 0.1% of their total K^+ and about 0.7% of their total Na^+. At low temperature (2 C) the release rates for K^+ and Na^+ were very similar (Fig. 2). In comparison with the efflux rates at 20 C, the efflux rates for Na^+ were nearly the same, particularly when comparing the rates of the fourth to sixth replacements. The efflux

rates of K^+, however, were increased by a factor of 7 at low temperature. This result suggests that in some way, metabolism affects the K^+ release. In order to confirm this metabolic effect, the release was studied under aerobic and anaerobic conditions. The efflux medium was treated with N_2 or air, by bubbling through nitrogen gas or air for 30 min before the efflux procedure was started. Under anaerobic conditions the release rate of K^+ was about six times higher than under aerobic conditions (Fig. 3), whereas the release rates for Na^+ were not significantly influenced by aeration. At the seventh replacement, the metabolic conditions were reversed, so that roots that effluxed before into an aerobic medium were now exposed to an anaerobic medium and vice versa. The roots promptly reacted to this change of metabolic conditions with regard to their K^+ release, but Na^+ release was not affected. Under anaerobic conditions, the K^+ efflux rates were not as high as the rates for Na^+ efflux. In agreement with Marschner *et al.* (11), it can be assumed that in the roots of the anaerobic treatment some oxygen was still present, favoring oxidative processes. The efflux rates in this experiment (Fig. 3) were lower than those of the first experiments (Figs. 1 and 2). This

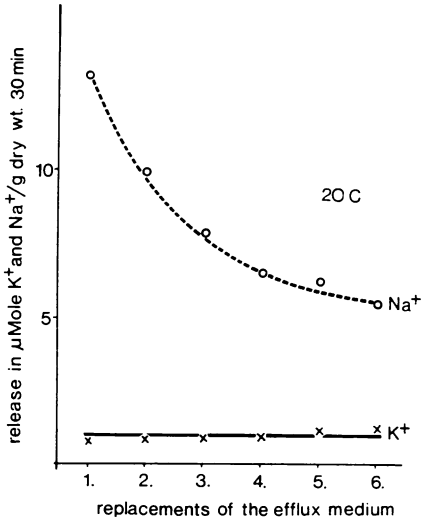

FIG. 1. Release of K^+ and Na^+ at 20 C during six replacements of the efflux medium. Initial content of the roots was 910 μmole K^+ and 701 μmole Na^+/g dry wt.

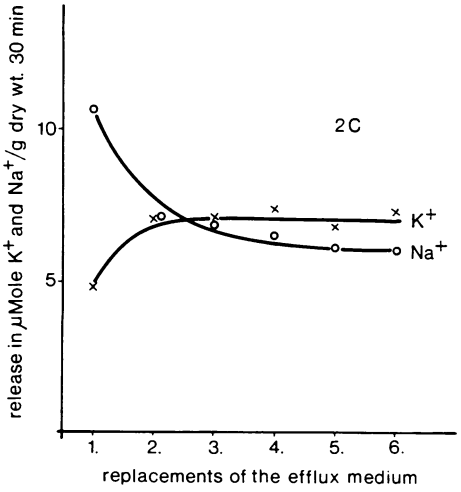

FIG. 2. Release of K^+ and Na^+ at 2 C during six replacements of the efflux medium. Initial content of the roots was 895 μmole K^+ and 741 μmole Na^+/g dry wt.

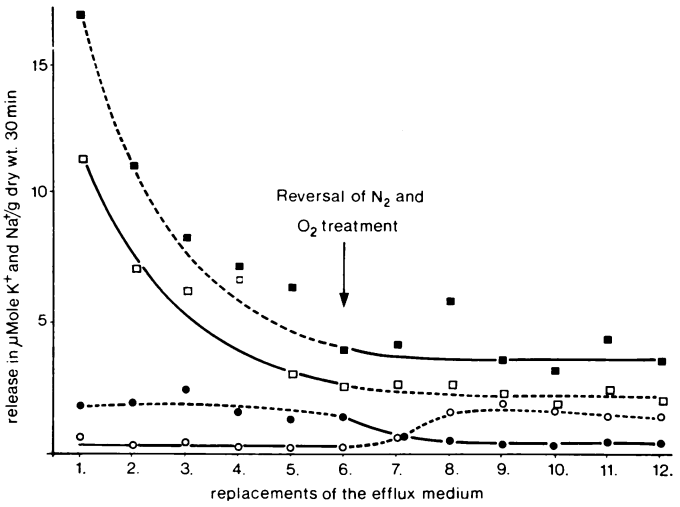

FIG. 3. Release of K^+ and Na^+ under aerobic (——) and anaerobic (-----) conditions during 12 replacements of the efflux medium. Initial content of the roots: 630 μmole K^+ (○), 452 μmole Na^+ (□), 540 μmole K^+ (●), 454 μmole Na^+ (■) per g dry wt.

Table I. *Release of K^+ and Na^+ into Two Efflux Mediums*

Comparisons were made between the release of K^+ and Na^+ into an efflux medium of 500 ml with no replacement during the efflux period (200 min) and K^+ and Na^+ release into an efflux medium of 25 ml replaced 20 times during the efflux period (200 min).

	Initial Root Content		Release		Release of the Root Content	
	K^+	Na^+	K^+	Na^+	K^+	Na^+
	μmole/g dry wt				%	
No replacement	1025	818	5.7	36.9	0.56	4.51
20 × replacements	1003	924	17.2	43.3	1.71	4.68

may have been due to the lower contents of K^+ and Na^+ in these roots. Nevertheless, the trends observed in the first experiment are also evident in the data of Figure 3.

Table I shows the quantities of K^+ and Na^+ released under the different efflux conditions. In one series (no replacement) the efflux medium of 500 ml was not replaced during the whole efflux period of 200 min; in the other series the efflux medium (25 ml solution) was replaced 20 times. The K^+ release into the "changing medium" was about three times higher than into the medium with no replacement, whereas the difference between the Na^+ quantities released under the two conditions was not dramatic, particularly when the Na^+ release is calculated as percentage of the Na^+ content of the root.

The release of Cl^- was of the same order of magnitude as the release of K^+ or Na^+ (Table II). The release rates of Cl^- were rather constant over the whole efflux period consisting of eight replacements of the efflux medium. This demonstrates that the free space of the roots was rather free of chloride at the beginning of the efflux period. Otherwise, the chloride quantities being released into the efflux medium should decrease during successive replacements. During the first replacements of the efflux medium, the quantities of chloride released did not equal the sum of the K^+ and Na^+ released. But beginning with the fifth replacement, the released chloride was equivalent to the $K^+ + Na^+$ released. Since the roots had been exposed to a rather high chloride concentration

Table II. *Release of K^+, Na^+, and Cl^- at 20 C*

Eight replacements of the efflux medium were made. Initial root content was 1702 μmole K^+, 1010 μmole Na^+, 1056 μmole Cl^-/g dry wt.

No. of Replacements	K^+	Na^+	$K^+ + Na^+$	Cl^-
	μmole/g dry wt·30 min			
1	4.0	11.7	15.7	8.3
2	5.0	8.0	13.0	8.9
3	5.2	6.5	11.7	8.6
4	4.9	6.3	11.2	10.3
5	4.0	5.1	9.1	8.9
6	4.2	5.0	9 2	9.5
7	4.6	4.2	8.8	9.0
8	3.8	3.9	7.7	7.8

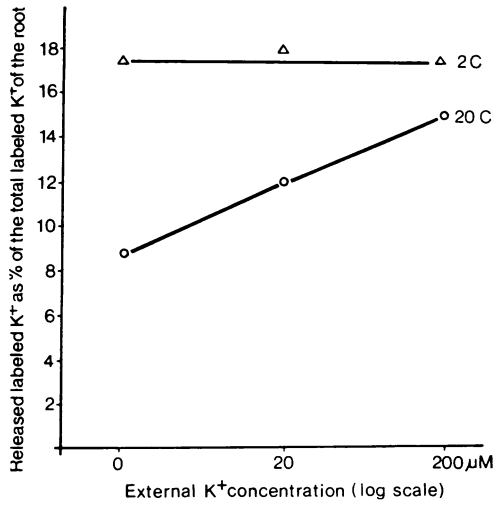

FIG. 4. Effect of the external K^+ concentration on the release of labeled K^+ (^{42}K) at different temperatures. The values represent the total amount of K^+ released in 5 hr. The external solution was replaced at intervals of 60 min.

(30 mM NaCl) before the efflux studies were carried out, the rather high quantities of chloride released could be expected.

Beginning with the fifth replacement in this experiment (Table II), the efflux rates for K^+ and Na^+ did not differ very much. But taking into consideration that the roots of this series had a considerably higher K^+ than Na^+ content, the relative release of Na^+ in this experiment was also higher than that of K^+, in good agreement with the results presented above.

The roots used in the experiment reported in Figure 4 were exposed to a labeled (^{42}K) 1 mM KCl solution for 14 hr. Then they were rinsed and washed with water and 0.5 mM $CaSO_4$ according to the procedure described in "Materials and Methods." Besides 0.5 mM $CaSO_4$, the efflux medium contained KCl in the following concentrations: 0, 20, and 200 μM. In one series the efflux was studied at 20 C, in the other at 2 C. The release at low temperature was not influenced by the concentration of unlabeled K^+ in the efflux medium, but at 20 C the release of labeled K^+ increased with the K^+ concentration of the outer solution (efflux medium).

DISCUSSION

The roots in the various experiments were from plants grown in a complete nutrient solution with a K^+ concentration of 3 mM which can be regarded as sufficiently high for a good K^+ supply. It is probable that during the Na^+ uptake period some of the root K^+ was exchanged for Na^+ of the external solution. In spite of this possibility, the K^+ contents of the roots for the efflux investigations were normal. All roots used were in good condition. They were not flaccid but had full turgor. We think they were in good metabolic condition, because they reacted so promptly to temperature and O_2 supply. Although during the pretreatment of the roots with 30 mM NaCl, no Ca^{2+} was present in the outer solution, it is not probable that this absence of Ca^{2+} had an irreversible, negative effect on the cell membranes. As was found by Mengel and Helal (16) the exchangeable Ca^{2+} of the roots rather than the Ca^{2+} of the outer solution is responsible for the permeability of the cell membranes. The roots used for the experiments were well supplied with Ca^{2+} during the growth, which justifies the assumption that they also had a sufficiently high content of exchangeable Ca^{2+}. According to previous experiments with corn (13), this exchangeable Ca^{2+} of the roots can be replaced by Na^+ with difficulty, but is easily exchanged by other divalent cations (Mg^{2+}, Ca^{2+}). If the cell membranes had been affected seriously by the NaCl pretreatment, the K content of the roots would not have been so high after the treatment. Also the prompt reaction of the roots to the different metabolic conditions indicates that the cell membranes were not damaged by the NaCl treatment.

We do not attribute the differences between K^+ and Na^+ release to a specific binding of K^+ to organic anions, because this binding should have worked also at low temperature. It is more likely that at the pH values prevailing in plant tissues, organic K and Na salts are completely dissociated. Further, it should be stressed that the effluxed K^+ and Na^+ measured in these experiments were not contaminated by xylem sap, due to the experimental setup we used.

The experimental data show that the rates of K^+ and Na^+ release were different under normal metabolic conditions, but were rather similar at low temperature. The high rates for Na^+ release during the first efflux periods (first to fourth replacement) probably were due to an additional Na^+ release from the free space of the roots, being a simple exchange reaction between the Ca^{2+} of the outer solution and the adsorbed Na^+ of the cell wall surfaces. This supposition is supported by the release of chloride, which was not equivalent to the release of $K^+ + Na^+$ during the first replacements (see Table II). But beginning with the fifth replacement it can be assumed that the released Na^+ came nearly entirely from the inner cell compartments (cytoplasm, vacuole). Under normal metabolic conditions, there was always a higher net release of Na^+ compared to K^+, although the K^+ contents of the roots were similar or even higher than the Na^+ contents. But when metabolism was inhibited by low temperature (Fig. 2), the rates of K^+ and Na^+ release were nearly equal. Under these conditions metabolic processes affecting the K^+ and Na^+ release can be ruled out. The release rate, the first replacement excluded, was about 7 μmole K^+ and 6 μmole Na^+/g dry wt·30 min, equivalent to 0.78% of the initial K^+ and 0.81% of the initial Na^+ content of the roots. As the initial K^+ content of the roots did not differ very much from the Na^+ content and as the release rates for K^+ and Na^+ expressed as percentage of the initial root contents are nearly identical, it can be assumed that the K^+ and Na^+ distribution over the various cell compartments was rather similar. If, for example, the Na^+ had been located predominantly in the cytoplasm and the K^+ in the vacuole, a higher net release of Na^+ should have been expected. It is, therefore, unlikely that the differences between the K^+ and Na^+ release rates at room temperature can be explained by a different distribution of K^+ and Na^+ over the various cell compartments.

It is more likely that this difference in some way was related to metabolism. This assumption is supported by the experimental data presented in Figure 3, showing that the K^+ release promptly reacted to aerobic or anaerobic conditions, whereas the Na^+ release was hardly affected. This effect may be caused by different metabolic processes. It is possible that under normal metabolic conditions, K^+ is in some way retained by the cell, maybe by a specific accumulation in cell organelles. On the other hand, it is also possible that K^+ and Na^+ effluxed under normal metabolic conditions at similar rates, but that some of the effluxed K^+ on its way out of the free space of the root was reabsorbed. For Na^+ the chance of reabsorption is low, because the uptake rates of maize for Na^+ are low compared with the uptake rates for K^+ (12). If reabsorption of K^+ rather than specific retention was the main cause for the reduced K^+ net efflux under normal metabolic conditions, then the conditions for reabsorption should influence the net release of K^+. The data of Table II demonstrate that the K^+ release was about three times higher in a changing efflux medium than in an unchanged efflux medium. A changing efflux medium improves K^+ migration out of the free space and, therefore, lowers reabsorption. The Na^+ release was only slightly influenced by the different efflux conditions, giving further evidence that Na^+ was not reabsorbed or was to only a lesser degree. The result of this experiment confirms our assumption that the influence of metabolism upon K^+ release is mainly attributed to a reabsorption of K^+ from the free space. We cannot completely rule out a specific retention of K^+, but we suppose that this is of minor importance in connection with the results presented here. As was shown by Hiatt and Lowe (6), the breakdown of organic acids can also influence the release of K^+. Since at low temperature such a decomposition of organic acids is blocked, the increase in K^+ release cannot be explained by a change in the content of organic acids.

The data of Figure 4 agree well with the above explanation. At 2 C there is no reabsorption, and the external K^+ had no influence on the K^+ release rate, whereas at 20 C the net release of labeled K^+ was higher, the higher the K^+ concentration of the outer solution. This result can be ascribed to a competition for carrier sites between the labeled effluxed K^+ and the unlabeled K^+ of the outer solution. The higher the concentration of the unlabeled K^+ in the free space, the more is the reabsorption of the effluxed labeled K^+ inhibited by competition.

It can be assumed, therefore, that a passive K^+ exchange between the cell K^+ and the external K^+ did not take place. At 20 C such a K^+ exchange cannot be ruled out, as the unlabeled K^+ can have been taken up actively, thus exchanging the labeled K^+ in the plant cells (19). By this means, the increased release of labeled K^+ can also be explained by the increasing unlabeled K^+ concentration of the outer solution. If this exchange process mainly was responsible for the K^+ release, a higher K^+ release at 20 C in comparison with the K^+ release at 2 C should be expected. But as can be seen from Figure 4, this is not the case. We, therefore, conclude that the results presented by Figure 4 should also be explained by a reabsorption of effluxed K^+.

The main conclusions that can be drawn from this investigation are that the efflux rates for K^+ and Na^+ are rather similar. But the net efflux of K^+ can be affected considerably by reabsorption, Efflux is a process which is not selective for either of the two cations, and it may be assumed that this efflux process is a diffussion through the cell membrane. It is unlikely that the Na^+ efflux is mediated by an outward Na^+ pump, because the Na^+ release is not influenced by different metabolic conditions. Differences in the net release of K^+, effected by metabolism, do not prove an active process for the K^+ release, because with maize roots the K^+ release is higher when metabolism is inhibited.

Acknowledgment—The authors gratefully acknowledge the assistance of Miss H. Kerstein.

LITERATURE CITED

1. Blount, R. W. and B. H. Levedahl. 1960. Active sodium and chloride transport in the single-celled marine alga *Halicystis ovalis*. Acta Physiol. Scand. 49: 1-9.
2. Dainty, J. 1962. Ion transport and electrical potentials in plant cells. Annu. Rev. Plant Physiol. 13: 379-403.
3. Epstein, E. 1961. The essential role of calcium in selective cation transport by plant cells. Plant Physiol. 36: 437-444.
4. Etherton, B. 1963. Relationships of cells transmembrane electropotential to potassium and sodium accumulation ratios in oat and pea seedlings. Plant Physiol. 38: 581-585.
5. Etherton, B. 1967. Steady state sodium and rubidium effluxes in *Pisum sativum* roots. Plant Physiol. 42: 685-690.
6. Hiatt, A. J. and R. H. Lowe. 1967. Loss of organic acids, amino acids, K and Cl from barley roots treated anaerobically and with metabolic inhibitors. Plant Physiol. 42: 1731-1736.
7. Higinbotham, N., B. Etherton, and R. J. Foster. 1967. Mineral ion contents and cell transmembrane electropotentials of pea and oat seedlings tissue. Plant Physiol. 42: 37-46.
8. Kylin, A. 1967. Further characterization of the sodium out-pump in *Scenedesmus*. *In:* Isotopes in Plant Nutrition and Physiology. International Atomic Energy Agency, Vienna. pp. 265-270.
9. Lowe, A. G. 1968. Enzyme mechanism for the active transport of sodium and potassium ions in animal cells. Nature 219: 934-936.
10. Marschner, H. 1964. Einfluss von Calcium auf die Na-Aufnahme und die Kaliumabgabe isolierter Gerstenwurzeln. Z. Pflanzenernähr., Düng., Bodenk. 107: 19-32.
11. Marschner, H., R. Handley, and R. Overstreet. 1966. Potassium loss and changes in the fine structure of corn root tips induced by H-ion. Plant Physiol. 41: 1725-1735.
12. Marschner, H. and W. Schafarczyk. 1967. Influx und Efflux von Natrium und Kalium bei Maisund Zuckerrübenpflanzen. Z. Pflanzenernähr. Bodenk. 118: 187-201.
13. Mengel, K. 1963. Die Bedeutung von Kationenkonkurrenzen im free space der Pflanzenwurzel für die aktive Kationenaufnahme. Agrochimica 7: 236-257.
14. Mengel, K. and K. Herwig. 1969. Der Einfluss der Temperatur auf die K-Retention, die Effluxrate und auf die Atmung junger abgeschnittener Getreidewurzeln. Z. Pflanzenphysiol. 60: 147-155.
15. Mengel, K. and B. Schneider. 1965. Die K-Aufnahme als Funktion der Influxrate und der Zellpermeabilität, mathematisch und experimentell an der K-Aufnahme junger Gerstnwurzeln dargestellt. Physiol. Plant. 18: 1105-1114.
16. Mengel, K. and M. Helal. 1967. Der Einfluss des austauschbaren Ca^{2+} junger Gerstenwurzeln auf den Flux von K^+ und Phosphat—eine Interpretation des Viets-Effektes. Z. Pflanzenphysiol. 57: 223-234.
17. Pallaghy, C. K. and B. I. H. Scott. 1969. The electrochemical state of cells of broad bean roots. Aust. J. Biol. Sci. 22: 585-600.
18. Pitman, M. G. 1969. Adaptation of barley roots to low oxygen supply and its relation to potassium and sodium uptake. Plant Physiol. 44: 1233-1240.
19. Poole, R. J. 1969. Carrier-mediated potassium efflux across the cell membrane of red beet. Plant Physiol. 44: 485-490.
20. Post, R. L., C. R. Merritt, C. R. Kinsolving, and C. D. Albright. 1960. Membrane adenosine triphosphatase as a participant in the active transport of sodium and potassium in the human erythrocyte. J. Biol. Chem. 235: 1796-1802.
21. Viets, F. G. 1944. Calcium and other polyvalent cations as accelerators of ion accumulation by excised barley roots. Plant Physiol. 19: 466-480.