Potassium and Nitrate Uptake and Cell Transmembrane
Electropotential in Excised Pea Epicotyls

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Abstract. In contrast to intact etiolated pea seedling tissue (Pisum sativum L.), excised segments immersed in a complete nutrient solution show marked increases in ion content, largely of K\(^+\) and NO\(_3^-\), over a 72-hour period. During this time there is increase in cell electropotential difference, PD. During the initial 6 to 8 hours there is a lag in ion uptake; cell PD, however, increases rapidly from approximately \(-50\) to \(-100\) mv then increases more slowly. The increase in PD precedes and thus may be a prerequisite for the rapid ion accumulation phase. Cell PD increases in either water or nutrient solution but eventually reaches higher levels in the latter. Following water pretreatment of sufficient duration K\(^+\) accumulation shows no lag period. The lag phase noted here appears dissimilar to that of storage tissues.

In an earlier study of excised seedling tissues (2), the Nernst equation was used as a criterion to evaluate the relationship between cell electropotential and ion concentration gradients. Of particular interest was the observation that shoot or coleoptile segments accumulated certain ions, e.g., K\(^+\) and NO\(_3^\text{-}\), in much greater amounts than did the intact seedling; consequently, an equilibrium was not attained as required for a proper test of conformity to the Nernst equation. The present study was undertaken to examine carefully the time course of K\(^+\) uptake of excised pea epicotyl segments to ascertain whether or not there is a close correlation of K\(^+\) and NO\(_3^\text{-}\) uptake to cell electropotential.

Materials and Methods

Pea seeds, Pisum sativum L., cultivar Alaska, were soaked for 2 hours prior to planting in trays of vermiculite well irrigated with nutrient solution. The seedlings were grown for 7 days in darkness at 25\(^\circ\), to the third internode stage.

Two 1-cm segments were excised from the third internode of each seedling after discarding the distal 1.5 cm. The segments were placed in deionized water following a pattern which gave samples each of about 0.4 g, containing equal numbers of first and second cm segments, and having the same mean period of immersion in water prior to blotting and weighing (from 1–2 hrs, depending on the number of samples required). Three samples were taken for the zero-time interval, and the remaining samples were placed each in 25 ml of nutrient solution in 125-ml Erlenmeyer flasks to be shaken for the periods indicated for each experiment. For longer time intervals, the nutrient solution was changed 12-hourly except in the experiment of 120 hours duration in which after 48 hours changes were 24-hourly. After the proper incubation time, samples were removed, blotted and weighed, and frozen for subsequent chemical analyses. Except when stated otherwise, 3 samples were used for each time interval. Both tissue preparation and incubation were carried out at 20\(^\circ\). Under these conditions the increase in fresh weight was small, ranging from 0 to 2 % generally but amounting to an average of 4.3 % in 1 test of 48 hours. Results of experiments involving influx are reported on the initial fresh weight basis and others on the final fresh weight basis (except for fig 4 which is to be compared with figs 2 and 3).

The nutrient solution used for both growing the seedlings and incubating the segments was of the same composition as that used in earlier work (1,2) and referred to as 1X. Its composition in mmoles/l was: KCl, 1.0; Ca(NO\(_3\))\(_2\)·4H\(_2\)O, 1.0; MgSO\(_4\)·7H\(_2\)O, 0.25; NaH\(_2\)PO\(_4\)·H\(_2\)O, 0.904; Na\(_2\)HPO\(_4\), 0.048. The pH value was 5.7. In some experiments this solution was used at 10-fold concentration (10X) at a pH of 5.3, again for both growing the seedlings and incubating the segments. Previous experiments have established that negligible changes in pH (0.0–0.3) occur in 24 hours in either concentration.

Hot water extracts of the frozen tissue samples were made as described previously (2). The extracts were then assayed for K\(^+\), and in some cases for NO\(_3^\text{-}\), to assess net change in content of these ions during incubation.

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Potassium was determined by flame photometry, using a Jarrel Ash "Dial Atom" atomic absorption flame spectrometer. Nitrate was determined colorimetrically by the phenoldisulfonic acid method. It is quite possible that NO₃⁻ reduction could have occurred but this was not investigated.

Apparent K⁺ influx (i.e. labeled influx uncorrected for efflux) was measured using ⁴²K as tracer. The ⁴²K was produced by irradiation of spectroscopically pure K₂CO₃ in the Washington State University reactor. Irradiation of 10 meq K₂CO₃ for 4 hours produced about 1 mc ⁴²K. The irradiated K₂CO₃ was converted to KCl by addition of the appropriate amount of 2 N HCl. This was used to supply all the KCl (1 mmole/l) in the 1X nutrient solution. For 10X solution, 4 mmoles KCl per liter were supplied from this source, carrier KCl being added to make up the balance.

Tissue segments were prepared and incubated in the usual way, but at harvesting the samples were placed in stainless steel planchets and dried to constant weight at 90°C. Counting was performed with the aid of a Nuclear Chicago automatic sample changer, and the counts were adjusted to an arbitrary zero time to correct for decay. Again each time interval was represented by 3 samples.

After allowing the activity to decay (7 days), the oven-dried samples were used to give hot water extracts, so that change in net K⁺ content could be determined by chemical analysis on the same samples.

Transmembrane electrical potential measurements were made in the same manner as reported earlier (7), except that the microelectrodes were connected to the high impedance electrometer via calomel half-cells instead of Ag-AgCl wires. This gave better stability to the measuring circuit. Each value reported is the mean of a total of 12 to 20 readings, made on 3 different segments.

**Results**

When pea epicotyl segments are incubated in a complete nutrient solution, there is a marked increase in the content of both cations and inorganic anions. In 1X solution over a period of 48 hours, cation content increases from about 25 μeq/g fresh weight in the freshly cut tissue to about 70 μeq/g. Of this increase, about 90 % is due to the accumulation of K⁺. Inorganic anion content increases over the same period from about 20 μeq/g fresh weight to 62 μeq/g, and 50 % of this increase is accounted for by NO₃⁻ absorption (2). Examination of the time course of uptake of these 2 ions (K⁺ and NO₃⁻) from 1X solution over a period of 5 days (fig 1) shows that accumulation continues for about 72 hours from the start of incubation, before equilibrium is approached. NO₃⁻ increasing from a barely detectable level to 40 μeq/g and K⁺ approaching equilibrium at about 100 μeq/g fresh weight.

There was a suggestion from experiments of this kind (fig 1) and from the results reported by Higinbotham et al. (2) that some delay in the onset of K⁺ and NO₃⁻ absorption occurred when freshly cut segments were immersed in the incubating solution. Consideration of this possibility, in combination with the observation that the cell electropotential difference, PD, in freshly cut epicotyl segments changed sharply in time, led to a more detailed study of the time course of K⁺ uptake and the development of cell PD during incubation.

Apparent influx of K⁺ (using ⁴²K as tracer) and net increase in K⁺ content were measured on the same oven-dried samples (1X treatment in fig 2, 10X treatment in fig 3), and net increase in K⁺ and NO₃⁻ content were determined from parallel samples which had been fresh frozen (fig 4). The exigencies of this experiment were such that the PD values shown in figures 2 and 3 had to be obtained from a separate batch of plants, grown and incubated under identical conditions. However, where PD and K⁺ content could be measured on the same samples, we have established that the values reported here are typical for the tissue in these conditions.

These results confirm that there is indeed some delay before K⁺ and NO₃⁻ uptake becomes marked, reminiscent of the lag in absorption which is well known in some storage tissues, but of much shorter duration. The lag was particularly noticeable in 1X solution. It was found that cell potential quickly became more negative relative to the exterior during the 8-hour lag period before the onset of K⁺ uptake (fig 2). the 13 to 18 hour value of −119 mv reported earlier was confirmed, and a further slow increase in polarity was observed. A quantitative correlation of cell potentials to ion uptake was not found nor is it to be expected in these non-equilibrium conditions for a variety of reasons. However, it is significant that a greater negative potential developed prior to development of rapid uptake of K⁺ and NO₃⁻.

Segments grown and incubated in 10X solution showed a similar overall pattern. Initial content of K⁺ and NO₃⁻ were much the same as in 1X tissue, but reached rather higher levels of accumulation after 48 hours (figs 3 and 4). Cell potential quickly became more negative, although it began to level off at a lower value than in the 1X treatment, reaching −116 mv at 48 hours, compared with the 1X value of −137 mv. Although in the 10X treatment the lag in absorption of K⁺ and NO₃⁻ was less marked, a period similar to that found with 1X tissue elapsed before absorption reached its maximal rate. The fact that in figure 3 the curve for K⁺ content shows a less distinct lag than that for tracer uptake (apparent influx) may be attributed to experimental error.

Pretreatment of the segments in deionized water for 4 hours reduced the lag period by about 4 hours when the tissue was subsequently transferred to 1X solution (fig 5). The lag period was entirely eliminated by a water pretreatment of 24 hours.
FIG. 1. The time course of K⁺ and NO₃⁻ content of segments incubated for 5 days in 1× solution (solutions changed 12-hourly to 48 hrs then 24-hourly). Each point determined from 1 sample only.

Figs. 2 and 3. The time course of K⁺ content, apparent K⁺ influx and cell PD of segments incubated in the solutions indicated in each figure. The standard error is indicated for each point, where it is greater than the symbol size.

FIG. 4. The time course of K⁺ and NO₃⁻ content of segments incubated in 1× and 10× solutions. The standard error is shown where larger than symbol size.

(fig 6), for when the tissue was transferred to 1X solution, K⁺ uptake immediately assumed the maximal rate observed after 12 hours in tissue given no pretreatment. However, the lag in NO₃⁻ uptake, as reflected by change in content, appeared to persist in the face of water pretreatment. The development of a more negative polarity in the cells was largely independent of the incubation medium, showing no delay when the tissue was incubated in water. although no increase beyond -95 mv was evident until the tissue was transferred to 1X solution. This resulted in a small rapid increase in electronegativity and then the slower increase concomitant with increasing K⁺ content found earlier.

In these experiments the assumption has been made that the cell PD measurements of the cortical parenchyma are typical for the segments as a whole. Microscopic measurements of the 2 vascular and 2 fiber bundles were made and the volumes estimated as right cylinders. The cortex (and epidermis) constitutes 88.4 ± 0.4 % of the segment volume and the bundles, 11.6 ± 0.4%. It seems very likely that the ion absorption pattern of the segments as a whole is typical of the cortical tissue since very
large differences in uptake by the stele would be required to alter it significantly.

**Discussion**

Excised segments of pea epicotyl, upon immersion in nutrient solution, show, in time, marked increases of cell electropotential difference and salt accumulation rates. The changes are complex and, in the absence of an equilibrium, no quantitative correlations are evident. However, a major part of the increase in cell PD occurs during an early lag period of K⁺ and NO₃⁻ uptake from nutrient solution, and a similar increase in cell PD also occurs when the segments are immersed in water. After a sufficient pretreatment in water, transfer of segments to nutrient solution is followed by an immediate uptake of K⁺ at the maximum rate. These findings establish qualitatively that an initial increase in cell PD, unrelated to the external medium, precedes the relatively rapid phase of K⁺ and NO₃⁻ uptake, and, thus, may be a prerequisite for this uptake. It is not known, of course, whether the PD represents a K⁺ diffusion potential or may arise from an electrogenic ion pump, e.g. an inwardly directed anion pump: there is good evidence for the latter (2).

In the intact seedling there exist certain restraints on the epicotyl tissue with respect to K⁺ and NO₃⁻ accumulation, since these ions are far removed from "equilibrium" when epicotyl segments are immersed in the seedling nutrient solution. The data also suggest that the low cell PD encountered with newly excised segments may be typical of intact epicotyls and this may be correlated in some way with the relatively low K⁺ content of the epicotyls in the intact seedling. Unpublished work (of R. B. James and N. Higinbotham) has established that indoleacetic acid lowers the cell PD, and it may be a release from this effect which is observed in excised segments; excision, of course, would remove the segments from the auxin gradient normally occurring in whole plants. That auxin may significantly influence ion uptake in the intact epicotyl at certain stages is further suggested by earlier evidence of auxin effects on ion accumulation in pea epicotyl segments (3). Of course, excision may have other effects which could alter ion fluxes.

There is no reason at present to think that the lag in ion uptake of pea epicotyl segments found here, and noted previously by Palmer and Loughman in respect to phosphate uptake (8), corresponds to that reported for various storage tissues (5, 9, 10, 11). Indeed, the phenomena associated with the lag phase in the 2 types of tissue differ in several respects. Storage tissues are generally quiescent until sliced and subsequently exhibit a marked increase in respiration (4, 6), whereas in pea epicotyls there is evidence (8) that the respiration rate, which presumably is relatively high in the intact seedling, falls rather than increases following excision. The lag period in epicotyl tissue is relatively short and this would seem to preclude the possibility of a de novo synthesis of absorption capacity which, with respect to Cl⁻, at least, is considered to occur in red beetroot slices (5). The lag in net uptake of K⁺ by beetroot disks has been attributed to an initially high efflux of this ion, nullifying the operation of an inherent capacity for uptake (11). That this does not happen in epicotyl tissue is evident from the data in figures 2 and 3, which indicate that initial efflux must be very small. Finally, studies with potato tuber slices aged in water (7) have shown
that the rapid increase in cell PD, reported here for pea stem, does not occur in that tissue. These differences suggest that, although pretreatment in water for sufficient time eliminates the lag in both seedling and storage tissues and must represent internal physiological adjustments, the significance of the lag is not the same in the 2 types of tissue.

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Literature Cited