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Short Communication

Vacuolar and Cytoplasmic Potassium Concentrations in Pea Roots in Relation to Cell-to-Medium Electrical Potentials[1]

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The cause of the cell-to-medium electrical potentials of plant and animal cells is incompletely understood. In some cases, the potentials appear to be diffusion potentials in which potassium ions play a major role (3, 7.) This is indicated A) by potentials which are close to the Nernst potentials for potassium and B) by cell-to-medium potentials which become markedly less negative when the external potassium ion concentration is increased (2, 3, 5, 6, 8, 13).

Some evidence indicates that the cell-to-medium potentials are not potassium diffusion potentials. In the squid axon, for example, a large potential remains after the axoplasm has been replaced with a potassium-free solution (14) [There is conflicting evidence here however (1)]. Similar results have been found in the case of vacuolar perfusion of *Halicystis* and *Nitella* cells (2, 15).

The cause of the cell-to-medium electrical potentials of higher plants has not been extensively investigated. Evidence for *Avena* roots indicates that most of the potential difference occurs between the cytoplasm and the medium, that a negligible potential change occurs between the cytoplasm and the vacuole, and that the cell-to-medium potentials of *Avena* coleoptiles become less negative when the potassium concentration in the medium is increased (5). Also, flame photometric analysis of extracts from roots and tops of pea and oat seedlings show that the potassium concentrations in the cells are much higher than the concentrations of other inorganic ions (7). The above factors indicate that the cell-to-medium potentials could be diffusion potentials caused principally by the cell-to-medium potassium concentration ratios.

In pea roots and stems, however, the cellular potassium concentrations under some conditions did not appear to be high enough to be a major factor in a diffusion potential system (4). This finding has to be qualified, however, because only the average cellular potassium concentration was measured and not the specific cytoplasmic and vacuolar concentrations. Because the vacuole is large relative to the cytoplasm, the cellular potassium concentration probably approximates the concentration in the vacuole. If the major potential difference is between the cytoplasm and the medium, as the data for *Avena* roots suggest, the critical potassium concentration would be in the cytoplasm. In the experiments reported here, an attempt was made to measure this value.

Root tissues were obtained from dark-grown 7-day-old *Pisum sativum* cv. Alaska seedlings. They had grown for 4 days with their roots in an aerated nutrient solution containing (in mmoles per liter) KCl, 10; $Ca(NO_3)_2$, 10; NaH_2PO_4, 9.05; Na_2HPO_4, 0.48; $MgSO_4$, 2.5; (pH 5.3). Previous treatment included washing the seeds and soaking them for 4 hours in distilled water, followed by a 4-day germination period on moistened filter paper. The temperature was 25° for seedling growth and subsequent experimental operations.

The potassium concentrations in the cytoplasm and vacuoles of the root cells were derived from measurements of the potential difference between potassium-sensitive and reference microelectrodes which were inserted with micromanipulators into the cytoplasm or vacuole of cortical parenchyma cells. Electrode insertion was approximately perpendicular to the transverse cut surface (5 mm behind the tip) of a root which was in the above nutrient solution in a lucite chamber. Electrode placement was observed with a microscope having a 40X long-working-distance objective (Unitron Company).

The potassium-sensitive microelectrodes were prepared from Corning NAS 27-4 glass by a method similar to that used by Khuri and co-workers (9). The 1 mm glass tubing was made into microcapillaries with a microelectrode puller like the model PE-2 of Narishige Scientific Company. Open tips of the capillaries (diameter less than 1 μ) were sealed with a microforge and the tip was assumed to be sealed if water would not move into it by capillarity.

Electrodes were filled with an electrolyte (0.1 M KCl buffered at pH 7 with 1 mM tris) by placing

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them in a boiling solution for 15 minutes. Electrodes were stored in the filling solution for up to 2 weeks (longer storage resulted in decreased potassium selectivity).

Each electrode was insulated as follows: It was held, point up, in a micromanipulator; a platinum loop was positioned with another manipulator around the base of the electrode. Polystyrene coil dope (colored with Sudan IV dye) was placed on the loop and the electrode was lowered until it was coated to within 1 μ of its tip. All operations were observed under 400 X magnification. The electrode was calibrated and used immediately after its coating had air-dried for 15 minutes.

Calibration consisted in measuring the potential difference between each electrode and a reference electrode when both were in KCl solutions of known concentration. Electrodes were used if their response was from 40 to 59 mv per 10-fold change in the potassium concentration and if the potential in 10 mM KCl was within 5 mv of the potential in 10 mM KCl plus 10 mM NaCl.

Reference microelectrodes of the Ling and Gerard type (10) were prepared with a vertical microelectrode puller from 1 mm pyrex melting-point capillaries (Corning). In order to minimize tip potentials, the electrodes were first filled in boiling distilled water then placed in 3 M KCl overnight. Electrodes with tips larger than 1 μ or with tip potentials larger than 5 mv (in a 10 mM KCl solution) were discarded.

Silver chlorided silver wires connected the solutions in both the reference and potassium selective microelectrodes to the inputs of a Keithley model 603 electrometer amplifier.

Table I shows the measurements of the potassium concentrations in the cytoplasm and vacuoles of several cells. In many cases both measurements were obtained on a single cell. The average cytoplasmic and vacuolar concentrations were 43 mn and 122 mn respectively.

Table I. *Vacuolar and Cytoplasmic Potassium Ion Concentrations in Pea Root Cells Measured With a Potassium Sensitive Microelectrode*

Root no.	Cell no.	Potassium conc Cytoplasm	Vacuole
		mn	*mn*
1	1	53	100
	2	35	122
2	1	46	...
3	1	...	110
	2	35	...
	3	...	43
4	1	54	130
	2	28	165
	3	43	230
	4	43	105
5	1	...	105
	2	54	105
Mean		43	122
Standard deviation		±9	±49

These are critical data for ascertaining whether or not the vacuole-to-medium potential of −108 mv (obtained with tissues in the above nutrient solution) (4) could be the result of a potassium diffusion potential resulting from either the vacuole-to-medium concentration ratio or the cytoplasm-to-medium concentration ratio. According to the Nernst equation, the maximum diffusion potential which could result from the vacuole-to-medium potassium concentration ratio is −64 mv. The maximum diffusion potential which could be caused by the cytoplasm-to-medium potassium ratio is −37 mv. These data, then, support the view that neither the cytoplasm-to-medium nor the vacuole-to-medium potassium ion concentration ratios could be the cause of the vacuole-to-medium potential of pea root cells.

The use of potassium selective microelectrodes for measurements of potassium concentrations in the small compartments of small plant cells had several limitations and sources of error: First, it was difficult to see the exact location of the electrode tip. Confidence in its location was increased, however, by finding that measured values were distinctly different when the electrode appeared to be in the cytoplasm compared to when it appeared to be in the vacuole.

Another source of error was that these electrodes were sensitive to sodium ions, though to a lesser degree than to potassium ions. Generally, when the potassium concentration in cells exceeds that of sodium, the sodium interference is negligible. In critical work, the sodium concentration in cells can be measured with sodium-selective electrodes and the sodium error of the potassium-selective electrode can be corrected for.

Another problem was the possibility of potassium leakage from the reference electrode which could have increased the measured potassium concentration. This factor was minimized by first inserting the potassium-selective electrode into a cell and then measuring the potential as soon as possible after inserting the reference electrode. The last 2 sources of error cause over-estimates of cellular potassium concentrations and thus would not detract from the principle conclusions of this paper.

If, the cell-to-medium potentials of pea roots is not caused by a potassium concentration ratio, then what is its cause? One possibility is that the potential is composed of a cytoplasm-to-cell wall potential in series with a cell wall-to-medium Donnan potential. This kind of system has been found in *Nitella* (11, 13). Another possibility is that another ion, possibly an organic ion, is involved in a diffusion potential system. Finally, there is evidence for the theory that the potential is caused by an electrogenic transport system. This theory has been suggested by several workers and is supported by experiments in which inhibitors such as dinitrophenol and azide have made the cell-to-medium potential approach zero (5, 12).

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