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Separation of Tonoplast and Plasma Membrane Potential and Resistance in Cells of Oat Coleoptiles

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Summary. Membrane potential and resistance were recorded from parenchymal cells of oat (*Avena*) coleoptiles, using one and two intracellular electrodes. Membrane potential is largest (−100 mV) in impalements with low input resistance (2–4 MΩ), and is less negative (−50 mV) in penetrations with high input resistance (> 20 mΩ). The interpretation is that the electrode lodges in the vacuole, which is positive to the cytoplasm (but still negative to the external solution), and that measurements of net membrane potential are compromised to varying degrees by leakage shunts introduced across the high resistance vacuolar membrane by the electrode. This conclusion is supported by several additional lines of evidence. (1) It is possible to convert large-*R*/small-*V* impalements into small-*R*/large-*V* penetrations by passing excess current through the electrode or by briefly 'ringing' the capacitance neutralization circuit in the amplifier. The cells usually recover their resistance in a few minutes, with a concomitant decrease in the negativity of the membrane potential. (2) Changes in external [K] affect the measured potential by an amount that is independent of the input resistance of the impalement. This is consistent with an effect of $[K]_o$ on the potential of the plasma membrane and the occurrence of leakage shunts primarily at the tonoplast. (3) Quantitatively, the effects of a change in $[K]_o$ on resistance indicate that nearly 90 percent of the input resistance of unshunted cells resides in the tonoplast. (4) The effects of metabolic inhibitors (DNP, CN^-) on potential are smaller in large-*R* than in small-*R* impalements. This observation suggests there are electrogenic pumps contributing to the membrane potential at both the plasmalemma and tonoplast. Finally, we conclude that with an electrode in the vacuole it is possible to record potentials that are dominated by the contribution of the plasma membrane, provided care is taken to select impalements combining both large, negative potential and low input resistance.

Key words plant cells · *Avena* · vacuolar membrane · plasmalemma · membrane potential · membrane resistance

Introduction

The contents of most plant cells are partitioned between two membrane-limited intracellular compartments – a large central vacuole bounded by the tonoplast, and a peripheral layer of cytoplasm one or a few μm in thickness with occasional transvacuolar strands, surrounded by the plasma membrane. In order to arrive at a better understanding of mechanisms of ion uptake and accumulation, there is considerable interest in knowing the magnitude of the transmembrane potentials of both plasmalemma and tonoplast. Measuring the potentials, however, has proved difficult. The cells are encased in a tough cellulosic wall, and due to a higher internal concentration of solutes, they are under considerable turgor pressure. The final position of the tip of a microelectrode that is inserted into a plant cell is largely determined by these anatomical and physiological features. The pipette must first penetrate the elastic cell wall, and if the wall presents resistance and dimples before yielding, the tip will ultimately lodge several μm inside the cell. As the cytoplasmic layer is so thin, the tip will likely be in the vacuole and record across both the tonoplast and plasma membrane in series. In one study, dye injection confirmed that the microelectrodes penetrated the vacuole (Goldsmith, Fernández- & Goldsmith, 1972).

Obtaining unambiguous records from the cytoplasm is therefore a technically demanding problem. Occasional chance visual observations on favorable preparations in which an abrupt increase in negativity was seen to coincide with cytoplasm creeping back over the tip of a pipette only shallowly inserted in the vacuole have provided some useful information (Lüttge & Zirke, 1974; Mertz & Higinbotham, 1976), and results have been obtained by using centrifugation to concentrate the cytoplasm at one end of the cell (Lüttge & Zirke, 1974; Goldsmith & Cleland, 1978), or selecting cells with a high proportion of cytoplasm (e.g. root hairs, Etherton & Higinbotham, 1960; Greenham, 1966). Several abrupt steps in potential are commonly observed as microelectrodes are inserted into plant cells (Etherton, 1970; Ginsburg- & Ginzburg, 1974; Lüttge & Zirke, 1974; Dunlop, 1976; Rona, Pitman, Lüttge & Ball, 1980). Although the general consensus is that the vacuole is positive to the cytoplasm, there is little agreement about the

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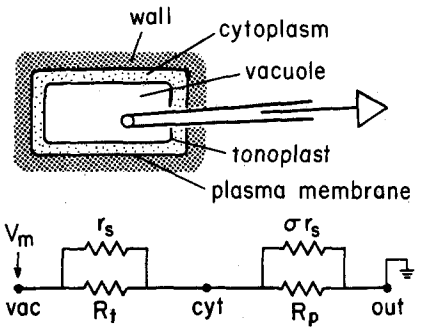

$$V_m = V_t\left(\frac{r_s}{r_s+R_t}\right) + V_p\left(\frac{\sigma r_s}{\sigma r_s+R_p}\right) \quad \cdots\cdots\cdots (1)$$

$$R_m = R_t\left(\frac{r_s}{r_s+R_t}\right) + \beta R_t\left(\frac{\sigma r_s}{\sigma r_s+\beta R_t}\right) \quad \cdots\cdots\cdots (2)$$

where $\beta = R_p/R_t$

Fig. 1. *Above:* Diagram of a coleoptile cell showing the relationship of the cytoplasm and vacuole and their surrounding membranes. In reality, the wall and cytoplasm are thinner than depicted, accounting for only a few percent of the width of the cell. In general, microelectrodes inserted into the cell enter the vacuole. *Below:* Equivalent circuit for the resistance to ground as seen from the tip of a micropipette that has passed through the cytoplasm (*cyt*) and entered the vacuole (*vac*). R_t, resistance of the tonoplast; R_p, resistance to ground from the cytoplasm of an unshunted cell (which is the parallel resistances of the plasma membrane and alternate paths to ground through plasmodesmata and neighboring cells); r_s, electrode-induced shunt resistance across the tonoplast; σr_s, electrode-induced shunt resistance across the plasma membrane, which may differ from r_s by a factor σ. Eq. (1) gives the voltage (V_m) measured across both membranes by an electrode in the vacuole. If both membranes seal around the micropipette so that the shunt resistance r_s is very large, the measured voltage is the sum of the membrane potentials at the tonoplast V_t, and the plasma membrane V_p. Thus in this limiting case, $V_m = V_t + V_p$. If, however, the shunt resistance r_s is not many times larger than R_t and R_p, V_t and V_p are attenuated by the terms in parentheses. One of the conclusions of the present paper is that shunting occurs commonly at the tonoplast, but that it is much less of a problem at the plasma membrane. In other words, in practice the coefficient σ in Eq. (1) assumes a large value. Eq. (2) describes the measured input resistance R_m in the presence of electrode-induced shunts. In the absence of substantial shunts (i.e., large r_s), Eq. (2) reduces to $R_m = R_t(1+\beta)$, where $\beta = R_p/R_t$ is the ratio of the input resistance of the cytoplasm to the resistance of the tonoplast. A second conclusion of this paper is that $\beta \simeq 1/8$

magnitude of the tonoplast potential. The interpretation of the voltage steps seen on advancing a microelectrode has proved both difficult and controversial (e.g. Lüttge & Zirke, 1974; Anderson & Higinbotham, 1975). The principal problem is in distinguishing the voltage contributions of different intracellular compartments from changes in tip potential of the electrode caused by deformation or transient plugging as wall or membrane material is carried in front of the electrode.

Plant physiologists have often tended to assume that higher plant cells will prove similar to the giant cells of charophytes (Hope & Walker, 1975, p. 91), where the tonoplast contributes little to the overall voltage and resistance of the cell. Goldsmith and Goldsmith (1978) have pointed out, however, that discrepancies between different published measurements on coleoptile cells of *Avena* could be understood if the specific resistance of the tonoplast is higher than that of the plasma membrane, and if significant leakage shunts are introduced by the electrode. If high input resistance is used as the criterion for an acceptable penetration, the electrode will measure the algebraic sum of the voltages across the plasma membrane and tonoplast, whereas if large membrane potentials are the criterion, the contribution of the tonoplast to the measured resistance and potential will be partially shunted by leaks. In this interpretation, the true input resistance of the vacuole is several times higher than the input resistance of the cytoplasm; therefore, leakage shunts introduced by the electrode reduce disproportionately the contribution of the tonoplast to the measured values of voltage and resistance. These relationships were developed quantitatively (Goldsmith & Goldsmith, 1978) and are summarized in Fig. 1.

Support for this hypothesis was obtained by Goldsmith and Cleland (1978). By arresting cytoplasmic streaming with cytochalasin B and centrifuging the cytoplasm of coleoptile cells to one end, they were able to put electrodes into the cytoplasm and vacuole of the same cell and show that the input resistance of the vacuole was several times larger than the input resistance of the cytoplasmic compartment.

In the present paper we explore these ideas further. We first demonstrate that over a considerable range there is an inverse relationship between membrane voltage and input resistance of coleoptile cells. We then show that in response to local disturbance at the site of penetration, changes in external potassium concentration, and metabolic inhibitors, the relationship between recorded membrane potential and input resistance is in accord with the model of Fig. 1. Moreover, these experiments lead to some quantitative conclusions about the magnitudes of the transmembrane potentials at the plasma membrane and tonoplast and the relative input reistances of the cytoplasm and vacuole, as well as the intracellular distribution of electrogenic pumps.

Materials and Methods

Plant Material

Oat seeds (*Avena sativa* L. cv. Victory; Swedish Seed Assoc., Svalöf, Sweden) were soaked in distilled water for about 3 hr and

then planted in washed damp vermiculite. The seedlings were germinated and grown in the dark for four days. Coleoptiles 2–3 cm in length were separated from the leaf. The apical 2 mm of the coleoptiles were removed and the coleoptiles were split lengthwise, with a new razor blade, through the sides of the coleoptile not containing vascular bundles. The split coleoptile sections were bathed in a medium consisting of 1 mM KCl, 0.1 mM $CaCl_2$ buffered with 1 mM 2(N-morpholino)ethane sulfonic acid (MES; Sigma, St. Louis, Mo.) and titrated to pH 6 with Tris(hydroxymethyl) aminomethane (Trizma Base; Sigma). The magnitude of the membrane potential, especially in cells adjacent to the cut surface, increased in tissue that was soaked for several hours after splitting. Thus most of the data presented here are from tissues that had been soaked in the medium for at least 3 hr prior to recording. The tissue was illuminated during experiments by a tungsten microscope lamp.

Electrical Recording

The recording chamber was a narrow Lucite trough, 40 mm in length and 6 mm in diameter, lined with Sylgard (Dow Corning Corp., Midland, Mich.). The section was placed in the middle, with its cut surface upward, and secured to the Sylgard by tiny platinum staples looped over the tissue and pushed into the Sylgard. Fresh solution was introduced into one end of the bath through a plastic manifold that allowed rapid alteration of the composition of the bath. The solution was removed from the opposite end of the bath by means of an aspirator, which allowed a constant flow of fresh solution through the bath. The recording bath contained about 0.5 ml of solution, which was replaced every 15 sec. The section was observed from above at 150 × magnification with a compound microscope and prism to reinvert the image. The reference electrode (Ag/AgCl connected via a 3 M KCl-agar bridge) was at the downstream end of the bath.

The micropipettes were drawn from fiber-filled thin-walled glass capillaries (No. 30-30-0, Federick Haer and Co., Brunswick, Me.) and filled with 3 M KCl by conventional techniques. They had resistances of 6–16 MΩ as measured in 1 mM KCl. The micropipettes were connected to a high impedance amplifier (Electronics for Life Sciences, Rockville, Md.) by a Ag/AgCl wire. The voltage record was displayed on an oscilloscope, photographed, and simultaneously recorded on a chart recorder. Membrane resistances were measured by passing 1 nA of current through the recording electrode and using the bridge circuit in the amplifier or by using two electrodes, one to pass current and the other to record voltage changes. Electrode resistance was monitored regularly.

Results

Relation Between Membrane Potential and Input Resistance

When many cells are sampled, the recorded input resistances R_m vary by a factor of more than 30. Moreover, there is an unexpected relationship between membrane potential V_m and input resistance R_m: impalements that exhibit lower input resistances have larger membrane potentials, which is opposite from the usual expectation based on considerations of leakage shunts (Fig. 2). This result is readily understood, however, if the cytoplasm is about −100 mV with respect to the outside of the cell, the vacuole is at least +40 mV to the cytoplasm, and $R_t \gg R_p$ [Fig. 1, $\beta \ll 1$ in Eq. (2)]. High resistance impalements would be those with a better seal between the membrane and the electrode [large r_s in Eqs. (1) and (2)], and in which V_m approximates the algebraic sum of plasmalemma and tonoplast potentials. Low resistance impalements, on the other hand, would have significant leaks. But with $R_t \gg R_p$, even equal leaks would shunt V_t more completely than V_p, and to a first approximation, a pipette tip located in the vacuole would be electrically in the cytoplasm. If the vacuole were negative to the cytoplasm, or if most of the input resistance of the cell were associated with the plasma membrane, the most negative potentials would be associated with the greatest input resistances. Neither of these alternatives is therefore consistent with the results in Fig. 2.

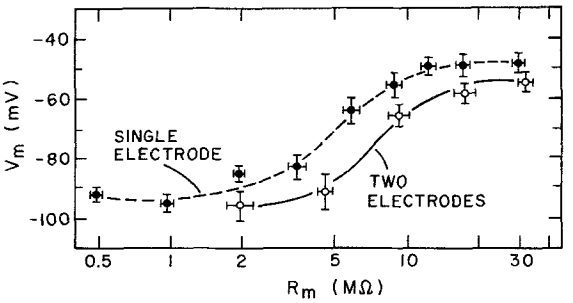

Fig. 2. An inverse relationship between net membrane potential V_m and input resistance R_m is observed when many cells are sampled. Oat coleoptile cells were bathed in 1 mM KCl, 0.1 mM $CaCl_2$, 1 mM MES at pH 6, and the membrane potentials and input resistances were recorded with intracellular electrodes. The results were lumped into nonoverlapping classes of input resistances for the purpose of calculating standard errors and plotting the data. The dashed curve (422 cells) shows the results when R_m was measured with a single electrode and bridge circuit. The solid curve (36 cells) was measured with two micropipettes in the cell, one for passing current and the second for recording voltage changes

The experimental results in Fig. 2 were obtained in two ways. Many of the cells were penetrated with a single electrode, and R_m was measured with a bridge circuit (dashed curve, 422 cells). Because this technique is not reliable for cells with the lowest values of input resistance, a second series of measurements was made with two electrodes in each cell, one for passing current and the other for measuring changes in membrane voltage (solid curve, 36 cells). With two electrodes, input resistances of less than 1 MΩ were not observed. However, the dashed and solid curves show the same sigmoid relationship between V_m and log R_m, demonstrating that this relation does not depend on the method used to measure input resistance. The curves are displaced by 10 mV on the voltage axis, due to the fact that the second set of measurements was done after a longer presoaking of the tissue, which results in more negative membrane potentials, particularly of the surface cells.

The curves were drawn through the data points by eye; reasons for the sigmoid shape will be treated in the discussion.

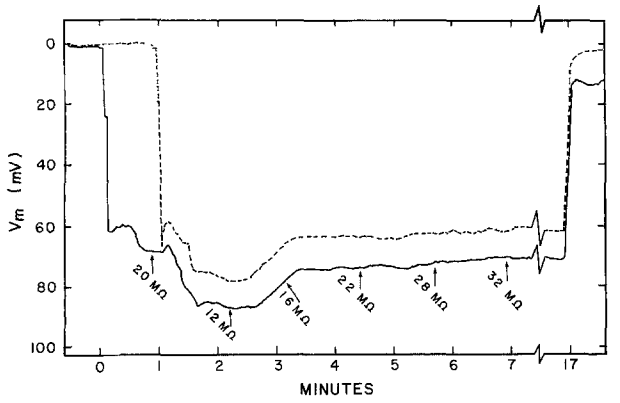

Fig. 3. Inserting a second micropipette into a cell usually causes a decrease in input resistance and an increase in membrane potential. This Figure shows the voltages recorded by two electrodes that were inserted into the same cell at approximately 0 and 1 min on the time axis. Values of input resistance were read at intervals from the oscilloscope records at the times indicated. Prior to the insertion of the second electrode, the input resistance was determined by the bridge method. All subsequent values were measured with two electrodes. V_m is inside negative

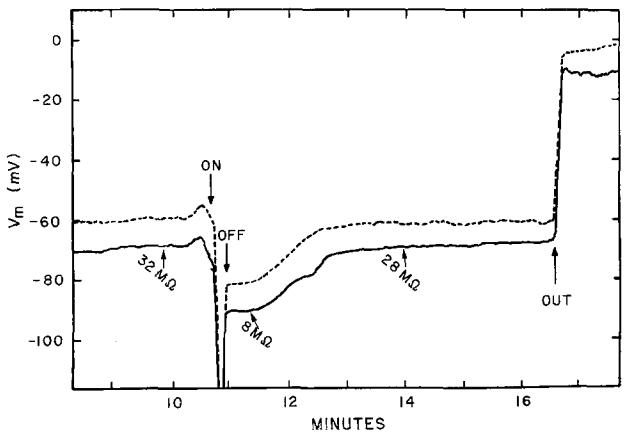

Fig. 4. A disturbance that causes a decrease in R_m results in an increased negativity of V_m. This is a continuation of the recording shown in Fig. 3. Between the arrows at 'on' and 'off', a 10-sec, 20-Hz train of large (*ca.* 30 namp), 48 msec, hyperpolarizing pulses was passed through one electrode. Both before and after the pulse train, R_m was measured at intervals by passing 1-namp pulses through one of the electrodes and recording the voltage change with the other. About 30 sec after the current stopped, the membrane began to seal, and the cell recovered during the following 90 sec

Experimentally Induced Changes in V_m and R_m Observed in Single Cells

The inverse relationship between V_m and R_m can also be observed in an individual cell. For example, introduction of a second electrode (Fig. 3, broken trace, at 1 min) usually caused a fall in input resistance accompanied by an increase in the size of the membrane potential. In the example of Fig. 3 the cell then recovered starting at about 2.5 min. (Note that the tip of the first electrode (solid trace) developed a further 10-mV negativity about 40 sec after entry, which persisted after the pipette was withdrawn from the cell. Spontaneous changes in electrode properties are commonly seen with this material.)

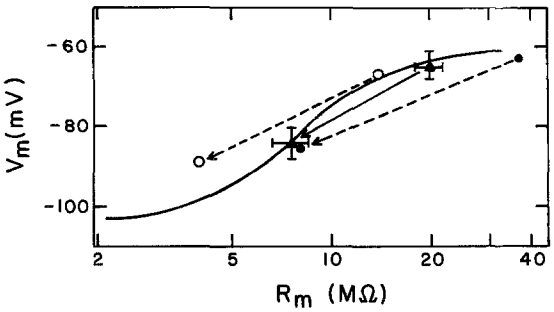

Fig. 5. The filled triangles are a summary of 15 experiments showing the net effect on V_m of decreasing R_m by local disturbance. The arrows show the direction of change of R_m and V_m, and the curve is a replot of the solid curve in Fig. 2. Error bars show ± 1 standard error of the mean. The two pairs of data points shown with circles are examples of two of the individual cells that contributed to the average. The open circles are an experiment in which R_m was altered by overcompensating the capacitance neutralization circuit; the solid circles show the response to a train of large, hyperpolarizing current pulses, like the example shown in Fig. 4

Other kinds of disturbance can convert large-R/small-V cells into their small-R/large-V counterparts. The cell of Fig. 4 was impaled with two electrodes. At about 11 min a large hyperpolarizing current was passed through one of the pipettes for several seconds. When the current stopped, the input resistance had dropped from 32 to 8 MΩ and the membrane potential had increased in magnitude by about 20 mV. During the next 1.5 min, the cell recovered resistance and the membrane potential became less negative. Similar results can be observed by overcompensating the capacitance neutralization circuit in the electrometer until it rings.

Figure 5 shows the average results of 15 experiments, as well as an example of an experiment of each type. The solid curve is a replot of the lower curve in Fig. 2. The solid circles are a case in which the changes in R and V were caused by large currents; the open circles show an experiment in which the capacitance neutralization circuit in the amplifier was made to ring briefly. In most cases the cells recovered in a few minutes. The mechanism of a similar electrical breakdown in algal cell membranes has been discussed by Coster and Zimmermann (1975).

Changes in External K^+ Affect the Potential at the Plasma Membrane but not the Tonoplast

Because only the plasma membrane and not the tonoplast is directly exposed to the external solution, changes in external potassium concentration should alter V_p but not V_t. Consequently, changes in $[K]_o$

Table 1. Effect of respiratory inhibitors on membrane potential

Input resistance MΩ	Membrane potential (mV)					
	−DNP	+DNP	Change	−KCN	+KCN	Change
0.5–2	−101±6	−39±3	+62	−96±6	−38±3	+58
>10	− 60±4	−25±6	+35	−64±4	−31±2	+33

Cells were impaled in 1 mM KCl, 0.1 mM $CaCl_2$, 1 mM MES buffer at pH 6, and V_m and R_m were recorded with a single micropipette. Then the bath medium was changed to one which additionally included 1 mM DNP or 1 mM KCN and the resulting change in membrane potential was recorded. For the purpose of comparison the data were lumped into two nonoverlapping categories depending on the initial size of R_m.

should alter V_m by the same *absolute* amount in large-R and small-R cells. This prediction is borne out by the experiment in Fig. 6. Membrane potential was monitored as $[K]_o$ was increased from 0.3 mM by superfusion, and for analysis the cells were grouped into two nonoverlapping categories depending on their input resistance. A single curve based on the Goldman-Hodgkin-Katz expression (Goldman, 1943; Hodgkin & Katz, 1949)

$$\Delta V = \frac{RT}{n\mathcal{F}} \ln\left(\frac{0.3+c}{[K]_o+c}\right)$$

fits both sets of data points with only a vertical displacement on the voltage axis, where $c = 5.7$ mM is a constant. If the membrane potential were solely a diffusion potential, the simplest interpretation of c would be $(P_{Cl}/P_K)\,[Cl]_i$, the ratio of permeability coefficients times the internal chloride concentration. As there is evidence that an electrogenic pump contributes to the membrane potential, the physical interpretation of c is more complex.

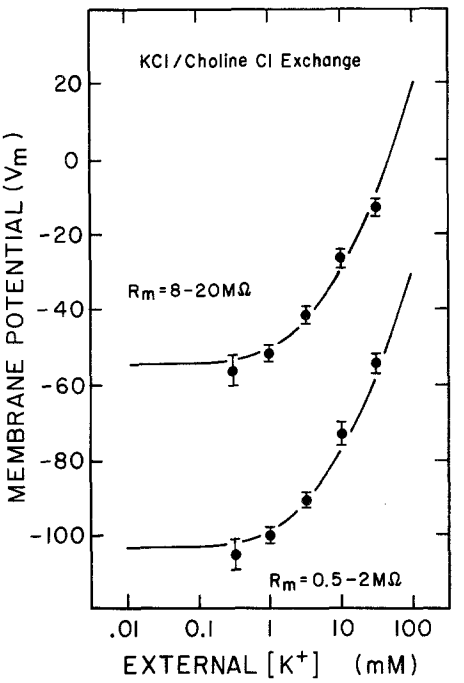

Fig. 6. Responses of high and low input resistance impalements to changes in $[K]_o$. Coleoptile sections were initially bathed in a solution containing 0.3 mM KCl, 29.7 mM choline Cl, and when V_m had stabilized, the choline was replaced stepwise with K^+ by changing the superfusing medium, and the decrease in negativity of potential was recorded. All solutions also contained 0.1 mM $CaCl_2$, 1 mM MES at pH 6, and increases in $[K]_o$ were accomplished without changing $[Cl]_o$, osmolarity, or total ionic concentration. Potential responses to changes in $[K]_o$ were complete in about 1 min and showed no transient overshoots. When the electrodes were inserted only in the bath, they showed <3 mV change in tip potential with alterations in $[K]_o$.

Metabolic Inhibitors Affect the Potential at Both Membranes

In the preceding experiment, the change in V_m caused by changes in $[K]_o$ was independent of the cell's resistance because only the plasma membrane was affected by $[K]_o$. By contrast, a treatment that alters both V_p and V_t should affect V_m to different extents in low and high resistance cells. Other evidence indicates that an electrogenic proton pump contributes to V_p, and as the vacuole is characteristically >1.5 pH units acid and 40–50 mV positive to the cytoplasm, it would not be surprising if the tonoplast also pumped protons. If so, a metabolic inhibitor that deprives electrogenic pumps of their source of energy should influence both V_p and V_t.

Table 1 shows the effects of dinitrophenol (DNP) and cyanide on the measured membrane potential of small- and large-R cells. Unlike an increase in $[K]_o$, both DNP and CN^- cause a change in V_m whose magnitude depends on the input resistance R_m of the cell. Thus these inhibitors appear to affect the potential at both the plasma membrane and tonoplast. Specifically, the effect of DNP and CN^- on V_m is larger in low-resistance cells. This is because in small-R cells the DNP- and CN^--inhibitable contribution to V_t is reduced by shunting, and the effect of DNP and CN^- on V_p is largely unmasked.

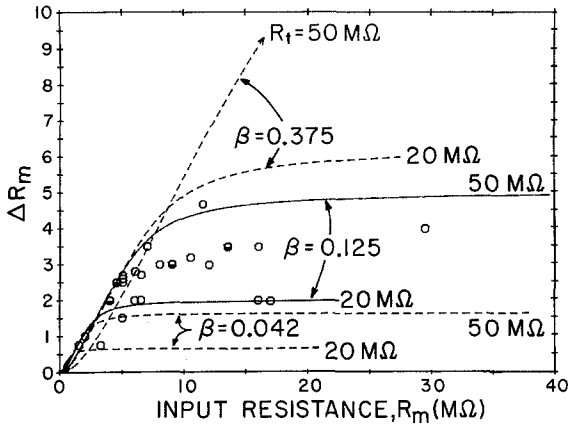

Fig. 7. Response of R_m to 300 X increase in $[K]_o$ in the bathing medium, plotted as a function of R_m in the low [K] solution. R_m was determined by the two-electrode method. Cells were impaled in a bathing medium containing 0.1 mM KCl, and once a steady resistance and potential were recorded, the bathing medium was exchanged for one containing 30 mM KCl and the change in R_m was measured. Half-filled circles indicate identical measurements from two cells. The solutions also contained 0.1 mM $CaCl_2$, 1 mM MES at pH 6, and were balanced osmotically with mannitol. Theoretical curves calculated with $\beta=0.125$ and $\sigma=10$ bracket the experimental points for R_t between 20 and 50 MΩ. *See Discussion* for a further description of the theoretical curves

Effect of $[K]_o$ on Input Resistance

Changes in $[K]_o$ should alter the input resistance of the cytoplasmic compartment R_p, but not the resistance of the tonoplast R_t. Consequently, changes in $[K]_o$ should have a proportionally greater effect on small-R than or large-R impalements, where most of the resistance is due to the tonoplast. Cells were impaled with pairs of electrodes and the input resistance measured by passing inward pulses of current while the cells were immersed in 0.1 mM KCl. The concentration of KCl in the bath was then increased to 30 mM by superfusion, and the resistance remeasured. In Fig. 7 the decrease in resistance is plotted for 28 cells versus the input resistance of the cell as originally measured in 0.1 mM KCl. Figure 7 shows that the change in R_m caused by a 300 X increase in $[K]_o$ approaches a constant 4 MΩ with cells of high input resistance. This plateauing of the data is to be expected if only R_p is influenced by the change in $[K]_o$ and if R_t dominates the input resistance of impalements with high R_m. The results are analyzed further in the Discussion to yield an estimate of R_p/R_t. A maximum value of about 4 MΩ for the cytoplasmic compartment is also consistent with the data in Fig. 2, where membrane voltage starts to become less negative when R_m exceeds a value of about 4 MΩ, indicating an increasing contribution from the tonoplast.

Discussion

Estimate of V_p and V_t

The data presented above support the hypothesis that the input resistance of the vacuole is larger than the input resistance of the cytoplasm, and that the large variation in measured input resistance is due in part to the variable size of leakage shunts. The results in Fig. 2 indicate that these leakage shunts intrude primarily at the tonoplast. If the absolute value of the leak were always the same at both membranes. Eq. (1) predicts that the cells with the very lowest input resistance would also have small membrane potentials (*see also* Fig. 3 in Goldsmith & Goldsmith, 1978). In other words, the curves in Fig. 2 should turn toward the origin at their left end, with V_m becoming less negative for impalements with the smallest values of R_m. Figure 2 shows, however, that the cells with the lowest input resistances have the most negative (largest) membrane potentials. This is partly because in small-R cells, a substantial membrane potential is the only available indicator of a successful penetration, so impalements with both small R_m and small V_m would be routinely rejected by the experimenter. The first conclusion to be drawn from Fig. 2 is that in small-R/large-V impalements, leakage shunts at the plasma membrane are not seriously degrading V_p; i.e., σ in Eq. (1) is large (probably at least 10–100, *see below*), and the measured voltage V_m approaches the potential across the plasmalemma V_p. To study V_p, one should therefore concentrate attention on small-R/large-V penetrations.

The collapse in V_m observed with higher input resistance impalements reflects the presence of tighter seals between the electrode and the tonoplast. As V_t is oppositely oriented with respect to V_p, the net voltage difference between the tip of the pipette and the external solution becomes less negative as the shunting of V_t decreases. What is less obvious is why the curves in Fig. 2 tend to flatten again at high values of R_m, because with large σ, Eq. (1) describes a monotonic decrease in the magnitude of V_m (to the limiting value of V_p+V_t) as shunt resistance r_s increases. The sigmoid nature of the curve is to be expected, however, because the cells have a distribution of sizes (and thus input resistances), with the larger and more visible cells represented more frequently in the sample from which recordings are made.

These conclusions about the basis for the sigmoid shape of the curves in Fig. 2 were reached after using a computer to model a population of cells with a distribution of values of tonoplast resistance, each

subject to an indeterminate amount of electrode-induced shunting. Theoretical curves showed the necessary shape at the low resistance end only with minimum values of σ approaching 100. As described below, the experiment of Fig. 7 also indicates that $\sigma > 10$. The conclusion that there is relatively little shunting at the plasma membrane is therefore firmly supported by two independent kinds of experiment. Similarly, the decrease in slope at the high resistance end of the curve was only obtained when the frequency distribution of R_t was weighted to favor smaller values (i.e., larger cells). However, as we do not have an independent measure of the frequency distribution of R_t in the population of cells sampled in Fig. 2, we will conclude only that a skewed distribution is a sufficient condition to cause the curves to flatten above $R_m = 20$ MΩ.

If we assume that at the right-hand end of the curve, $V_m = -55$ mV $\simeq V_p + V_t$, then, under the conditions of these experiments, with the absolute value of $V_p > 95$ mV (cytoplasm negative), $V_t > 40$ mV (vacuole positive to cytoplasm). Note that −95 and 40 mV are minimal values for the sizes of these membrane potentials; the computer simulation suggests that each could be greater in magnitude by as much as 10 mV.

Consideration of an Alternative Explanation

If neighboring cells were pumping ions electrogenically at very different rates, in a large sample one might observe an inverse relationship between V_m and input resistance. In some cases, cells in which ion pumping has been blocked by respiratory inhibitors may have less negative potentials and greater resistances than uninhibited cells (Anderson, Hendrix & Higinbotham, 1974; Keifer & Spanswick, 1978, 1979; Drake, 1979). Thus a population of cells in different physiological states might be the basis for an inverse relationship between V_m and R_m. This interpretation, however, is not supported by other data. The large-R/small-V impalements can, in the same cell, be reversibly converted to small-R/large-V impalements by experimentally decreasing R_m (Figs. 3–5). As it is more reasonable to suppose that the experimental treatments increase leakage shunts rather than stimulate an electrogenic pump, the results of this experiment indicate that the inverse relationship between R_m and V_m is based on the presence of two intracellular compartments with opposite potential polarities and not a distribution of physiological states in the population of cells sampled.

Estimate of R_p/R_t

Cells with the lowest input resistance have the most negative membrane potentials because of three conditions: (i) $\beta = R_p/R_t \ll 1$; (*ii*) the vacuole is positive to the cytoplasm; and (*iii*) many impalements have substantial leakage shunts across the tonoplast. We shall now show how the effect of $[K]_o$ on R_m (Fig. 7) leads to an estimate of β.

The curves in Fig. 7 are based on Eq. (2). If a number of cells with a fixed tonoplast resistance are sampled and there is a random variation in shunt resistance r_s, the measured values of input resistance will range downward from a maximum of $R_m = R_t(1+\beta)$. As we shall see, the data on the effect of $[K]_o$ are well fit if R_t for most cells lies in the range 20–50 MΩ. This is in the range of values observed in an earlier study in which large resistance was used as the criterion for an acceptable penetration (Goldsmith et al., 1972).

Because only the plasmalemma is exposed to the external solution, the effect on R_m of changing $[K]_o$ is primarily on R_p (rather than on R_t). Since we know the relationship between V_p and $[K]_o$ (Fig. 6), we can calculate the dependence of R_p $(=\beta R_t)$ on $[K]_o$ for various values of β from constant field theory (Eq. 6.0 in Hodgkin & Katz, 1949). The effect of $[K]_o$ on R_m also depends on r_s [Eq. (2)]. The solid curves in Fig. 7 show the expected change in R_m on increasing $[K]_o$ from 0.1 to 30 mM, plotted as function of R_m (as measured in 0.1 mM K^+), for two values of R_t (20 and 50 MΩ) and $\beta = 0.125$. R_m (abscissa) and ΔR_m (ordinate) both depend on the leakage resistance r_s, which was the independent variable in the calculations.

There are several conclusions to be drawn from this analysis. (1) Some spread of the data is to be expected from the distribution of cell sizes, but nearly all the data points are embraced by the curves for $R_t = 50$ MΩ and $R_t = 20$ MΩ, with $\beta = 0.125$. (2) The calculations are sensitive to the parameter β; as shown by the broken curves, threefold increases or decreases in the size of β give very poor fits. (3) It is not possible to play off increases in β against decreases in R_t and still observe a satisfactory fit to the experimental results. As proportionally more of the input resistance is assigned to R_p, the equations predict larger changes in ΔR_m than are actually observed. (4) Although it is not shown in Fig. 7, in order for the steeply rising limbs of the curves to follow the data points convincingly, σ must be at least 10–100. As mentioned above, this experiment therefore provides independent evidence that the leakage shunts are substantially larger at the tonoplast than at the plasma membrane.

In summary, the presence of cells with input resistance in excess of 20 MΩ (*see also* Goldsmith et al., 1972), coupled with the effects of $[K]_o$ on input resistance, lead to the conclusion that most of the input resistance of unshunted cells resides in the tonoplast, with the input resistance of the vacuole about 8 times larger than the input resistance measured from the cytoplasm. The value of $\beta=0.125$ lies between previous estimates of 0.35 from measurements on centrifuged cells (Goldsmith & Cleland, 1978), and 0.05–0.1 based on a theoretical argument having to do with the detection of intercellular coupling (Goldsmith- & Goldsmith, 1978). A value of $\beta=0.125$, however, does not necessarily mean that the specific resistance of the tonoplast is 8 times greater than that of the plasma membrane. The low input resistance of the cytoplasmic compared to the vacuolar compartment could be due in part to intercellular coupling through plasmodesmata (Spanswick, 1972; Drake, Carr & Anderson, 1978).

Origin of the Membrane Potential

Although the primary purpose of these experiments was not to study the origin of the membrane potential, the results have some bearing on the question. In agreement with earlier studies (Etherton & Higinbotham, 1960; Higinbotham, Etherton & Foster, 1964) the effects of changes in external [K] indicate the presence of an ionic diffusion potential at the plasma membrane, and with $[K]_o > 5$ mM, the passive fluxes of K^+ are substantial relative to the fluxes of other ions present. The effects of metabolic inhibitors that interfere with the availability of internal ATP (Table 1) are most simply interpreted as a decrease in the activity of electrogenic ion pumps. An electrogenic efflux of protons is present at the plasma membrane (Higinbotham, Graves & Davis, 1970; Cleland & Lomax, 1977; Cleland, Prins, Harper & Higinbotham, 1977; Poole, 1978; Marrè, 1979) and our experiments suggest a similar transport occurs from cytoplasm to vacuole. Fischer, Lüttge and Higinbotham (1976) concluded that the electrogenic pump in moss cells resides exclusively at the plasmalemma; however, this conclusion might need to be revised if shunting of the tonoplast potential is also a problem in these cells. Further work is required to address the origins of V_p and V_t more directly.

Comparison with Other Work on Higher Plants

Previous measurements of input resistances of coleoptile (Higinbotham, Hope & Findlay, 1964; Spanswick, 1972; Etherton, Keifer & Spanswick, 1977) as well as other higher plant cells (Spanswick, 1972, Anderson, Hendrix & Higinbotham, 1974; Prins, Harper & Higinbotham, 1980; Rona et al., 1980) fall at the low end of the range of values we record. In these other cases, however, highly negative membrane potentials were used as the criterion for successful impalements. Our analysis indicates that such impalements reflect the properties primarily of the plasma membrane, not because the tonoplast has negligible resistance and potential, but because it has been largly short-circuited by leakage. Other measurements of V_t reported for higher plants range from 0 to +60 mV (Etherton & Higinbotham, 1960; Ginsburg & Ginzburg, 1974; Lüttge & Zirke, 1974; Dunlop, 1976; Mertz & Higinbotham, 1976; Rona et al., 1980); moreover, values of β that can be inferred from previous work on other plants range from 1 to 5 (Greenham, 1966; Rona et al., 1980). Some of these discrepancies may reflect the use of different tissues or species, but the possibility that shunting at the tonoplast accounts for much of the variation calls for further examination.

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