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Electrical Potentials in Stomatal Complexes[1]

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ABSTRACT

Guard cells of several species, but predominantly *Commelina communis*, were impaled by micropipette electrodes and potential differences measured that occurred between cell compartments and the flowing bathing medium. The wall developed a Donnan potential that was between −60 and −70 millivolt in 30 millimolar KCl at pH 7. The density of the fixed charges ranged from 0.3 to 0.5 molar; its dependence on pH was almost identical with the titration curve of authentic polygalacturonic acid. The vacuolar potential of guard cells of *Commelina communis* L., *Zea mays* L., *Nicotiana glauca* Graham, *Allium cepa* L., and *Vicia faba* L. was between −40 and −50 millivolt in 30 millimolar KCl when stomata were open and about −30 millivolt when stomata were closed. The vacuolar potential of guard cells of *C. communis* was almost linearly related to stomatal aperture and responded to changes in the ionic strength in the bathing medium in a Nernstian manner. No specificity for any alkali ion (except Li^+), ammonium, or choline appeared. Lithium caused hyperpolarization. Calcium in concentrations between 1 and 100 millimolar in the medium led to stomatal closure, also caused hyperpolarization, and triggered transient oscillations in the intracellular potential. Gradients in the electrical potential existed across stomatal complexes with open pores. When stomata closed, these gradients almost disappeared or slightly reverted; all epidermal cells were then at potentials near −30 millivolt in 30 millimolar KCl.

Stomatal movement is the result of a transfer of inorganic ions, including H^+, between guard cells and the tissue surrounding them, and of the metabolism of organic acids within the guard cells. We do not know the metabolic event which occurs first in stomatal opening nor do we know the process initiating closing. Inasmuch as in each case transfer of electrolytes is involved, we can hope that measurements of electrical potentials in guard cells and their neighbors will provide some indication which processes might be primary and which might be secondary. Electrical potentials have already been measured in stomatal cells, but the body of results is not consistent. Penny and Bowling (14) determined that no electrical potential differences existed between the cells of a stomatal complex of *Commelina communis*, but Gunar *et al.* (6) reported that a potential gradient was present across the stomatal complex of *Tradescantia albiflora*, a species that like *C. communis* belongs to the spiderwort family. Penny and Bowling concluded from their results that K^+ as well as Cl^- were actively transferred during stomatal opening and closing. Gunar *et al.* (6) determined that guard cells were more negative than subsidiary cells by about 30 mv, which could indicate that only anions are transported actively during stomatal opening and K^+ follows passively. Pallaghy (12) found that guard cells of tobacco stomata with open pores were negatively charged relative to the bathing solution and that the potential difference changed in a Nernstian pattern when the ionic strength of KCl and NaCl in the solution was varied. Apparently, the permeability of the guard cell membranes for cations exceeded that for chloride, and a corresponding diffusion potential was established when epidermal strips were brought into solutions of alkali chlorides. Alternatively, the observed potentials could have been Donnan effects. Although Moody and Zeiger (10) observed light-dependent, transient potential changes in onion guard cells which they interpreted as being manifestations of an electrogenic mechanism, the evidence for an electrogenic component of the potential difference between guard cells and their bathing medium is still equivocal.

We felt that a new electrophysiological exploration of epidermal cells in general and of stomatal cells in particular was necessary. We did not succeed in keeping a micropipette in place in a cell of a moving stoma, so we restricted our measurements to cells of stomata that stayed either open or closed; our results apply only to stomata that are in the quasistationary state.

MATERIALS AND METHODS

Plants and Preparation of Epidermal Samples. Plants of *C. communis* L. were grown in growth chambers (22/20 C day/night; 85% RH; 14.5 h light; 85 w m^{-2} from fluorescent tubes, General Electric FR96T12-CW-1500). The first and second fully expanded leaves were removed from the tip of a branch and the basal half of each leaf was cut into two longitudinal sections. These sections were floated, with the abaxial side up, on distilled H_2O for 3 to 6 h either in the light (85 w m^{-2} from mercury vapor lamps, General Electric H 400 RD X 33-1) and CO_2-free air to allow stomata to open or in ambient air and darkness to prevent stomata from opening. Using watchmaker's forceps, part of the abaxial epidermis was removed as a strip of about 5 × 10 mm^2, rinsed with distilled H_2O, and mounted, cuticle side down, onto a Lucite block of 12 × 4 × 4 mm^3 that was wrapped with Parafilm. The epidermal sample was secured in place by wrapping three overlapping Parafilm strips over the edges of the tissue and around the Lucite block. The Lucite block, with mounted epidermis, was then placed in a snuggly fitting pocket in a 57 × 7 × 7 mm^3 channel milled into a Lucite plate. Bathing solution (about 3 ml) was pipetted into the channel such that the tissue was covered with a thin layer of solution. Unless otherwise mentioned, the solution was continuously flushed at a flow rate of 0.5 ml/min by a peristaltic pump. Channel and pump formed a closed loop of 226 ml capacity. Solutions were changed by momentarily stopping the flow and switching the liquid in the reservoir and part of the tubing. Peeling of epidermis and mounting damaged some of the ordinary epidermal cells; damaged cells were collapsed and could be easily recognized. All stomatal cells, however, remained inflated. Between the time of harvest and mounting, leaves and epidermal

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strips were in room light (about 0.5 w m^{-2} from fluorescent tubes, General Electric F 400 w). Under the microscope, the tissue received additional illumination from a tungsten lamp through a glass-fiber bundle and a blue filter. The filter was present to enhance visibility of the tissue; filtering the light had no noticeable effect on the electrical properties of the stomatal cells.

Plants of *Vicia faba* L. (Improved Long Pod, Lagomarsino Seed Inc., Sacramento, CA), *Nicotiana glauca* Graham (Clyde Robin Seed Co. Inc., Castro Valley, CA), and *Allium cepa* L. (Stuttgarter, sets from Joseph Harris Seed Co., Rochester, NY) were grown under the same conditions as those of *C. communis*, and epidermal strips were prepared and mounted in a similar manner. In the case of *A. cepa*, epidermal strips were obtained by making two transverse cuts around the midsection of the second or third expanded leaf, after which the epidermis was teased away from the mesophyll between the transverse cuts. Plants of *Zea mays* L. (Michigan 500, Department of Crop and Soil Sciences, Michigan State University) were grown in growth chambers in a 16-h day; light intensity increasing during the day from 80 to 400 w m^{-2} and then again decreasing; 27/17 C day/night; 50% RH). Leaf sections were taken at a distance of 10 to 20 from the base of the fifth leaf of 4-week-old plants and subsequently treated like *C. communis* leaf sections.

All plants in the growth chamber were fertilized with half-strength Hoagland solution which was 25 μM with respect to Cl^- and had a pH of 6.5.

Solutions. In isolated epidermal strips exposed to simple media, stomatal complexes of *C. communis* retained their ability to function if Ca^{2+} and other multivalent cations were held to a minimum. In general the bathing solution contained in mEq/l or mmol/l : 30.0 K^+; 29.8 Cl^-; 1.0 Mops.[4] The pH of this solution was 7.0. The solution contained a few μeq/l of Ca^{2+} as a contaminant of the glassware and the reagent grade acids and salts. For the study of the effects of various treatments upon the electrical properties of stomatal cells, additions were made to the bathing solution to give the following concentrations: for inhibitor studies 10 μM DNP, 5 μM CCCP, or 1 mM KCN; for alteration of protoplasts or their membrane properties, 2.2 M *t*-butyl alcohol, 1% (w/v) SDS, or 1% (v/v) Triton X-100; for plasmolysis studies, 0 to 1.5 osmolal sucrose or mannitol; and for cytorrhysis experiments, 1.5 osmolal PEG 6000 (Aquacide III from Calbiochem). For the study of pH effects upon the electrical potentials in guard cells, the pH of the bathing solution was adjusted to HCl which resulted in a change in the Cl^- concentration of 0.1 mM or less between pH 3 and 7 and a 5 and 10 mM increase at pH 2.5 and 2.0, respectively. Solutions at pH 6.0 were buffered with 1 mM Mes instead of Mops; bathing solutions of pH < 6.0 were not buffered.

For production of guard cell protoplasts, the procedure of Zeiger and Hepler (21) was followed with some modification. The digestion of the cell walls of guard cells from mounted epidermal strips of *C. communis* was carried out in bathing solution supplemented with 0.24 osmolal mannitol or sucrose and 4% (w/v) crude cellulase-pectinase (Onoguka R-10 from Kinki Yakult Mfg. Co. Ltd., 8–21 Shingikan-Cho, Nishinomiya, Japan). After digestion periods of 0, 1.5, and 3 to 5 h, electrical potential measurements of guard cells were performed in an identical bathing solution minus enzyme, then returned to the enzyme solution for further digestion, if any. For impalement by micropipettes, guard cell protoplasts were wedged between cotton fibers.

Electrical Measurements. Epidermal samples were allowed to equilibrate with each solution for at least 30 min before electrical measurements were begun, even though electrical adjustments of guard cells to changes in the external solution were complete within 5 min. In most cases, solutions were changed while the micropipette was withdrawn from the cell, but sometimes the pipette was left in the cell. Identical results were obtained by the two procedures. With the exception of protoplast isolation experiments, electrical measurements were completed within 5 h after peeling the epidermal strip from the leaf. In material not subjected to changes in the bathing medium, potentials stayed constant within this time.

Borosilicate capillary tubing (Corning 7740 and Kwik Fil from W-P Instruments, New Haven, CN) was drawn into glass capillaries with tip diameters less than 0.8 μm on a vertical pipette puller (David Kopf Instruments, Tujunga, CA). By replacing the heater coil of the pipette puller with a 2.5-turn steel coil, which had been snuggly wrapped around a stainless steel tube having a 2-mm inner diameter, micropipettes could be produced which sealed into the impaled cells better and had greater structural rigidity and uniformity, had lower tip potentials and resistances, and were electrically less noisy than micropipettes prepared using conventional heater coils. Pipettes were filled with 3 M KCl. The resistance of the micropipettes before cellular penetrations ranged between 15 and 35 MΩ and between 10 and 15 MΩ after withdrawal. Micropipettes were connected via a Ag:AgCl half cell to a W-P Instruments (New Haven, CN) model M-701 electrometer (input resistance 10^{11} Ω) which allowed current injection from a function generator (model F-33, Interstate Electronics Corporation, Anaheim, CA). A capillary in the bathing solution served as reference electrode; it was connected via a matched Ag:AgCl half cell to the grounded input of the electrometer. The reference electrode dipped up to 1 mm into the solution. The recorded potential was not sensitive to variations in the depth of immersion. One voltage output of the electrometer was connected to a strip chart recorder (E 1101 E, Esterline Angus Instrument Co., Indianapolis, IN), the second output as well as the output for monitoring injected current pulses were connected to a dual-beam storage oscilloscope (5103 N, Tektronix, Beaverton, OR). All electrical equipment was calibrated once a week with a Leeds and Northrup (Philadelphia, PA) type K-5 potentiometer using a Weston cell as the standard voltage source. The microscope and tissue chamber were inside a Faraday cage. The micropipettes were inserted into the tissue with a Leitz micromanipulator and observed at 400× magnification with a Leitz Laborlux II, fixed-stage microscope, fitted with a long working distance objective L32x/0.40 N.A.

Membrane resistance was monitored regularly. We found it very difficult to insert routinely two capillaries into one guard cell. We, therefore, resorted to a single micropipette method (5) although we are aware of its possible lack of reliability (4). We felt we should attempt to obtain some information at least on the order of magnitude of the electrical resistances that occur in guard cells. Briefly, square wave pulses with a frequency between 1 and 1,000 Hz and an amplitude of 1 namp were passed for a few seconds between the microelectrode and the reference electrode. The voltage change across the micropipette was compensated by the bridge circuit in the electrometer. The voltage change across the membranes was monitored and found to contain a fast component (time constant 10–20 μs) and a slow component (time constant 0.1–1.3 ms). In earlier studies, the fast component has been associated with the micropipette resistance while the slow component has been attributed to the membrane resistances (5). We followed this interpretation.

Tip potentials of the micropipette were determined in KCl solutions immediately before and after tissue penetration. Micropipettes having a tip potential difference greater than 10 mv between immersion in 30 mM and 3.0 M KCl solutions were discarded. Corrections for tip potential changes upon penetrations of guard cells were made assuming that the protoplasm of guard cells from open and closed stomata was approximately equivalent to 500 and 100 mM KCl, respectively (14, 15). The corrections were usually of the order of 2 to 3 mv, rarely of 4 or 5 mv.

[4] Abbreviations: Mops, morpholinopropanesulfonic acid; CCCP; carbonyl cyanide *m*-chlorophenylhydrazone; DNP; dinitrophenol.

Electrical recordings were discarded if: (a) the resistance (and associated tip potential) of the micropipette increased during the course of the experiment; (b) the tip potential measurements of the micropipette in KCl solutions before and after tissue penetrations differed by more than 2 mv; (c) the micropipette entered a cell near a chloroplast or penetrated a chloroplast or the nucleus; (d) the penetrated cell collapsed; or (e) cyclosis stopped.

Each electrical potential value given in this report represents the average of at least 10 measurements, generally many more. Most experiments were repeated at least twice. In each case, one standard deviation of a potential measurement was smaller than 4 mv.

RESULTS

Electrical Potentials Recorded During Penetrations of Epidermal Cells. Most of the electrophysiological measurements reported in this paper were made on cells of epidermal strips of *C. communis*. A stomatal complex of the abaxial epidermis of this species is shown in Figure 1. When a micropipette was inserted into a guard cell of an open stoma, the potential difference between microelectrode and reference electrode changed, following a pattern depicted in Figure 2. When the micropipette touched a cuticular surface in the stomatal pore, a positive deflection appeared and the resistance of the micropipette increased to a large value (>30 MΩ). The magnitudes of the changes in potential and resistance were positively correlated with each other. In a bathing solution of flowing 30 mM KCl, the potential change was >30 mv; it was zero when a guard cell was approached through a neighboring subsidiary cell, *i.e.* through a surface lacking a cuticle. Positive potential difference and high pipette resistance disappeared when the micropipette was withdrawn from the cell or when it was further advanced into the cell. In the latter case, a stable negative potential of about 64 mv appeared, whereas the resistance of the micropipette either remained unchanged or decreased relative to its value in the bathing solution. No resistance change occurred that could be attributed to the penetration of a membrane. The magnitude of the potential did not change when coarse-tipped pipettes (about 1 μm tip diameter, 5–10 MΩ reistance) were substituted for the fine-tipped ones (<0.8 μm tip diameter, 15–35 MΩ resistance). During this measurement, the surface of the guard cell appeared slightly depressed at the point of contact with the micropipette. We believe that this initial negative potential corresponds to the cell-wall potential of Nagai and Kishimoto (11). We will refer to it as cell wall potential in this paper. When the base plate of the micromanipulator was tapped lightly with the finger, an immediate decrease in negativity (depolarization) to about −42 mv occurred and the resistance increased to about 65 MΩ (corresponding to a unit area resistance of about 1.3 kΩ cm^2 on the assumption that the active surface of a guard cell was about 2×10^{-5} cm^2). We presume that the tip of the micropipette was now in the vacuole. In KCl solutions of ≤10 mM, the potential difference remained constant after penetration of a guard cell, but in 30 mM KCl the potential difference began to increase with a lag of only seconds and reached a stable value of about −50 mv after 1.5 to 3 min (Fig. 2 shows a particularly smooth trace). Simultaneously, cytoplasm collected in the region around the tip of the micropipette, and the resistance fell to about 4 MΩ. Measurement of the resistance of the pipette after withdrawal from the cell showed that the decrease in resistance was not caused by breakage of the pipette tip. In solutions of ≥100 mM, the nucleus of the guard cells migrated over the micropipette and plugged it. When this happened the resistance of the external measuring circuit rose abruptly. Based on these visual observations, and on the resistance measurements, we classify the negative potential that occurs after depolarization and before cytoplasmic redistribution and hyperpolarization as the vacuolar potential. We further presume that the stable negative potential that appears after formation of a cytoplasmic sheath around the micropipette and after decrease in resistance and hyperpolarization is the cytoplasmic potential. The fall in resistance indicates to us that a potential resulting from the formation of a new plasmalemma is not involved (20). There were many cases in which we were uncertain whether the tip of the micropipette was in the vacuole or the cytoplasm. If merely a high resistance of the measuring circuit indicated the presence of a membrane of high resistance in the electrical path and sheath formation was not clearly seen we called the measured potential differences intracellular potentials.

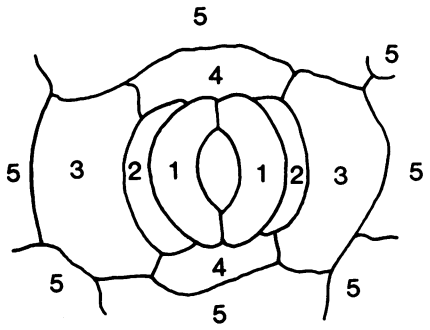

FIG. 1. Stomatal complex of *C. communis*, about 720X. 1, guard cells; 2, inner lateral subsidiary cells; 3, outer lateral subsidiary cells; 4, polar subsidiary cells; 5, ordinary epidermal cells.

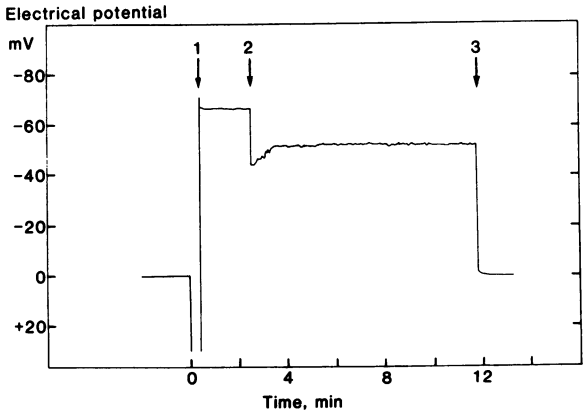

FIG. 2. Recording of the electrical potential of a guard cell, made during gradual insertion and withdrawal of a micropipette. An epidermal strip of *C. communis* was bathed in a 30 mM KCl solution. At 0 min, the micropipette touched the surface of the guard cell. The first and second arrows mark points at which the micropipette was inserted and then advanced further into the guard cell. At the third arrow, the micropipette was withdrawn. In our interpretation of the trace, the cell wall potential appears between arrows 1 and 2, followed by a brief indication of the vacuolar potential; then the cytoplasm moved over the tip of the electrode, and the cytoplasmic potential began to be recorded before minute 4 had elapsed.

Electrical recordings made during shallow penetrations of subsidiary and other epidermal cells resembled those made on guards cells in pattern but not in magnitude. Hyperpolarization following deep penetration occurred only in guard cells. In all cases, the state of inflation of the cells was maintained during penetrations and intracellular measurements, *i.e.* micropipettes withstood estimated intracellular pressures of up to 30 bars; this was confirmed by insertion of pipettes into a pressure bomb.

Magnitude of the Electrical Potentials in Epidermal Cells. When the stomata were open, an electrical gradient existed from the vacuoles of the ordinary epidermal cells across the stomatal complex to the vacuoles of guard cells (Table I). The cytoplasmic

Table I. *Vacuolar Potentials of Epidermal Cells*

Epidermal strips of *C. communis* were bathed in 30 mM KCl, 1 mM Mops (pH 7.0). The apertures of the open stomata were 12 to 14 μm wide.

Cell Type	Vacuolar Potential	
	Stomata open	Stomata closed
	mv	
Guard cells	−45.1	−30.4
Inner lateral subsidiary cells	−30.1	−30.1
Polar subsidiary cells	−29.7	−30.7
Outer lateral subsidiary cells	−29.5	−31.0
Ordinary epidermal cells	−28.3	−32.4

Table II. *Vacuolar Potentials in Epidermal Strips With Open Stomata of Various Species*

Epidermal strips were bathed in 30 mM KCl, 1 mM Mops (pH 7.0).

Plant Species	Stomatal Aperture	Vacuolar Potential		
		Guard cells	Subsidiary cells	Ordinary epidermal cells
	μm		*mv*	
Vicia faba	13	−42.1	–[a]	−26.9
Nicotiana glauca	8	−50.3	–	−23.4
Allium cepa	8	−39.0	–	−26.5
Zea mays	8	−50.5	−28.9	−30.5

[a] Dashes represent species without subsidiary cells.

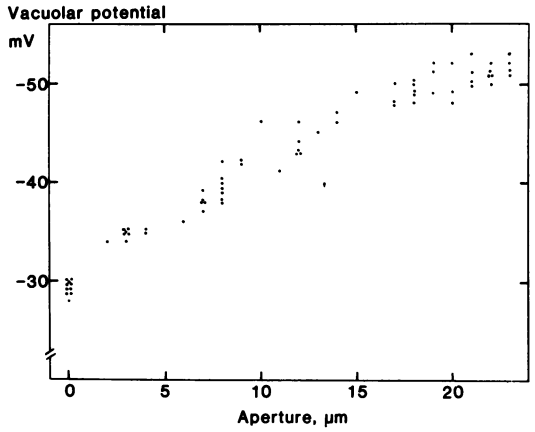

FIG. 3. Vacuolar potentials of guard cells related to stomatal aperture. The natural variation in stomatal aperture was used as it occurred in epidermal strips of *C. communis* bathed in 30 mM KCl, 1 mM Mops (pH 7.0). Each point represents a measurement taken on one guard cell.

potentials, measured under the same conditions, were −32 mv in ordinary epidermal cells and subsidiary cells, and −52 mv in the guard cells. Potential differences of on the average 19 mv occurred between the vacuoles of guard cells and of ordinary epidermal cells also in samples form *V. faba, N. glauca, A. cepa,* and *Z. mays* (Table II). The potential gradient vanished when stomata closed (Table I), and in all epidermal cells the cytoplasmic potentials were then between 2 and 5 mv more negative than the vacuolar potentials. The electric potential in the vacuoles of guard cells had a component that was almost linearly related to stomatal apertures (Fig. 3).

In 30 mM KCl solution, the cell wall potentials were generally around −64 mv. This was true for all types of epidermal cells in all five species tested. Although hardly any gradient in wall potential existed across stomatal complexes of *C. communis*, the potential difference between wall and medium decreased with the physiological age of the leaf (Table III). A good correlation existed between wall potential and stomatal aperture. However, this correlation does not indicate a causal relationship between wall potential and aperture, it rather indicates that both parameters change with leaf age. In leaves of the same age, vacuolar potential can vary with stomatal aperture while the wall potential does not. For instance, in Figure 3, wall potentials were at about −62 mv for all apertures recorded between 0 and 23 μm.

Effect of Metabolic Inhibitors on Electrical Properties of Epidermal Cells. Exposure of epidermal strips to a concentration as high as 5 μM of the uncoupler, CCCP, for 1 h or longer hardly affected stomatal aperture, whether initially narrow or wide, and did not affect cell wall or vacuolar potentials of any epidermal cell of *C. communis.* The membrane resistance of guard cells increased after exposure to CCCP by 4 to 8 MΩ, whereas the membrane resistances of the other epidermal cells increased only slightly or not at all. In the presence of CCCP, a cytoplasmic sheath did not form in guard cells around the tip of the micropipette. The influence of CCCP on the cytoplasmic potential could therefore not be determined. DNP and KCN, at concentrations of 10 μM and 1 mM, respectively, did not affect the cell wall potential or the vacuolar potential. Cyanide, like CCCP, increased the membrane resistance of guard cells while DNP did not.

Influence of External Ionic Composition and Ionic Strength on the Intracellular Potentials of Guard Cells. The vacuolar potential of guard cells responded linearly and reversibly to the logarithm of the external KCl concentration when the latter was changed between 1 and 300 mM; the average slope was 47 mv/decade (Table IV). The chlorides of Cs^+, Rb^+, and Na^+ produced almost identical effects. When the external salt concentration was

Table III. *Electrical Potentials of Guard Cells Related to Physiological Age*

Epidermal strips of *C. communis* were bathed in 30 mM KCl, 1 mM Mops (pH 7.0)

Developmental Stage of Leaf	Stomatal Aperture	Electrical Potential		
		Cell wall	Cytoplasm	Vacuole
	μm		*mv*	
Expanding	18	−68	−54	−48
First fully expanded	13	−64	−50	−42
Second fully expanded	10	−61	−48	−40
Third fully expanded	8[a]	−56	−45	−38

[a] As the range of stomatal apertures was large (2–10 μm) in leaves of this stage, guard cells of stomata having an aperture near the median aperture of 8 μm were selected for the electrical measurements.

Table IV. *Electrical Potentials of Guard Cells in Epidermal Strips of C. communis Bathed in KCl Solutions of Various Concentrations*

Solutions buffered at pH 7.0 with 1 mM Mops.

KCl	Stomatal Aperture	Cell Wall Potential	Vacuolar Potential
mM	μm	*mv*	
800	0–4[a]	0.0	−2.4
300	20–22[a]	−7.2	−2.3
100	18–22[a]	−40.0	−27.2
30	11–13	−63.6	−41.6
10	6	−93.0	−65.6
3	4–6	−118.1	−94.0
1	2–4	−155.0	−120.3

[a] Subsidiary cells were plasmolyzed.

changed at levels below 100 mM, overshoots occurred in the intracellular potential. These transients lasted up to 6 min. To investigate the dependence of the vacuolar potential of guard cells on the cationic composition of the bathing solution further, the K^+ concentration of the bathing solution was reduced while maintaining the external ionic strength and osmolarity by an equivalent increase in the concentration of another monovalent cation. A level of 30 mM was chosen because 30 meq/l was found to be the lowest concentration of K^+ at which stomata maintained their aperture in contact with a flowing solution. Substitutions of K^+ with Rb^+, Na^+, Cs^+, NH_4^+, and choline had no influence on the cytoplasmic or vacuolar potentials of guard cells of *C. communis*, whereas substituting Li^+ for K^+ caused a hyperpolarization by 15 mv (Table V). There was no indication that NH_4^+ was toxic to guard cells. Replacement of Cl^- by the presumably nonpermeating anion iminodiacetate did not affect stomatal responses to changes in the K^+ content of the bathing solution, except at concentrations ≥300 mM (Table VI). When Ca^{2+} was added to the bathing solution of 30 mM KCl to give a concentration of 1 mM $CaCl_2$, open stomata of *C. communis* closed and the membrane resistance of guard cells increased from 1.2 to 4.6 kΩ cm^2. The potential of the cell walls changed from −64 to −47 mv. The vacuolar potential responded in two steps. First, its value decreased from −42 to −27 mv. Then, when stomata closed from 13 to 15 μm to 2 to 4 μm, the potential difference declined to −12 mv within 5 min, indicating a loss of solutes from the guard cell vacuoles. Concentrations of $CaCl_2$ <1 mM produced similar but smaller effects. Stomata that had closed in response to Ca^{2+} could not be reopened by increasing the external concentration of K^+. Oscillations in the electrical potentials were triggered when guard cells of *C. communis* were penetrated in 30 mM $CaCl_2$ (Fig. 4). In one-third of the cases, only the initial overshoot occurred; in the other cases, oscillations followed that either decayed, were sustained, or even increased in amplitude.

Table V. *Influence of Alkali Cations, NH_4^+, and Choline on the Electrical Potentials of Guard Cells from Epidermal Strips of C. communis*

Strips were bathed in Cl^- salt solutions buffered at pH 7.0 with 1 mM Mops.

Chloride Treatment	Stomatal Aperture	Cytoplasmic Potential	Cell Wall Potential
mM	*μm*	*mv*	
30 K + 3 Na	11–13	−47.4	−60.5
3 K + 30 Na	12–14	−49.2	−62.1
30 K + 3 Li	12–14	−49.4	−57.7
3 K + 30 Li	13–16	−64.3	−58.0
30 K + 3 Rb	12–14	−46.6	−61.8
3 K + 30 Rb	12–14	−47.4	−58.5
30 K + 3 Cs	10–13	−44.7	−58.3
3 K + 30 Cs	10–13	−46.6	−57.8
30 K + 3 NH_4	12–16	−47.2	−60.6
3 K + 30 NH_4	12–16	−47.0	−61.1
30 K + 3 Choline	14–16	−53.1	−64.0
3 K + 30 Choline	14–16	−52.6	−63.8

Table VI. *Effect of Replacement of Chloride in the Bathing Solution by Iminodiacetate on Stomatal Aperture and Vacuolar Potentials of Guard Cells*

Epidermal strips of *C. communis* bathed in solutions buffered at pH 7.0 with 1 mM Mops.

Concentration of K Salt	KCl		K Iminodiacetate	
	Stomatal aperture	Vacuolar potential	Stomatal aperture	Vacuolar potential
mM	*μm*	*mv*	*μm*	*mv*
800	0–4	−2.4	0–2	−0.1
300	20–22	−2.3	0–2	−11.1
100	18–22	−27.2	16–20	−28.0
30	11–13	−41.6	10–13	−40.8
10	6	−65.6	4–6	−65.1
3	4–6	−94.0	4–6	−88.0

Cell wall resistances could not be measured reliably in high Ca treatments because the physical properties of the walls had changed. We observed changes in the appearance of the guard cell nuclei. In a medium that was 30 mM Ca^{2+}, they were swollen; in 100 mM Ca^{2+}, nuclei could no longer be seen.

Influence of External Ionic Composition and Ionic Strength on the Cell Wall Potential of Guard Cells. The cell wall potential of guard cells of *C. communis* was characterized by a linear response to the logarithm of the KCl concentration in the range between 1 and 300 mM; the average slope was 59 mv/decade (Table IV). A sharp decrease in the response occurred when concentrations were raised from 300 to 800 mM. Substitution of K^+ by Na^+, Rb^+, Cs^+, Li^+, NH_4^+, and choline did not bring out an effect that had been specific for a particular monovalent cation (Table V). The cell wall behaved like an ion exchanger. The possibility that the wall potential was that of a Donnan space was tested in several experiments whose description follows.

Effects on Electrical Potentials of Treatments That Alter Membrane Permeability, and of Changes in External pH. The wall potential remained undiminished when guard cells of *C. communis* were treated with alcohol or detergents, or were ruptured or frozen (Table VII), whereas the intracellular potentials were virtually eliminated. If the wall potential is produced by the fixed negative charges of polygalacturonic acid, the potential should decline with the pH of the bathing solution. Figure 5A shows that this was the case. The potentials measured can be used to determine the density of fixed charges in the wall. The Donnan ratio, r, is defined by $r = [K_s^+]/[K_w^+]$, where $[K_s^+]$ is the activity of K^+ in the solution and $[K_w^+]$ that in the wall. The Donnan ratio can be calculated from the potential difference, E, between wall and solution: $E = (RT/F)\ln r$, where R, T, and F are the gas constant, absolute temperature, and the Faraday unit, respectively. The density, z, of the charges in the walls is then $z = [K_s^+]\,(1/r - r)$.

Figure 5B shows charge densities derived from the data of Figure 5A. Half-maximal density occurred at an external pH of 4.

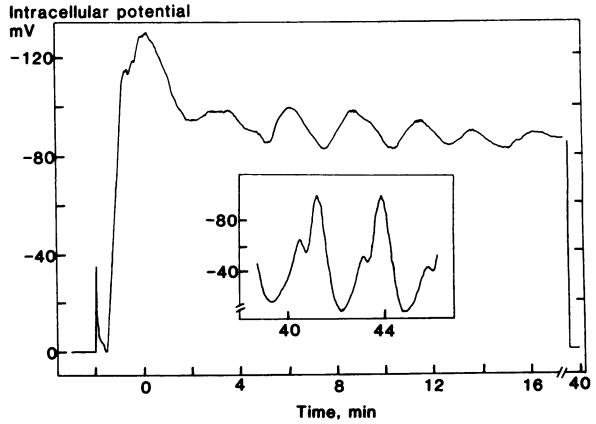

FIG. 4. Oscillations of the intracellular potential of a guard cell in an epidermal strip of *C. communis* bathed in 30 mM $CaCl_2$. Insertion of a micropipette was the trigger. Insert shows another example with a secondary peak in the hyperpolarizing phase of each cycle.

Table VII. *Influence of Various Tissue Treatments Which Kill Plant Cells or Alter Membrane Permeability on the Electrical Potentials of Guard Cells*

Epidermal strips of *C. communis* bathed in 30 mM KCl buffered at pH 7.0 with 1 mM Mops. Stomatal apertures were between 12 and 14 μm before treatments.

Treatment	Cell Wall Potential	Intracellular Potential
	mv	
Control	−68.8	−50.5
10 min in 2.2 M *t*-butyl alcohol	−68.2	
Control	−62.8	−47.4
15 s in liquid N_2	−63.4	−1.2
Control	−62.2	−46.4
10 min in 1% SDS	−63.0	−1.0
Control	−62.3	−49.2
30 min in 1% Triton X-100	−61.3	−2.3
Control	−64.4	−49.5
Rupture of cells by repeated penetration with electrode	−63.1	−4.1

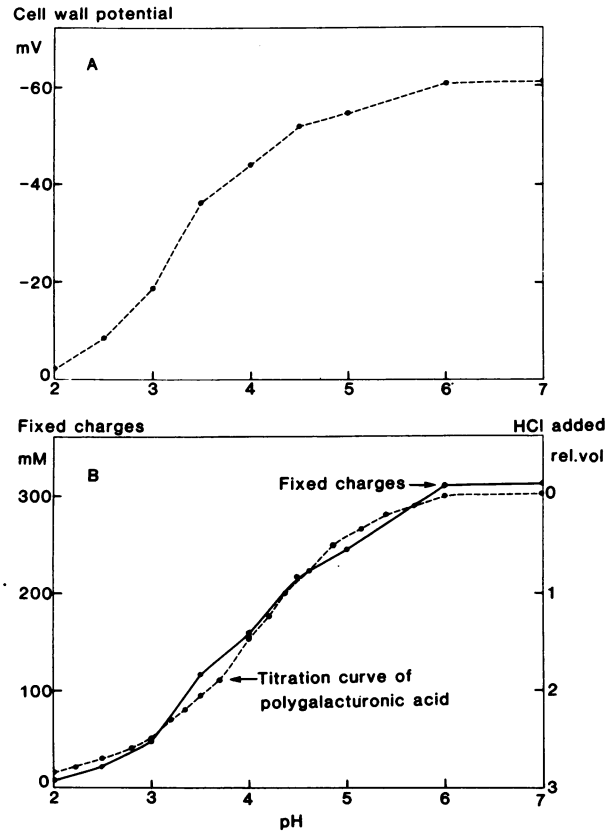

FIG. 5. The influence of the external pH on the electrical potential (A) and density of fixed charges (B) in the wall of guard cells of *C. communis*. Epidermal strips were bathed in 30 mM KCl (activity 28.3); the pH was adjusted by addition of HCl. B, shows also the titration curve of 1% (w/v) polygalacturonic acid with 0.1 N HCl as the titrant.

Since in the ideal case the Donnan ratios are identical for all monovalent cations in the Donnan system, the H^+ activity in the cell wall was about 0.55 mM at an external pH of 4, which corresponds to a pH of 3.3 in the Donnan space of the wall. The pattern of the pH dependence of the charge density in the wall was almost identical with the titration curve of polygalacturonic acid (Fig. 5B). Evaluation of all data collected during the course of this investigation showed that the density of fixed charges in the guard cell walls of *C. communis* varied between 250 and 480 mM at a pH of 7 of the bathing medium.

Changes in external pH hardly affected the vacuolar and cytoplasmic potentials of guard cells, as long as the pH remained above 4 (Fig. 6). Below pH 4, the micropipettes penetrated the cell walls more easily than at pH 4; apertures increased temporarily to 20 μm and then approached 6 μm. When stomata closed, potential differences disappeared and the chloroplasts in the guard cells disintegrated.

Effects of Plasmolysis, Cytorrhysis, and Wall Digestion on Guard Cell Potentials. Exposure of guard cells of open stomata to sucrose solutions of high osmolarity did not affect wall potentials and intracellular potentials but a reduction of the intracellular potential set in as soon as the plasmalemma separated from the cell wall (Table VIII). The magnitude of the reduction in intracellular potential was correlated with the extent to which the area of contact between plasmalemma and cell wall was reduced. No loss of potential occurred when an osmoticum of large molecular size was used that could not penetrate cell walls and, therefore, led to a collapse of the guard cells without separation of the plasmalemma from the wall (cytorrhysis; Table VIII). Intracellular potentials of guard cells of *C. communis* decreased when the cell walls were removed by enzymic digestion (Table IX). A correlation existed between the loss of potential and the degree of separation of the protoplast from the remaining cell wall. The intracellular potential of free-floating protoplasts was about zero.

DISCUSSION

This investigation was restricted to stomata whose aperture did not change while potentials were being measured. In the quasi-stationary state, potential differences between the vacuoles of guard cells and the bathing medium changed with the electrolyte activity in the medium in a Nernstian manner, as potentials of Donnan spaces with sequestered negative charges would have done (Table IV). The same applied to the cell walls: they behaved like ion exchangers with fixed anions. The charge densities in vacuoles and walls of guard cells were of equal magnitude. A nearly parallel response of vacuolar wall potentials to changes in the ionic strength of the medium was the result, as if vacuole and

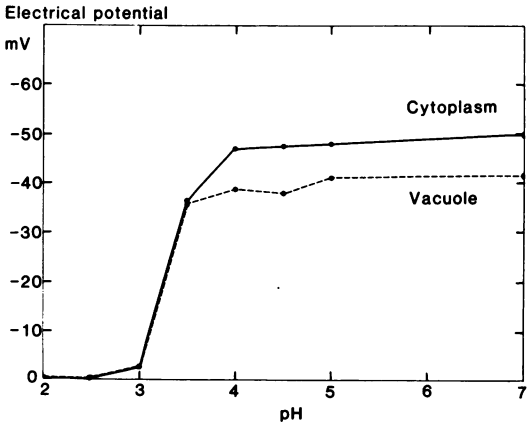

FIG. 6. The influence of the external pH on vacuolar and cytoplasmic potentials of epidermal strips of *C. communis* with initially open stomata (14 μm). 30 mM KCl, pH adjusted by addition of HCl. At pH 4.5, stomata opened to 24 μm; at pH ≤4, stomata opened temporarily to apertures >20 μm and then closed to 6 μm. At pH 3.5, stomata closed with 1 h; at pH ≤2.5 they closed with almost no delay.

Table VIII. *Effects of Plasmolysis and Cytorrhysis on the Electrical Potentials of Guard Cells*

Epidermal strips of *C. communis* bathed in 20 mM KCl buffered at pH 7.0 with 1 mM Mops.

Treatment	Condition of Guard Cells	Stomatal Aperture	Electrical Potential	
			Intracellular	Cell wall
		μm	mv	
Plasmolysis				
30 mM KCl	Turgid, stomata open	12–15	−41.3	−63.7
↓				
Addition of sucrose to give	No plasmolysis, stomata closed	0–2	−38.8	−64.0
↓				
30 mM KCl +1.5 osmolal sucrose	Plasmolysis, stomata closed	0	−8.7	−63.1
Cytorrhysis				
30 mM KCl	Turgid, stomata open	13–14	−42.1	−65.2
↓				
Addition of PEG to give	No cytorrhysis, stomata closed	0	−41.7	−65.0
↓				
30 mM KCl +1.5 osmolal PEG 6000	Cytorrhysis, stomata closed	0	−40.1	−66.1

Table IX. *Intracellular Potential of Guard Cells of C. communis During Enzymic Digestion of Cell Walls*

Epidermal strips treated with 4% (w/v) cellulase-pectinase, 30 mM KCl, 1 mM Mops, 0.23 M mannitol. Potentials were measured in flowing 30 mM KCl, 1 mM Mops (pH 7), 0.23 M mannitol; the solution was 0.24 osmolal.

Enzyme Treatment	Condition of Guard Cells	Intracellular Potential
h		mv
0	Intact cell wall, stomatal aperture 10–12 μm	−38.1
1.5	Partially digested cell wall, guard cells curled in pairs	−25.1
3.0	Partially digested cell wall, protoplasts partially separated from cell wall	−11.4
3.5–5.0	Protoplasts adhering to surface of partially digested cell wall	−7.7
3.5–5.0	Protoplasts free from surface of partially digested cell wall	−0.3

wall had been electrically coupled (Table IV). If it is true that a micropipette surrounded by a cytoplasmic sheath indicates the potential of the cytoplasm, then our data show that even the cytoplasmic potential changed in parallel with the vacuolar and wall potentials, with the cytoplasmic potential being always about 7 mv below that of the vacuole (Figs. 2 and 6; Table III). In the following discussion we shall deal with each potential separately.

Cell Wall Potential. A decrease in potential upon contact between micropipette and cell wall has been observed before, for instance in tissue of oat coleoptiles by Goldsmith *et al.* (5), and thought to have resulted from an interaction between the tip of the micropipette and the polyanionic wall material. We believe the first decrease in potential that we measured during impalement of epidermal cells was not an artefact but represented a Donnan potential: no increase in resistance occurred when the pipette was inserted, and the slope of 59 mv/decade concentration change in the bathing medium could be measured with high reproducibility (for instance, the values in Table IV and Fig. 5). The computed charge density of the wall (0.3–0.5 M) appears high but is lower than that reported for cell walls of *Chara australis* (0.8 M) (2) and about equal to that determined for leaf tissue of *Hordeum vulgaris* (16). Possibly, we even underestimated the charge density because of the probable presence of Ca^{2+}. The apparent pK of the cell wall was similar to that of authentic polygalacturonic acid (Fig. 5). The wall potential declined as the tissue aged, probably as a result of an accumulation of Ca^{2+} or of an esterification of the galacturonate (Table III). Alternatively, the presumed wall potential could have been caused by a penetration of the micropipette into cytoplasm or vacuole, but the measured potentials remained unaffected by plasmolysis (Table VIII), application of metabolic inhibitors, or cell death (Table VII). The initial negative potential disappeared when the cell walls were enzymically digested (Table IX). We, therefore, interpret the first decrease in potential occurring during the penetration of epidermal cells, including guard cells, as being caused by the fixed negative charges in the cell walls.

Vacuolar Potentials. At first sight, the potential differences we measured appear to be lower than the ones reported by other workers in the field; their values range between −72 and −166 mv (6, 10, 12). However, the other investigations were conducted with KCl concentrations between 1 and 10 mM in the bathing solution. If we refer to the corresponding data in Table IV, we see that there is no serious disagreement between the results of our measurements and those of earlier investigations.

We were surprised that the potential difference between vacuole and bathing medium had no obvious electrogenic component. Perhaps transients indicated electrogenic activity that, in the quasistationary, could not be recognized. Temporary changes in the potential difference occurred in response to alterations in the ionic activity in the medium (if KCl was <100 mM), in response to high external Ca^{2+} concentrations (Fig. 4), or in response to changes in illumination. One could argue that leakage of electrolytes around the micropipette prevented us from recording electrogenic potentials. We do not think that that was the case: all cells maintained turgor after impalement, and stomatal apertures remained constant. Maintenance of potential difference, turgor, and aperture in the presence of leakage would require the operation of a strong compensatory electroneutral transport mechanism. No evidence for such a metabolically supported import during the measurement appeared. Application of CCCP (known to prevent stomatal opening) (13) or CN^- did not cause a change in potential difference, loss of turgor, or stomatal closure. The suspicion that the inhibitors

had not reached the cell membranes was unfounded. Although the potential difference remained unchanged, the membrane resistance increased (as reported for pea root cells and cells of *Chara corallina*) (1, 9) indicating that the inhibitors had elicited a change in at least one membrane property. Further support for the view that we measured vacuolar potentials comes from the observation that Li^+ caused a measurable, specific hyperpolarization that had no parallel in the ion exchange potential of the walls (Table V). Before we relate vacuolar potentials to stomatal aperture and ion content we feel it necessary to discuss potentials that represented cytoplasmic potentials.

Cytoplasmic Potential. We are not certain whether the tip of a micropipette was situated in the cytoplasm when a visible sheath formed around the capillary. However, the considerable reduction in resistance from about 65 MΩ to about 4 MΩ that occurred during sheath formation indicates the elimination of the tonoplast from the electrical path. If this interpretation is correct, then the resistance of the plasmalemma of a guard cell appears to be extremely small. This conclusion needs corroboration once a method for the unequivocal measurement of the plasmalemma potential in guard cells has become available. As in other instances (*e.g.* 19), the presumed cytoplasmic potential was below the vacuolar potential but slightly above wall potential (Figs. 2 and 6; Table III). With the exception of Li^+, all species of monovalent cations present in the bathing medium had the same effect on the cytoplasmic potential (Table V). That means that all monovalent cations except Li^+ had about the same mobility on the path from cell wall to cytoplasm. A similar low cation specificity was reported to be typical for cells of the salt glands of *Limonium* (8).

Vacuolar and Intracellular Potentials in Relation to Stomatal Aperture and Ion Content. Donnan potentials of spaces surrounded by membranes are boundary cases of diffusion potentials that apply when the membrane permeability for one sequestered ion is zero or is very low (3). The average slope of the response of the cell wall potential to a 10-fold change in external salt concentration was 59 mv, that of the vacuolar potential was 47 mv (Table IV). Electrically, the cell wall behaved like an ideal cation exchanger, the vacuole in combination with the cytoplasm nearly like one. Exact compliance with the theory cannot be expected in the latter case because variation in stomatal aperture indicates that the ion content of the guard cells increased with increasing external KCl concentration and thereby caused a reduction of the apparent Nernstian slope. If we assume that all potentials measured indeed has been Donnan potentials, we obtain from the data in Tables I, II, IV, V, VII, and in Figures 3 and 6 the following representative densities of nondiffusible negative charges (in Eq l^{-1}) in guard cells:

Cell walls: 0.300 to 0.500
Cytoplasm: 0.095 (stomata closed) to 0.240 (stomata open)
Vacuole: 0.090 (stomata closed) to 0.230 (stomata open)

We do not know whether the approximate equality of the charge density in vacuole and cytoplasm of guard cells of open stomata and in their cell walls is fortuitous or of physiological significance.

When the estimated charge density in the vacuole of guard cells was related to stomatal opening, an almost perfect linearity emerged (plot not shown); the data presented in Figure 3 yielded the estimating equation $z = 87 + 6.25A$; $r^2 = 0.96$; $n = 71$; with z = charge density in mEq/l and A in μm. We can assume that the number of osmotically active particles in the cell is proportional to the number of nondiffusible charges. The linearity found leads to the conclusion that in *C. communis*, solute content and stomatal aperture are linearly related to each other, much like in guard cells of *V. faba* for which the same conclusion was reached from results of an investigation employing a method quite different from the one used here (18).

If we assume that organic anions are completely sequestered in the vacuoles of the guard cells and provide the Donnan charges, we can use the data of Penny *et al.* (14, 15) to compute the dependence of the Donnan potential of guard cells on the external KCl concentration and compare it with the measured values. Estimates (not shown) and measurements did not agree too well. Poor agreement was not surprising in view of the experience that malate contents of guard cells can either be high or low at the same stomatal aperture, depending on the availability of Cl^- (18). If vacuolar or intracellular potentials are potentials resulting from the diffusion of K^+ and Cl^- and if the permeability from Cl^- is smaller (*e.g.* by a factor of 0.2) than that for K^+ we obtain estimates which agree with a few measured values but not over the whole range of concentrations of external solutes we used. Not all of our data are, therefore, consistent with the view that variation in guard cell turgor is the effect of controlled transfer and production of negative charges. Obviously, in the future, ion activities, electrical potentials, osmotic pressures and stomatal apertures need to be determined on the same material simultaneously. Measurements on subsidiary and the ordinary epidermal cells should be included because our potential measurements (Table I) did not reflect the differences in ionic activities Penny *et al.* (14, 15) determined between cells of open and those of closed stomata.

So far we attempted to relate vacuolar potentials in the guard cells to ion activities. Referring to Figure 3, one could argue equally well that not activity differences but turgor determined the electrical potentials in guard cells. When turgor was changed by submersion of epidermal samples with open stomata into hypertonic solutions, potentials stayed virtually constant, as long as guard cells were not plasmolyzed. Only when the cytoplasm separated from the cell walls, the potential difference disappeared (Table VIII). When separation was prevented by repeating the experiment with PEG 6000 as an osmoticum that cannot penetrate cell walls, the potential difference was maintained even when cytorrhysis indicated that turgor was lost. Apparently, not turgor but a contact between plasmalemma and wall is required to maintain a negative potential in the vacuole of guard cells. Experiments with wall-digesting enzymes gave results consistent with this view (Table IX). Potential loss as a result of wall digestion has been observed on cells of other tissues (*e.g.* 17, 19).

Salt Leakage From Guard Cells in Epidermal Strips. Stomata became narrower or even closed when epidermal strips with open stomata were exposed to flowing KCl solutions of a concentration <100 mM. A histochemical test with Na cobaltinitrite showed that K^+ was lost from guard cells during stomatal closure. Closing could be partially or entirely prevented by stoppage of solution flow and the concomitant increase in boundary layer resistance. Obviously, solutions in contact with epidermal strips often constitute a sink for salts and not a source (18) and the concentration of salts customarily offered to epidermal strips (*e.g.* 10 mM) may be too low if stirring of the solution enhances loss more than supply.

Calcium as Inhibitor. Stomata stopped functioning when epidermal strips of *C. communis* were exposed to Ca^{2+} salts at a concentration as low as 1 mM.[5] Earlier observations of suppression of stomatal opening by Ca^{2+} and enhancement by presumed chelation of Ca^{2+} (reviewed in 18) are consistent with our experience. In *C. communis*, and many other species, all epidermal cells accumulate crystals of Ca oxalate, except the guard cells. Stomata begin to respond sluggishly when the appearance of oxalate crystals in the inner lateral subsidiary cells indicates an increase in the Ca^{2+} concentration in the vicinity of the guard cells as a result of an exhaustion of the capacity of the outer subsidiary cells and the ordinary epidermal cells to act as a screen from high Ca^{2+} levels. Meristems and phloem appear to be protected from Ca^{2+}

[5] In recent unpublished experiments, stomatal activity in epidermal strips of *C. communis* was less severely affected by Ca^{2+} than it was during the investigation we report on here.

by the precipitation of oxalate (7). In addition, the transient rises and oscillations in intracellular potentials that occurred in guard cells when they were impaled in Ca^{2+} solutions (Fig. 4) could have been manifestations of a mechanism for the extrusion of Ca^{2+}.

Concluding Interpretation. The results obtained during this investigation, in combination with knowledge obtained before (see ref. 18 for review) let guard cells appear to be leaky compartments whose turgor is built up by production and transfer of anions into the vacuoles and associated nonspecific migration of monovalent cations along their electrochemical gradients. Import of anions and cations into guard cells follows, together with the production of organic acids. This view includes the possibility that, in the case of limited availability of cations, a process of active uptake of K^+ into guard cells will determine the rate of stomatal opening and the degree of aperture reached.

Guard cells lose turgor by controlled increase in leakage of ions into the apoplast of the epidermis. An active uptake of ions from there into subsidiary cells and ordinary epidermal cells very likely occurs during closure, but electrophysiological evidence for it did not appear. Epidermal strips floating on solutions are exposed to artificial conditions in which the normally restricted ion exchange between guard cell wall and the rest of the epidermal apoplast is replaced by solute loss into the solution (or gain of solutes from there). In the whole leaf, ion exchange between the wall of a guard cell (which occupies a considerable fraction of the total volume of the cell) and the vacuole may constitute the major portion of ion transfer of these cells. Cations could be released from the wall in exchange for H^+. However, how Donnan exclusion of anions from cell walls is overcome and how changes in the electrical field of the wall in the proximity of the plasmalemma affect stomatal functioning remain problems to be investigated.

The electrical potentials recorded in guard cells provide little evidence for the operation of an electrogenic pump; perhaps metabolically generated potential changes are rapidly and effectively neutralized by easy diffusion of counter charges. However, oscillations in the intracellular potential of guard cells in response to high Ca^{2+} concentrations could have had an electrogenic cause. In further investigations, attention will have to be paid to potential changes that are small.

The potential difference between the guard cell's interior and the solution, as well as the electrical resistance of this path, was affected by the presence or absence of Ca^{2+}. Ca^{2+} could have a role in the regulation of guard cell turgor.

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LITERATURE CITED

1. Anderson WP, DL Hendrix, N Higinbotham 1974 The effect of cyanide and carbon monoxide on the electrical potential and resistance of cell membranes. Plant Physiol 54: 712–716
2. Dainty J, AB Hope, C Denby 1960 Ionic relations of cells of *Chara australis*. II. The indiffusible anions of the cell wall. Aust J Biol Sci 13: 267–276
3. Donnan FG 1911 Theorie der Membrangleichgewichte und Membranpotentiale bei Vorhandensein von nicht dialysierenden Elektrolyten. Ein Beitrag zur physikalisch-chemischen Physiologie. Z Elektrochemie 17: 572–581
4. Etherton B, DW Keifer, RM Spanswick 1977 Comparison of three methods for measuring electrical resistances of plant cell membranes. Plant Physiol 60: 684–688
5. Goldsmith MHM, HR Fernández, TH Goldsmith 1972 Electrical properties of parenchymal cell membranes in the oat coleoptile. Planta 102: 302–323
6. Gunar II, IF Zlotnikova, LA Panichkin 1975 Electrophysiological investigations of cells of the stomatal complex of spiderwort. Sov Plant Physiol 22: 704–707
7. Hanger BC 1974 Relationship between calcium oxalate crystal deposition and the ontogeny of subterranean clover leaves. *In* RL Bieleski, AR Ferguson, MM Cresswell, eds, Mechanisms of Regulation of Plant Growth, Bull 12. The Royal Society of New Zealand, Wellington, pp 619–624
8. Hill BS, AE Hill 1973 ATP-driven chloride pumping and ATPase activity in the *Limonium* salt gland. J Membr Biol 12: 145–158
9. Keifer DW, RM Spanswick 1979 Correlation of adenosine triphosphate levels in *Chara corallina* with the activity of the electrogenic pump. Plant Physiol 64: 165–168
10. Moody W, E Zeiger 1978 Electrophysiological properties of onion guard cells. Planta 139: 159–165
11. Nagai R, U Kishimoto 1964 Cell wall potential in *Nitella*. Plant Cell Physiol 5: 21–31
12. Pallaghy CK 1968 Electrophysiological studies in guard cells of tobacco. Planta 80: 147–153
13. Pallaghy CK, RA Fischer 1974 Metabolic aspects of stomatal opening and ion accumulation by guard cells in *Vicia faba*. Z Pflanzenphysiol 71: 332–344
14. Penny MG, DJ Bowling 1974 A study of potassium gradients in the epidermis of intact leaves of *Commelina communis* L. in relation to stomatal opening. Planta 119: 17–25
15. Penny MG, LS Kelday, DC Bowling 1976 Active chloride transport of the leaf epidermis of *Commelina communis* in relation to stomatal activity. Planta 130: 291–294
16. Pitman MG, U Lüttge, D Kramer, E Bell 1974 Free space characteristics of barley leaf slices. Aust J Plant Physiol 1: 65–75
17. Racusen RH, AM Kinnersley, AW Galston 1977 Osmotically induced changes in electrical properties of plant protoplast membranes. Science 198: 405–407
18. Raschke K 1979 Movements of stomata. *In* W Haupt, ME Feinleib, eds, Physiology of Movements, Encyclopedia of Plant Physiology, New Series, Vol 7. Springer-Verlag, Berlin, pp 383–441
19. Rona JP, D Cornel, R Heller 1977 Direct measurement of the potential difference between the cytoplasm of free cells of *Acer pseudoplatanus* L. and the external medium. Bioelectrochem Bioenerget 4: 185–194
20. Walker NA 1955 Microelectrode experiments on *Nitella*. Aust J Biol Sci 8: 476–489
21. Zeiger E, PK Hepler 1976 Production of guard cell protoplasts from onion and tobacco. Plant Physiol 58: 492–498