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Mechanism of the Seismonastic Reaction in Mimosa pudica[1]

Robert D. Allen[2]

Biophysics Department, University of California at Los Angeles, Los Angeles, California 90024

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Abstract. The efflux of K^+ from the pulvinar cells of *Mimosa pudica* was shown to increase substantially during the seismonastic reaction. This result is shown to indicate a decrease in σ (reflection coefficient) of pulvinar cell membrane for potassium salts which could account for the pulvinar cell turgor decrease during the seismonastic reaction.

Membrane potentials and concentrations of Ca^{2+}, K^+, Cl^-, S, and P were measured in the top and bottom halves of pulvini. Pulvinar cells showed a large negative membrane potential, cell vacuole relative to external solution, but no significant difference in membrane potential could be detected between upper and lower pulvinar cells. A large difference in K^+ and Cl^- concentration between top and bottom pulvinar halves was evident in reactive pulvini but not in unreactive pulvini. The effect of K^+ concentration on plant growth and leaf reactivity was also investigated.

One of the most fascinating phenomena in the plant kingdom is the seismonastic, *i.e.* rapid, leaf movement exhibited by *Mimosa pudica*. Movement can be initiated by stimuli such as electrical shock or touch, and is effected by the main pulvinus at the base of the petiole. It is generally accepted that this stimulus causes a loss of turgor in the cells composing the lower side of the pulvinus (20); the leaf then moves downward completing the reaction in 1 to 2 sec. Weintraub (20) thoroughly reviews the 3 principal theories (protoplasmic contraction, permeability increase, and intracellular osmotic pressure decrease) proposed to explain the sudden turgor loss in the lower pulvinar cells. But in spite of extensive investigation of the seismonastic reaction, little evidence has been obtained on which to base an explanation of the turgor loss. This is the general aim of my work.

In this type of phenomenon, where a volume as well as solute efflux occurs, the correct parameter describing water movement is the reflection coefficient (σ) derived from the theory of irreversible thermodynamics and defined in equation I (5, 15).

$$J_v \text{ (volume flow)} = Lp\ (\Delta P - \sigma RT\Delta C_s) \qquad \text{I}$$

$$J_s \text{ (solute flow)} = \omega\, RT\Delta C_s + (1-\sigma)\,\overline{C_s} J_v \qquad \text{II}$$

Where:

L_p = Hydraulic conductivity, a volume (mostly water) permeability coefficient.

ΔP = Difference in hydrostatic pressure across the cell membrane.

$RT\Delta C_s$ = Thermodynamic osmotic pressure difference across the cell membrane.

σ = The reflection coefficient, the relative membrane permeability to solutes and water. When $\sigma = 1$, the membrane is semipermeable; when $\sigma = 0$, the membrane is completely nonselective to passage of solutes and water. Note that when $J_v = 0$, $\sigma = \dfrac{\Delta P}{RT\Delta C_s}$; σ expresses the ratio between the observed osmotic pressure and calculated osmotic pressure.

$\overline{C_s}$ = A mean solute concentration.

ω = A solute permeability. When $J_v = 0$, equation II takes the form:

$$J_s = \omega\, RT\Delta C_s \qquad \text{III}$$

Then, $\omega\, RT = P_s$, the conventional solute permeability coefficient.

These equations have been developed for uncharged solutes only, but may be applied to ions if the cation and anion are considered as an electroneutral salt.

During the steady-state, cell volume is constant and thus $J_v = 0$. Under these conditions ΔP (the hydrostatic pressure) will be equal to $\sigma\, RT\Delta C_s$ (the osmotic pressure). If σ decreases, $\sigma\, RT\Delta C_s$ will then be less than ΔP and volume will flow from the cell. This volume efflux will continue until ΔP and $\sigma\, RT\Delta C_s$ are again equal. If σ decreases rapidly, a water efflux and hydrostatic pressure decrease will occur almost as rapidly. Thus, a decrease in σ could explain a rapid turgor decrease in plant cells.

It should be pointed out that σ is a thermodynamic quantity and the detection of a change in σ will give no information about molecular events. For

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[2] Present address: Department of Anatomy, Faculty of Medicine, University of British Columbia, Vancouver 8, B.C., Canada.

instance, σ may decrease by the opening of pores in the membrane or the enhancement of an outwardly directed salt pump, but no decision may be made regarding which event is more probable.

When one considers the rapid flux increases for electrolytes in both plant [*Chara* (9)] and animal [nerve axons (13)] cells upon stimulation, a σ decrease seems to be the most likely theory to explain the turgor decrease in the seismonastic reaction.

The experiments reported in this paper attempt to describe the steady-state conditions for pulvinar cell membrane potential and ion concentration and to show a decrease in σ by measuring the relative radioisotope efflux rates from the pulvinar cells during the seismonastic reaction. $^{42}K^+$ was used in the efflux measurements because K^+ shows rapid flux increases in other excitable cells, and Toryama (18, 19) presents evidence that K^+ leaves the pulvinar cells during the seismonastic reaction.

Materials and Methods

Mimosa pudica seed was planted in coarse sand and watered daily. Two-week-old seedlings were transferred to one-gallon jars containing Hoagland's solution II (12), modified for most experiments (table I), as the fundamental nutrient solution. In 6 weeks, the matured plants had grown about 1 m tall and had attained a bushy appearance. Mature leaves were as large as 12 cm across, had a deep green, healthy appearance, and showed normal sensitivity.

Ion Analysis. Usually 10 to 20 pulvini were collected in a single sample. Pulvini were split into upper and lower halves for separate analysis when measuring pulvinar ion concentrations. Any ion concentration difference may be related to the different functions upper and lower pulvinar cells show during the seismonastic reaction. Samples were placed in double distilled water for 15 min to remove ions from the water free space. These pulvinar tissue samples were then blotted and weighed to obtain wet weights. They were lyophilized and weighed again to obtain dry weights, and stored in a desiccator until the final ion analysis.

Reactive pulvini were collected from the 2 youngest mature leaves on K^+-deficient plant and on plants grown in normal nutrient solution. Unreactive pulvini were collected from very young leaves, defined as those leaves whose pinnules had not opened, and from pulvini of older K^+-deficient plants whose pinnules had begun to turn brown. Plants used for ion analysis had 5 mM Cl^- added to the nutrient solutions (table I) to determine if the common anion unnecessary for normal growth was partitioned in different parts of the pulvinus.

X-ray fluorescence spectroscopy was used for the analysis of ions in pulvini. This technique permitted determinations of calcium, potassium, chloride, sulfur, and phosphorus (1, 2).

Table I. *Composition of Nutrient Solutions*

	Normal	5 ppm K^+	Minus K^+
$NH_4H_2PO_4$ (mM)	1.00	1.00	1.00
$Ca(NO_3)_2$ (mM)	4.00	7.00	7.00
KNO_3 (mM)	6.00	0.13	...
$MgSO_4$ (mM)	2.00	2.00	2.00
[1]KCl (mM)	5.00	...	...
[1]$MgCl_2$ (mM)	...	2.50	...
Micronutrients (μM)	1.00	1.00	1.00
Iron Chelate (mM)	0.10	0.10	0.10

[1]5 mM Cl^- was added to the basic Hoagland's solution II for ion analysis experiments described later and was not necesasry for normal healthy plants.

$^{42}K^+$ Efflux Experiments. The efflux of $^{42}K^+$ from pulvinar cells was measured during steady-state conditions and during the seismonastic reaction. A pulvinus was removed from a plant by cutting the stem above and below the leaf and by cutting the petiole 4 mm from the end of the pulvinus. The short piece of stem with attached pulvinus was fastened securely in a polyethylene cup. Five ml of the 10 mM KCl solution used in microelectrode experiments were labeled with 0.1 mc $^{42}K^+$ and added to the cup so that the pulvinus and short piece of petiole were completely immersed. To facilitate better contact between the solution and pulvinar cells, part of the epidermis was cut away from each side of the pulvinus. Uptake of $^{42}K^+$ by the cut end of the stem below the pulvinus was prevented by coating the cut surface with silicone grease.

The pulvinus was left in the $^{42}K^+$ solution for 4 hr to permit exchange of $^{42}K^+$ with intracellular K^+. During this period it was stimulated every 30 min. If the pulvinus did not show good reactivity, it was discarded. The specific activity of $^{42}K^+$ in the pulvinar cells at the start of the experiment was within a factor of 4 of the $^{42}K^+$ specific activity in the bathing solution; thus high enough to permit the steady-state flux experiments to be performed. After the 4-hr labeling period, the efflux of $^{42}K^+$ from the pulvinar cells to an inactive KCl solution was measured in a "washing-out" experiment using the following procedure. The ^{24}KCl solution was first replaced with inactive KCl bathing solution. At 5-min intervals over the next 90 min, bathing solution samples were collected by withdrawing the solution with a syringe and placing it in a counting tube. Fresh KCl solution was then poured into the cup. About 40 min after the washing-out procedure was begun, the petiole was braced in an attempt to prevent downward movement and thus limit pulvinar cell volume change, and the pulvinus was stimulated. The brace was removed 25 min later, and the pulvinus was stimulated again. By counting the radioactivity in each bathing solution sample, the efflux of $^{42}K^+$ from the pulvinar cell was measured as a function of time. In 4 of the washing-out experiments, the amount of $^{42}K^+$ remaining in the pulvinus at the end

of the experiment was determined by digesting the pulvinus in HNO_3 and counting this sample.

Samples were counted using a Nuclear-Chicago well type scintillation counter (Model 181A) with a NaI crystal detector (DS202V). A single channel differential pulse height analyzer (Model 1810) measured gamma radiation over a 60 kev range centered at 1.516 mev, the principal energy peak of ^{42}K.

Cellular Potential Measurements. Membrane potentials between cell vacuoles and external solution were measured using glass microelectrodes with a 1 μ tip diameter and filled with 3 M KCl.

Ag-AgCl electrodes connected the microelectrode to the measuring circuit consisting of Hewlett-Packard non-inverting follower unit (DY 2460A) with input resistance greater than 10^{10} Ω and a Hewlett-Packard vacuum tube voltmeter (Model 412A).

A pulvinus with short piece of attached stem was prepared for this experiment in the same manner as in the $^{42}K^+$ experiments. Using a rack and pinion gear arrangement, this pulvinus preparation could be positioned in a small plexiglass container through a small hole in 1 side (Fig. 1). Prior to insertion the microelectrode resistance and tip potential were measured. If the tip potential was initially less than 10 mv and resistance less than 100 MΩ, the electrode was judged acceptable. Insertions were always alternated between the top and bottom half of the pulvinus by repositioning the pulvinus after each insertion.

Reactivity of the pulvinus was checked before and after a series of potential measurements. If a good reaction occurred, the readings were accepted; if not, all readings were discarded.

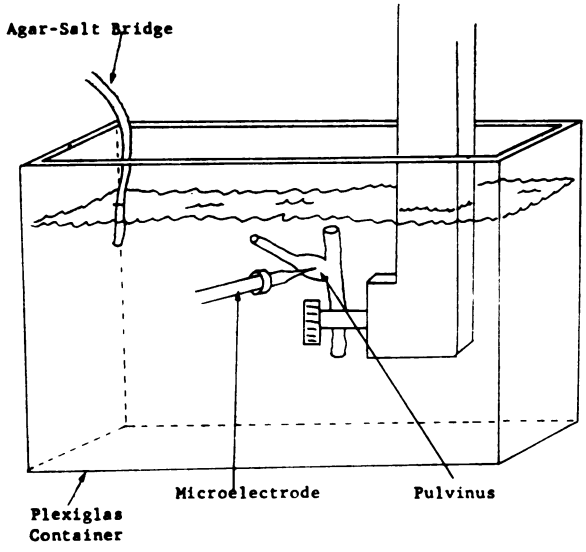

FIG. 1. Experimental arrangement used for measuring membrane potentials.

Results

Variation of K^+ in Nutrient Solution. Control plants grown in 6 mM K^+ solution grew to about 1 m in height in 2 months. The leaves remained a healthy dark green and retained normal reactivity for a number of weeks.

Plants grown in 5 ppm (0.13 mM) K^+ solution grew to about 30 cm in height in 2 months. The 2 youngest mature leaves remained green for a week or 2 and showed fair reactivity. Older leaves tended to turn brown and lose most of their reactivity.

The plants grown in solutions with no K^+ showed only about 8 cm of growth in 2 months. The leaves exhibited no sensitivity and turned brown within a few days.

Ion Analysis. An analysis of unreactive pulvini (very young leaves and old K^+-deficient leaves) was compared with an analysis of reactive pulvini (control plants and young K^+-deficient leaves). Potassium and chloride concentration differences between upper and lower pulvinar halves of reactive pulvini were always greater than the concentration differences for unreactive pulvini. In the unreactive pulvini from older K^+-deficient leaves, the difference in K^+ and Cl^- concentrations between top and bottom pulvinar halves was not significant ($P>0.05$) (table II). Unreactive pulvini from very young leaves

Table II. *Analysis of Unreactive Pulvini*

Results expressed as μmoles/g fresh weight $\pm$ standard error. Δ = Concentration in bottom—concentration in top. Δ is calculated for each individual sample, and these values averaged for entry in table. "Very Young Leaves" are leaves in which the pinnules have not fully opened. "K^+-deficient Leaves" are older leaves from normal plants in which the pinnules have begun to turn brown. Numbers in parenthesis are numbers of replicate analyses.

	Ca^{2+}	K^+	Cl^-	S	P
		μ moles/g fresh wt			
Very young leaves (11)					
Top	5.35 ± 0.2	82.4 ± 2.4	39.3 ± 1.1	9.7 ± 0.3	20.0 ± 0.8
Bottom	5.54 ± 0.2	93.5 ± 2.8	39.2 ± 0.9	9.5 ± 0.4	17.6 ± 0.6
Δ	0.19 ± 0.3	11.1 ± 3.3	—0.1 ± 1.2	—0.2 ± 0.5	—2.4 ± 0.1
K^+-deficient leaves (9)					
Top	26.1 ± 4.8	15.4 ± 2.5	97.1 ±13.0	8.6 ± 1.8	22.2 ± 2.9
Bottom	18.1 ± 1.6	19.6 ± 2.2	109.1 ± 8.3	7.6 ± 1.2	19.9 ± 1.8
Δ	—8.0 ± 5.3	4.2 ± 3.3	12.0 ±17.3	—1.0 ± 2.0	—2.3 ± 3.4

Table III. *Analysis of Reactive Pulvini*

Results expressed as μmoles/g fresh weight ± standard error. Top and bottom denote pulvinar halves. Δ = Concentration in bottom—concentration in top. Δ is calculated for each individual sample, and these values averaged for entry in the table. "Normal Tips" refers to pulvini of 2 youngest mature leaves from plants grown in K^+-deficient nutrient solution. "K^+-deficient Tips" refers to pulvini "K^+-deficient Leaves" are older leaves from normal plants in which the pinnules have begun to turn brown. Numbers in parenthesis are numbers of replicate analyses.

	Ca^{2+}	K^+	Cl^-	S	P
		μ moles/g fresh wt			
K^+-deficient tip (8)					
Top	13.5 ± 0.8	20.6 ± 2.1	56.1 ± 2.6	5.8 ± 3.2	20.9 ± 1.0
Bottom	10.9 ± 0.8	26.8 ± 2.8	86.2 ± 5.6	7.6 ± 0.6	19.3 ± 1.7
Δ	—2.6 ± 1.0	6.2 ± 2.1	30.1 ± 6.3	1.8 ± 0.9	—1.6 ± 2.2
Normal tip (10)					
Top	13.4 ± 0.8	121.0 ± 6.3	52.5 ± 2.8	...	...
Bottom	10.2 ± 0.4	160.8 ± 4.4	73.2 ± 3.0	...	...
Δ	—3.2 ± 0.9	39.8 ± 7.1	20.7 ± 4.4	...	...

exhibited a significant difference in K^+ concentration between top and bottom pulvinar halves, but concentration differences for Cl^- were not significant. In the reactive pulvini collected from the K^+-deficient plants (K^+-deficient tips) and plants grown in normal solution (normal tips), the difference in K^+ and Cl^- concentrations between top and bottom pulvinar halves was highly significant ($P<0.01$) (table III).

These data also indicate reactivity is not related to the absolute K^+ content of the pulvinus. Reactive K^+-deficient pulvini are low in K^+, while unreactive very young pulvini contain high concentrations of K^+. On the other hand, reactive normal pulvini are high in K^+, while K^+ concentration is low in older K^+-deficient unreactive pulvini. Similar results are noted for Cl^- content in the pulvini.

The difference in Ca^{2+} concentration between pulvinar halves was significant ($P<0.05$) for both normal and K^+-deficient pulvini. This was probably due to the fact that cell walls where Ca^{2+} is more concentrated than in the cytoplasm are thicker in the top half of the pulvinus.

Steady-State K^+ Efflux. The efflux of $^{42}K^+$ from the pulvinar cells was calculated using equation IV (7, 10).

$$J\ (flux) = \frac{\Delta\ ln\ C_i^*}{\Delta t} \quad \frac{V\ C_i}{A} \qquad \text{IV}$$

Where:

C_i^* = Concentration of $^{42}K^+$ remaining in pulvinus.

V = Cell volume to area ratio.

$\frac{V}{A}$ = $\frac{\text{Cell radius}}{3}$ (assuming cells are spherical)

C_i = Total K^+ concentration in pulvinus.

t = Time.

The logarithm of radioactivity in the pulvinus was plotted against time, and the slope of this plot, $\frac{\Delta\ ln\ C_i^*}{\Delta t}$, was used in equation IV to calculate $^{42}K^+$ efflux (table IV).

Table IV. *Steady-State $^{42}K^+$ Efflux From Pulvinar Cells*

Expt. No.	Efflux
	moles/cm² sec
6-20	2.82×10^{-12}
6-14	8.75×10^{-12}
6-28	7.75×10^{-11}
6-27	140×10^{-11}

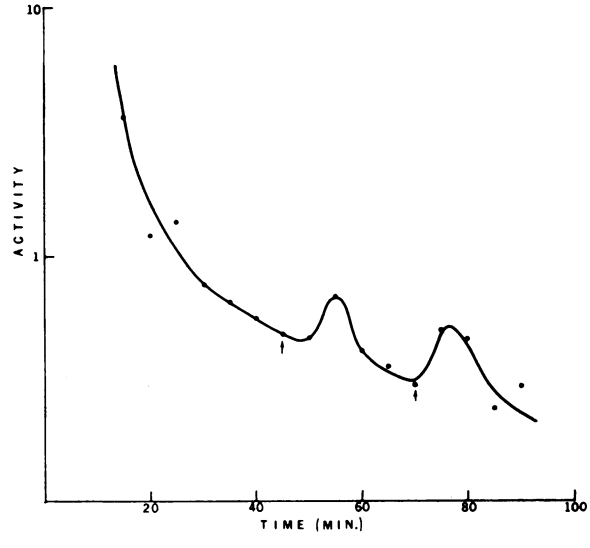

FIG. 2. Exchange of radioactive potassium; activity appearing in successive "wash-out" fractions plotted logarithmically against time. Stimulation of pulvini in braced condition indicated by first arrow and in unbraced condition by second arrow.

Table V. *Increase in $^{42}K^+$ Efflux During Stimulation of Braced Pulvini Compared to Stimulation of Unbraced Pulvini*

Expt. No.	Increase[1] braced	Increase[1] unbraced
	%	%
5-16	6	240
6-7	77	82
6-13	175	120
6-14	0	125
6-20	60	17
6-27	290	200

[1] % increase in effluxes was calculated as the increase in counts from the extrapolated washout curve.

$^{42}K^+$ Efflux During Stimulation. A large increase of $^{42}K^+$ efflux occurred when the pulvini were stimulated. These pulvini were either braced to prevent movement or not braced and allowed to move; in both cases, a large increase in efflux was observed (Fig. 2). The increase in efflux from the pulvinar cells during a single seismonastic reaction was as high as 290 % in the braced pulvini and 240 % in the unbraced pulvini (table V). A comparison of the $^{42}K^+$ efflux when the petiole was braced and stimulated and when the pulvinus was allowed to move indicates that the difference between these 2 values is not constant. Preventing pulvinar movement by merely bracing the petiole probably does not prevent the volume decrease of the lower pulvinar cells during the seismonastic reaction.

Membrane Potential Measurements. Pulvinar cell membrane potentials were measured in 3, 10, and 100 mM KCl bathing solutions. Large negative membrane potentials, as high as −134 mv (vacuole relative to bathing solution), were recorded in pulvinar cells (table VI). Ion concentrations for K^+ and Cl^- were also measured in the pulvini used in these experiments. The transmembrane potential decreased by about 33 mv when 10 mM KCl solution was replaced with 100 mM KCl solution, but only by about 8 mv when 10 mM KCl was replaced with 3 mM KCl. Note that the K^+ and Cl^- concentrations in the top pulvinar half in 10 mM KCl were much lower than the concentrations in the top pulvinar half in the other bathing solutions.

The difference in membrane potentials between cells of the top and bottom pulvinar halves was also checked (table VI). This difference was never significant ($P>0.05$). The possible significance of these data in interpreting the steady-state permeability properties of the cell membrane is discussed later.

Discussion

One of the more striking results of the ion analysis is the dependence of pulvinar reactivity on a highly significant K^+ and Cl^- concentration difference between upper and lower pulvinar cells. The dependence of reactivity on a concentration difference rather than absolute concentration may indicate that an osmotic pressure and thus turgor pressure difference between upper and lower pulvinar cells determines pulvinar reactivity.

The concentration difference cannot be accounted for by a difference in electrochemical potential gradients since cellular membrane potentials are equal in top and bottom pulvinar cells. Possibly selective active transport mechanisms exist in the cells composing one or both sides of the pulvinus.

The decrease in cellular membrane potential when the external solution was increased from 10 to 100 mM KCl is in the direction predicted by a membrane more permeable to K^+ than Cl^-. The decrease of 33 mv reported here is close to the decrease of 25 mv reported for oat coleoptile cells (11).

The decrease in membrane potential when going from 10 mM KCl to 3 mM KCl was significant ($P<0.05$) for the bottom pulvinar half but not for the top. In this concentration range, the decrease in membrane potential with decreasing external KCl concentration would indicate a membrane more permeable to anions than K^+ at least for the bottom

Table VI. *Membrane Potentials and K^+ and Cl^- Concentrations in* Mimosa pudica *Pulvini*

Membrane potentials were measured as vacuole relative to bathing solution and represent the average of all measurements made on 4 pulvini in each bathing solution. Numbers in parenthesis denote number of measurements. T and B refer to top and bottom pulvinar halves respectively. The difference in membrane potential between top and bottom halves was calculated from successive measurements only. In addition to KCl all bathing solutions contained 1 mM $NH_4H_2PO_4$, 4 mM $Ca(NO_3)_2$ and 2 mM $MgSO_4$.

KCl Concn in bath	3 mM		10 mM		100 mM	
position in pulvinus	T (22)	B (23)	T (23)	B (24)	T (22)	B (24)
Membrane Potential ± Standard Error	128.8 ± 2.7	122.9 ± 2.3	134.2 ± 2.7	132.7 ± 2.5	97.4 ± 3.5	99.8 ± 3.4
Ion Concentration K^+	104.5	103.0	79.7	106.5	130.8	152.0
μmoles/g fresh wt Cl^-	40.1	**45.2**	**39.6**	80.9	68.6	82.1
Difference in membrane potential between top and bottom halves ± SE	3.8 ± 2.1 (17)		1.3 ± 2.4 (9)		0.1 ± 2.8 (20)	

pulvinar cells. Possibly the pulvinar cell membrane is highly permeable to K^+ in the 10 to 100 mM KCl range, but in the 3 to 10 mM KCl range K^+ permeability decreases to less than Cl^- permeability. A decrease in K^+ permeability at low external K^+ concentrations has previously been proposed for the squid axon membrane (3).

Steady-State $^{42}K^+$ Efflux. The efflux values measured for *Mimosa pudica* are probably larger than the real values due to some $^{42}K^+$ efflux through the epidermis of the stem and petiole. However, the epidermis presents a much larger diffusion barrier than the pulvinar cell walls, and it is unlikely that the measured fluxes are markedly different from the real fluxes.

The K^+ efflux values of from 8.7 to 77.5 p moles/cm^2 sec measured in this work are similar to those reported for *Valonia ventricosa* (10). However, fluxes are smaller in other marine plants (7), in fresh-water plants (8, 14, 16), and in land plants (17).

Detection of a Reflection Coefficient Change. As pointed out in the Introduction, a decrease in σ could account for the pulvinar cell turgor loss during the seismonastic reaction. The value of σ for K^+ and its counterions can be estimated from equation V derived by Dainty and Ginzburg (6).

$$\sigma = 1 - \frac{\omega \bar{V}_s}{Lp} - \text{friction term} \qquad \text{V}$$

Where:

V_s = Partial molar volume of solute.

Frictional term—accounts for the frictional interaction between solute and water and between solute and membrane.

If the $\frac{\omega \bar{V}_s}{Lp}$ term in equation V is small, *i.e.* Lp is much larger than $\omega \bar{V}_s$, then the frictional term is probably small. In calculations below, the value of $\omega \bar{V}_s$ in the steady state is shown to be much less than Lp. Thus, the frictional term will be disregarded in this work when calculating σ for the steady state. Obviously any change in σ will result in a change in ω and *vice versa*. For the calculation of σ, a reasonable estimate of Lp may be obtained from the literature (4) and ω calculated from equation III by considering the K^+ and Cl^- ions as the electroneutral salt (KCl).

$$J_s = \omega RT \triangle C_s \qquad \text{III}$$

$\triangle C_s$ = 0.1 moles/liter — Calculated from data in table III average of values given in table IV

J_s = 2.5 × 10^{-11} moles/cm^2 sec.

$$\omega = \frac{2.5 \times 10^{-11}}{(8 \times 10^{-2})\ (3 \times 10^{2})\ (0.1)}$$

$$= 1 \times 10^{-11} \text{ moles/atm cm}^2 \text{ sec.}$$

Using this value in equation V:

$$\sigma = 1 - \frac{\omega \bar{V}_s}{Lp} - \text{friction term} \qquad \text{V}$$

$\bar{V}_s$ = 26 cm^3/mole for KCl

$$\sigma = 1 - \frac{(1 \times 10^{-11})\ (2.6 \times 10^{1})}{10^{-6}}$$

− frictional term

$$\sigma = 1 - 2.6 \times 10^{-4} - \text{frictional term}$$

Since $\frac{\omega \bar{V}_s}{Lp}$ is small, the frictional term is probably small and may be neglected.

σ = 1. Thus, σ = 1 for KCl under steady-state conditions.

Equation II indicates that if σ = 1, $(1-\sigma)\ \bar{C}_s J_v$ will be very small and J_v should make little or no contribution to J_s. Since σ = 1 for $^{42}K^+$ in the steady state, the volume flow occurring during the seismonastic reaction should not result in any increased $^{42}K^+$ efflux unless σ decreases. The results of the $^{42}K^+$ efflux experiments (table V and Fig. 2) show that a large efflux of $^{42}K^+$ occurred when the pulvinus was stimulated, indicating that a decrease in σ did occur.

This decrease in σ can be seen readily by referring to equations I and II. A volume efflux would occur with the decrease in σ (equation I). Then an increase in $^{42}K^+$ efflux would result from the decrease in σ and from the increase in J_v (equation II). Presumably, σ decreases only in the lower pulvinar cells although separate efflux experiments could not be performed on upper and lower pulvinar halves to check this point.

It should be pointed out that no details of the σ decrease can be deduced from these results. However, some possibilities can be suggested. Possibly a nonselective reflection coefficient change occurs for all or most cell solutes, and the hydrostatic pressure forces water and solutes from the cell. On the other hand, a selective reflection coefficient change might occur. Perhaps σ first decreases for anions and then for K^+, similar to the events occurring during the *Chara* action potential (9). More studies must be made before any details of the σ decrease indicated by this work can be determined.

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