

Migration of Calcium and Its Role in the Regulation of Seismonasty in the Motor Cell of *Mimosa pudica* L.¹

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ABSTRACT

Volume and conformational changes of the contractile tannin vacuoles of the abaxial motor cells of the primary pulvinus of *Mimosa pudica* L. parallel the seismonastic leaf movement. Since such changes in cells and organelles of animal systems are often regulated by calcium, we studied Ca²⁺ movement in the motor cells and tissue. By fixation with Lillie's neutral buffered formalin, followed by staining with alizarin red sulfate (ARS), calcium was localized in the tannin vacuoles of the motor cells of the primary pulvinus. After treatment with ethylenediaminetetraacetate, 8-hydroxyquinoline, and several other calcium-complexing or extracting agents, the color reaction due to alizarin red sulfonate was no longer present. By using an analytical method, it was shown that the effluent from stimulated pulvini has significantly more Ca²⁺ than that from unstimulated controls. Ten millimolar LaCl₃ inhibits recovery of the tannin vacuole *in vivo* in 10 mM CaCl₂ or in distilled water. Quantitative data obtained by microspectrophotometry demonstrated calcium migration during the bending movement of the primary pulvinus. In the adaxial motor cells a small amount of calcium migrates from the tannin vacuole, and calcium on the cell wall moves to the central vacuole. In the abaxial half, a large amount of calcium from the tannin vacuole moves to the central vacuole of the motor cell. It is probable that the calcium binds to the microfibrillar contents of the central vacuole. These observations support the contention that Ca²⁺ migrates between the surface of the tannin vacuole and the inside of the central vacuole. The recovery and maintenance of the tannin vacuole in the spherical form may play a role in maintaining turgor in the motor cells of the abaxial half of the primary pulvinus of *Mimosa*.

An abaxial motor cell of the primary pulvinus of *Mimosa pudica* contains a large central vacuole, within which, and abutting the side of the cell, is a large contractile tannin vacuole. Contraction and recovery of these tannin vacuoles parallel the bending movements of the pulvinus. By using fixed and stained preparations, morphological changes in the tannin vacuole (30, 32-34, 39, 40) as well as ultrastructural changes in the central vacuole (40), have been observed before and after tactile stimulation. Such observations have led to specu-

lation about the possible causative role of these organelles in the turgor changes of the motor cells that are responsible for the bending motion. These turgor changes are probably brought about by the rapid efflux of K⁺, which serves as the osmotic agent (2, 31).

The induced efflux of monovalent cations, such as potassium, has long been known to be associated with changes in cellular form and activity of animal cells and organelles. In addition, the intracellular movement of calcium ions has been correlated with such potassium fluxes, as well as with conformational changes in proteins (4, 12) and cells (21, 22). Since conformational changes of the tannin vacuoles and of the contents of the central vacuole can be correlated with the bending movement of the *Mimosa* pulvinus (1, 35, 36), it seemed appropriate to study the possible relation of Ca²⁺ to these changes. Generally, living cells possess ionized or bound calcium (18, 23), and early workers used sodium alizarin sulfonate to detect it cytochemically in the ionized or weakly bound form (5). More recently, alizarin red sulfate has been employed (7, 8, 19). We have coupled this chromogenic technique to that of microspectrophotometry to localize Ca²⁺ within the pulvinal motor cells and to follow its migration during seismonastic bending of the leaf.

MATERIALS AND METHODS

We used the primary pulvinus of *Mimosa pudica* L. The plants were grown in pots in the greenhouse and were transferred to the laboratory windowsill before use. In order to examine the motor cells before the bending movement, the plants were anaesthetized with ethyl ether vapor for 30 min (30, 32, 33).

For the microscopic localization of calcium in the motor cell, pulvini obtained before or after stimulation were fixed in Lillie's neutral buffered formalin (commercial formalin, 100 ml; distilled water, 900 ml; NaH₂PO₄·H₂O, 4 g; Na₂HPO₄, 6.5 g), at room temperature for 24 hr (17, 20). They were then washed in running water for several hours and dehydrated with a *t*-butanol series and embedded in paraffin. Thirty-micron sections were cut and mounted with Meyer's albumin adhesive.

Dahl's method was modified for the detection of calcium in the *Mimosa* motor cell (7). One percent ARS³ was made up in distilled water and brought to pH 5.5 with 2% aqueous NH₄OH. It is worth stressing that the staining reaction for calcium is delicately dependent upon the pH of the ARS solution (7, 19). This solution was stable in the refrigerator for 2 to 3 months. ARS satisfactory for use in this procedure was obtained from Industrial Chemicals Division, Allied Chemical Co., Morristown, New Jersey, and K & K Laboratories, Inc., Plainview, New York.

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³ Abbreviation: ARS: alizarin red sulfonate.

After staining for 2 hr, the sections were washed in distilled water for 20 sec, and then soaked in acetone for 25 sec. They were then placed in a jar of acetone-xylol (50:50) for about 25 sec, soaked in xylol for 40 to 60 sec, and mounted in xylol-balsam. To make the xylol-balsam solution, 12 ml of xylol was mixed with 40 ml of balsam and kept in an open beaker for 3 hr to allow excess xylol to evaporate. The remaining solution was then stored for use in a closed container. In successful preparations using this technique, the red color reaction due to the calcium-ARS complex is clearly observable.

The red colored complex was prepared *in vitro*, by mixing 10 ml of 10 mM CaCl₂ buffered with phosphate at pH 5.5 with 1 ml of 0.2% ARS (18), on ringed and covered glass slides for spectrophotometric analysis.

The stained intracellular elements, as well as the complex of Ca²⁺-ARS *in vitro*, were quantitatively analyzed with a Leitz M P V microspectrophotometer. This consisted of a Leitz Ortholux microscope fitted with a constant voltage light source, a series of second order interference filters (Bausch and Lomb Optical Co.), coupled with appropriate colored glass filters (Corning Glass Co., Corning, N. Y.). The optical arrangement, photomultiplier, and electronic instrumentation were designed for the microscope by the manufacturer (E. Leitz, Inc., New York, N.Y.). The instrument was housed and used in a temperature-controlled darkroom, since even the slightest stray light reflected to the stage was detected by the photomultiplier.

The effects of calcium-complexing reagents was studied with resting and stimulated pulvini. They were fixed as usual, but after the tap water wash, the sections on the glass slide were soaked in aqueous solutions of Ca-extracting or complexing agents at 20 C, for 20 to 120 min. The reagents that were used were 1 mM EDTA, 10 μM 8-hydroxyquinoline, 10 μM phosphotungstic acid, 0.1 mM ammonium molybdate, 1 mM ammonium phosphate, 10 mM ammonium sulfate, 5% acetic acid, and 0.25% nitric acid. The sections were then thoroughly washed in distilled water and stained with 1% ARS.

By using intact plants, the rate of leaf recovery was determined by measuring the abaxial angle at short intervals. Recovery of the tannin vacuole was measured on vacuoles of motor cells contained in thin free-hand sections of the primary pulvinus bather in buffer on a microscope slide. The dimensions of the vacuoles were measured at close intervals with a calibrated ocular micrometer.

Since lanthanum ions have been shown to affect the transmembrane movement of Ca²⁺ (21, 43, 44), we tested the effect of LaCl₃ upon the recovery process of tannin vacuoles *in vivo*. Living, stimulated motor tissues, in thin section, the motor cells of which had vacuoles that had been flattened against the side of the cell, were used. The length and width of tannin vacuoles in the ventral half of the primary pulvinus were measured immediately after stimulation and 20 to 30 min after soaking in 10 mM LaCl₃, 10 mM CaCl₂, or distilled water.

For the measurement of total Ca²⁺ efflux from resting or stimulated excised pulvini, the pinnae were stripped off and the sides of the pulvini trimmed off after the method of Blackman and Paine (3). Each pulvinus-petiole preparation was aligned on a glass microscope slide and held in place with petroleum jelly which also served to seal the wounds and prevent dehydration. The slide was held at the distal end by a clamp attached to a rack and pinion. The pulvini which hung loose below the slide could thus be raised or lowered into a plastic cup containing 5 ml of phosphate buffer, pH 6.5. The pulvini were allowed to remain at rest for several minutes, and then an aliquot of the bathing solution was taken for measurement. The tissue was stimulated by cutting the distal end of

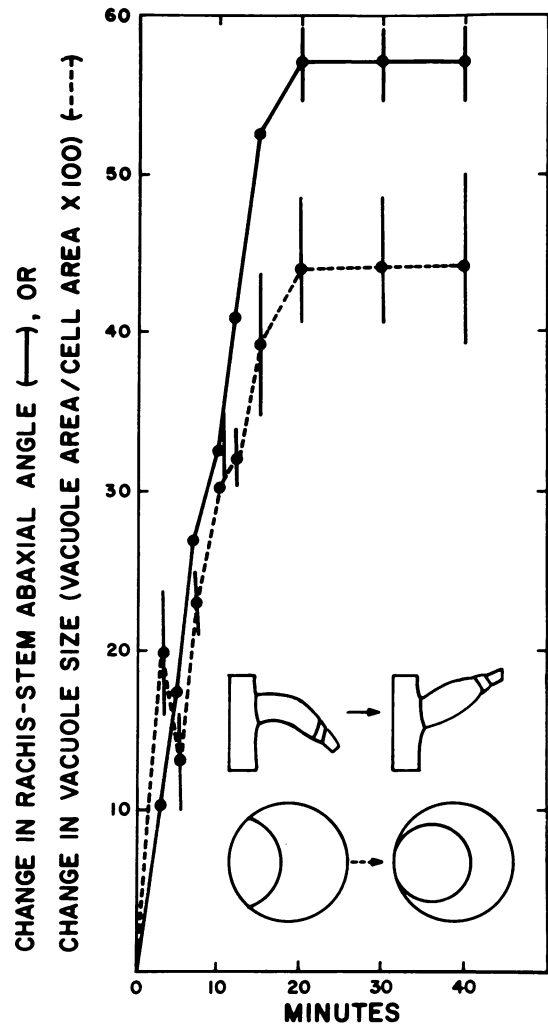


FIG. 1. Kinetics of recovery of the seismonastically stimulated leaf and of the tannin vacuoles in the primary pulvinus. Inserts show the changes in conformation of the primary pulvinus and of the tannin vacuole due to mechanical stimulation.

Table I. The Concentration of Calcium in the Bathing Solution of Primary Pulvini of *Mimosa pudica* L. before and after Several Mechanical Stimuli

Aliquots of the solution bathing the pulvini were analyzed for calcium by the chromogenic method of Golterman and Clymo (11).

Experiment No.	Pulvini No.	Stimuli No.	Amount of Ca ²⁺ before Stimuli	Amount of Ca ²⁺ after Stimuli	Change in Ca ²⁺
			<i>nmoles</i>		
1	5	4	5.5	8.5	3.0
2	5	2	0.5	1.5	1.0
3	4	3	5.0	9.5	4.5
4	3	3	5.5	8.5	3.5
5	3	3	1.0	4.0	3.0
6	3	3	3.0	5.5	2.5
Average	4	3	4.0	7.0	3.0

the petiole with a razor blade, and another aliquot of the bathing solution was taken. The amount of Ca²⁺ in the bathing solutions was measured by the chromogenic method of Golterman and Clymo (11), whereby the absorbance of a com-

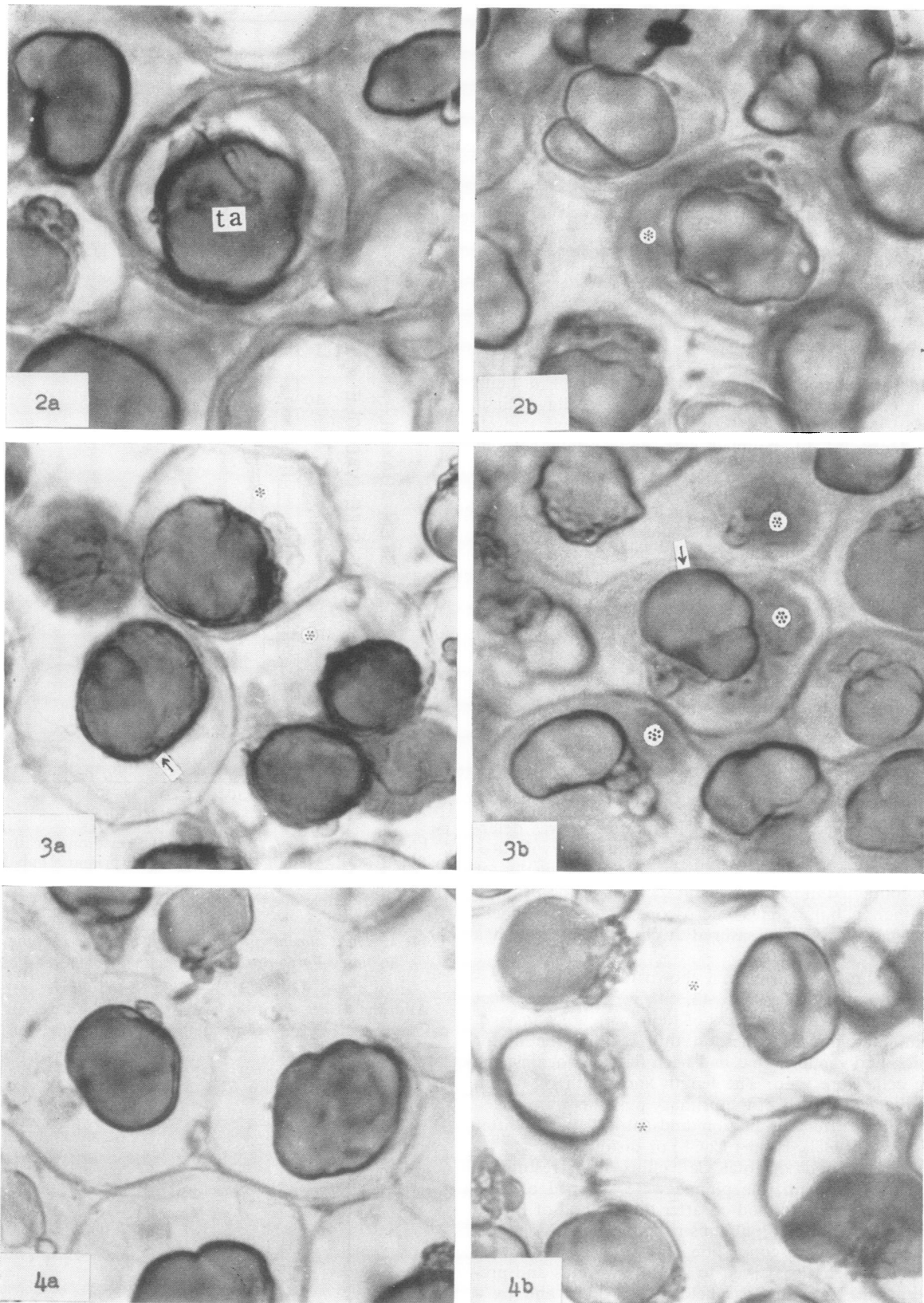


FIG. 2. Cytochemical detection of calcium in motor tissue-cells of the adaxial half of the primary pulvinus of *Mimosa pudica*. a: Before stimulation; b: after stimulation; ta: tannin vacuole; arrows indicate cell wall; asterisk indicates central vacuole. $\times 1000$.

FIG. 3. Cytochemical detection of calcium in motor tissue-cells of the abaxial half of the primary pulvinus of *Mimosa pudica*. a: Before stimulation; b: after stimulation; arrow indicates surface of tannin vacuole; asterisks indicate central vacuoles. $\times 1000$. Sections were fixed with Lillie's buffered neutral formalin and stained with alizarin red S.

FIG. 4. Motor tissue-cells of the abaxial half of the primary pulvinus of *Mimosa pudica*. a: Before stimulation. b: after stimulation. After treatment with acetic acid for 15 min and washing with water, the sections were stained with alizarin red S. $\times 1000$.

plex formed by Ca^{2+} and glyoxal *bis*-(2-hydroxyanil) is measured at 520 nm.

RESULTS

In order to correlate the changing form of the tannin vacuoles with the movement of the leaf we studied the recovery rates of the two processes. When the rachistem abaxial angle was measured during recovery from bending and compared to the kinetics of vacuole recovery, both curves follow the same time course, recovering in about 15 to 20 min (Fig. 1). Thus, the conformational changes of the tannin vacuoles provide an excellent cellular correlate of seismonastic leaf movement.

When the effluent from the primary pulvinus was processed with glyoxal *bis*-(2-hydroxyanil), a colored product was produced, indicating that Ca^{2+} was present. The amount of Ca^{2+} in the effluent taken after stimulation was greater than that taken before stimulation (Table I). However, this efflux of Ca^{2+} from the cells of the pulvinus might have come largely from the cut tissue, which is not normally present *in situ*. In the living plant, it is likely that this Ca^{2+} moves into the stem.

In addition to efflux of Ca^{2+} from the pulvinus, we studied Ca^{2+} migration within the motor cells. Using fixed and stained material, red color reactions, typical of Ca^{2+} complexes, were clearly observable (Figs. 2a-3b). There were obvious changes in the site of the color reaction in motor cells examined before and after stimulation. Although some of the Ca^{2+} was surely washed out of the tissue during fixation, Ca^{2+} was always present in the tannin vacuole before stimulation and in the central

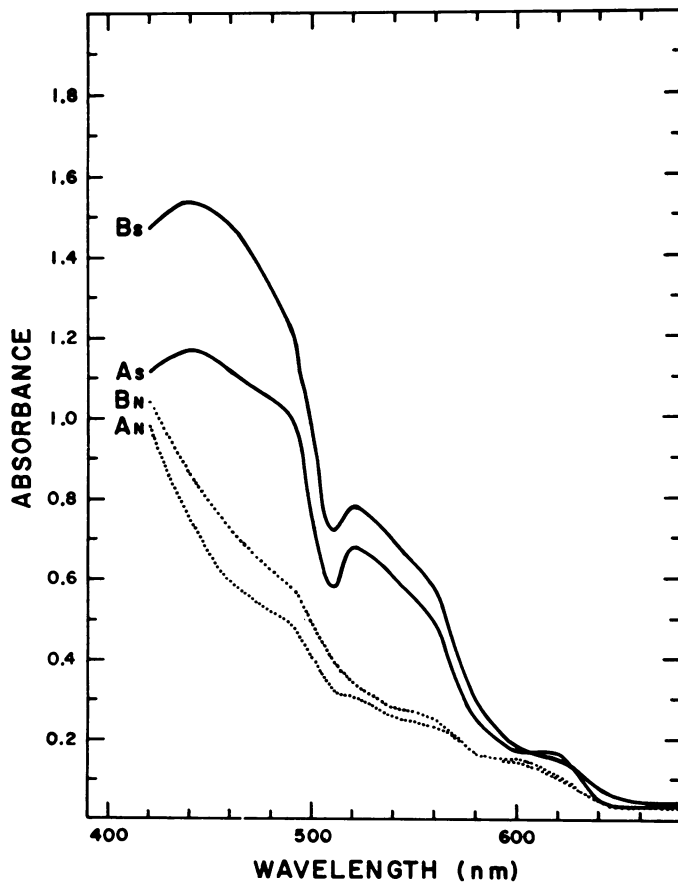


FIG. 5. Absorption spectra of tannin vacuoles in the adaxial *Mimosa* motor cells. Bs: Before stimulation and stained; As: after stimulation and stained; Bn: before stimulation and nonstained; An: after stimulation and nonstained.

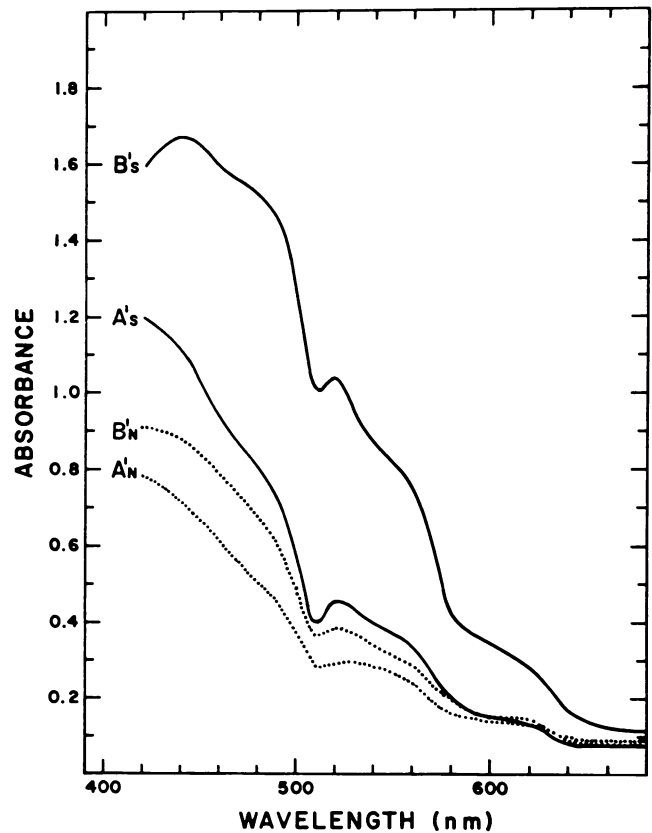


FIG. 6. Absorption spectra of tannin vacuoles in the abaxial *Mimosa* motor cells. B's: Before stimulation and stained; A's: after stimulation and stained; B'n: before stimulation and nonstained; A'n: after stimulation and nonstained.

Table II. *Microspectrophotometric Analysis of the ARS Staining Reaction of Tannin Vacuoles in the Motor Cells of the Primary Pulvinus*

Absorbance at 440 nm was computed from measurements made of both staining and unstained material. Each datum is the average of 100 measurements and is followed by its standard error.

	Absorbance at 440 nm		
	Before stimulus	After stimulus	Change
Adaxial half			
Stained	1.542 ± 0.077	1.160 ± 0.041	0.382 ± 0.059
Nonstained	0.862 ± 0.038	0.752 ± 0.053	0.110 ± 0.046
Abaxial half			
Stained	1.673 ± 0.054	1.106 ± 0.019	0.567 ± 0.037
Nonstained	0.885 ± 0.058	0.704 ± 0.024	0.181 ± 0.041

vacuole after stimulation, and was therefore at least weakly bound to those elements. Some of these weak bonds were apparently broken when the stain was introduced to the cells, so that the Ca^{2+} complexed with the ARS.

The Adaxial Motor Cells. In the motor cells of the adaxial half of the resting primary pulvinus, the ARS reaction was found in the tannin vacuoles and in the cell walls (Fig. 2a). After stimulation, the tannin vacuoles assumed an orange color, the cell wall did not stain red (Fig. 2b), and the central vacuole (indicated with an asterisk) showed only a slight red

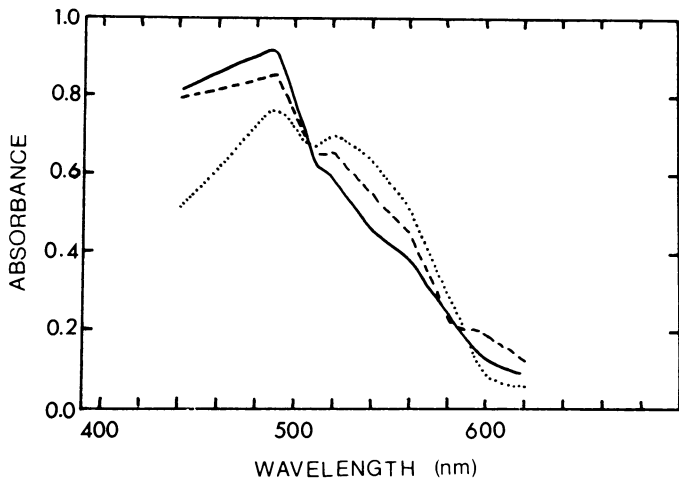


FIG. 7. Absorption spectrum of authentic CaCl_2 -ARS complex (\cdots) (10 mM CaCl_2 [2 ml] + 0.2% ARS [1 ml] at pH 5.5 with phosphate buffer) compared with the difference spectrum of the Ca^{2+} -ARS complex on the tannin vacuole of the abaxial motor cell before stimulation (----), and the difference spectrum of the Ca^{2+} -ARS complex in the central vacuole of the abaxial motor cell after stimulation (—). All of the spectra were obtained with the microspectrophotometer.

color. In unstained material, the tannin vacuole is yellow. The absorption spectra of nonstained tannin vacuoles in the adaxial half before (BN) and after stimulation (AN) are shown in Figure 5. These spectra displayed no peaks and were only slightly different. In stained material, however, absorption peaks were found at 440 and 520 nm before and after stimulus.

The Abaxial Motor Cells. On the abaxial half of the pulvinus, the picture that one sees is quite different. Before receiving a stimulus, the surface of the tannin vacuole (as indicated by the arrow in Fig. 3a) is round and stains red with ARS. The central vacuole (as indicated by the asterisks in Fig. 3a) does not stain. After receiving a stimulus, the tannin vacuole assumes a nonspherical shape, no longer stains with ARS, and appears yellow. At the same time, the central vacuole (indicated by the asterisks) assumes the ARS red color (Fig. 3b). There is no difference in the cell walls of the abaxial motor cells before and after stimulation (Fig. 3, a and b). Under these experimental conditions, the protoplasm could not be observed either before or after stimulation. The absorption spectra of the unstained abaxial tannin vacuoles before (B'N) and after stimulation (A'N) show only small absorbance differences (Fig. 6). The absorption spectra of the stained abaxial tannin vacuole fixed before (B'S) and after stimulation (A'S) showed absorption peaks at 520 nm and 440 nm.

Table II gives the absorption and absorption differences for tannin vacuoles on the adaxial and abaxial sides. The tannin vacuoles on the abaxial side had more Ca^{2+} before stimulation, and lost more Ca^{2+} due to stimulation. The absorption spectrum of the abaxial central vacuole after stimulation is indicated in Figure 7. We could measure the absorption spectra of both unstained (B'N) and stained (B'S) unstimulated ventral cells. The difference spectrum (B'S-B'N), which shows the amount of color complex on the tannin vacuole before stimulation, was computed (Fig. 7). This difference spectrum is almost identical to the absorption spectrum of the color complex found in the central vacuole after stimulation. In other words, the same amount of Ca^{2+} that is in the tannin vacuole before stimulation, is found in the central vacuole after stimulation. For comparison, an absorption spectrum of a Ca^{2+} -ARS com-

plex, measured *in vitro* with the microspectrophotometer, is also shown in Figure 7. Its similarity to the stained cellular material can readily be seen.

After treatment with each of the Ca^{2+} complexing or extracting agents, neither the tannin vacuoles in resting motor cells nor the contents of the central vacuoles in stimulated motor cells reacted to ARS (Fig. 4, a and b). It is probable that the Ca^{2+} on the tannin vacuole and/or in the central vacuole either was washed out by these reagents or rendered unreactive with ARS.

In the living motor cells, the tannin vacuoles are seen to be flattened on one side in the abaxial motor cells of the primary pulvinus within 3 to 4 min after receiving the stimulus (35, 36). On recovery, the tannin vacuole is restored to the spherical state after 15 to 20 min. The effects of the addition of La^{3+} and Ca^{2+} to living cells are shown in Figure 8 and Table III. In either distilled water or CaCl_2 , the length and width of the tannin vacuole became almost equal, and the recovery to spherical state was made in 20 to 30 min. However, the diameters of the tannin vacuoles in the motor cells soaked in distilled water were somewhat larger than those which had been treated with CaCl_2 . The lengths and widths of the tannin vacuoles from cells treated with both LaCl_3 and CaCl_2 are shown in Figure 8d. The effect of CaCl_2 upon the tannin vacuole was inhibited by the LaCl_3 . As can be seen in Table III, both the length and width of the recovering tannin vacuole always increased. However, where LaCl_3 was added, the width did not increase as much as the length, resulting in a form that was not spherical (Fig. 8).

In the electron microscopic study, the abaxial central vacuoles were seen to have a fine fibrillar content before stimulation. After stimulation, however, the contents appeared contracted and clumped together (Fig. 9).

DISCUSSION

Although the volume changes of the tannin vacuoles in the abaxial motor cells are of interest *per se*, they are significant because the time course of vacuolar recovery is the same as that of the seismonastic recovery motion of the leaf. The combination of Lillie's neutral formalin and ARS gave excellent results in the fixation of the tannin vacuole as well as in demonstrating calcium. The validity of identification and detection of calcium was supported both by the removal of calcium by Ca^{2+} -extracting agents, and the observation that ARS will not produce the red color with other cations tested. This is reinforced by the fact that the absorption spectrum of a known Ca^{2+} -ARS complex was similar to the spectra of stained cellular elements. As a result of the study of effluxing Ca^{2+} , it is obvious that some of the Ca^{2+} that moves in the cells also leaves the tissue. Therefore, the Ca^{2+} that ultimately complexes with the ARS is probably bound and not free to move within or out of the motor cells after fixation. Dissolved Ca^{2+} , however, probably does get washed out of the cells during fixation. Thus, we propose that the stained Ca^{2+} is bound to the tannin vacuole membrane in the resting motor cells, and to something else (possibly microfibrils) in the central vacuoles of stimulated motor cells. Implicit in this hypothesis is the assumption that the bonds holding the Ca^{2+} to the intracellular elements are relatively weak, and that the Ca^{2+} can preferentially bind to the ARS at a pH of 5.5. This assumption is supported by other studies (7, 19).

The general structure of the motor cell of the primary pulvinus is shown in Figure 10a. Each motor cell has a thin cytoplasmic layer at its periphery and diffused fine fibrillar contents distributed in the central vacuole (Fig. 9a).

Upon receiving a stimulus, the adaxial half of the pulvinus

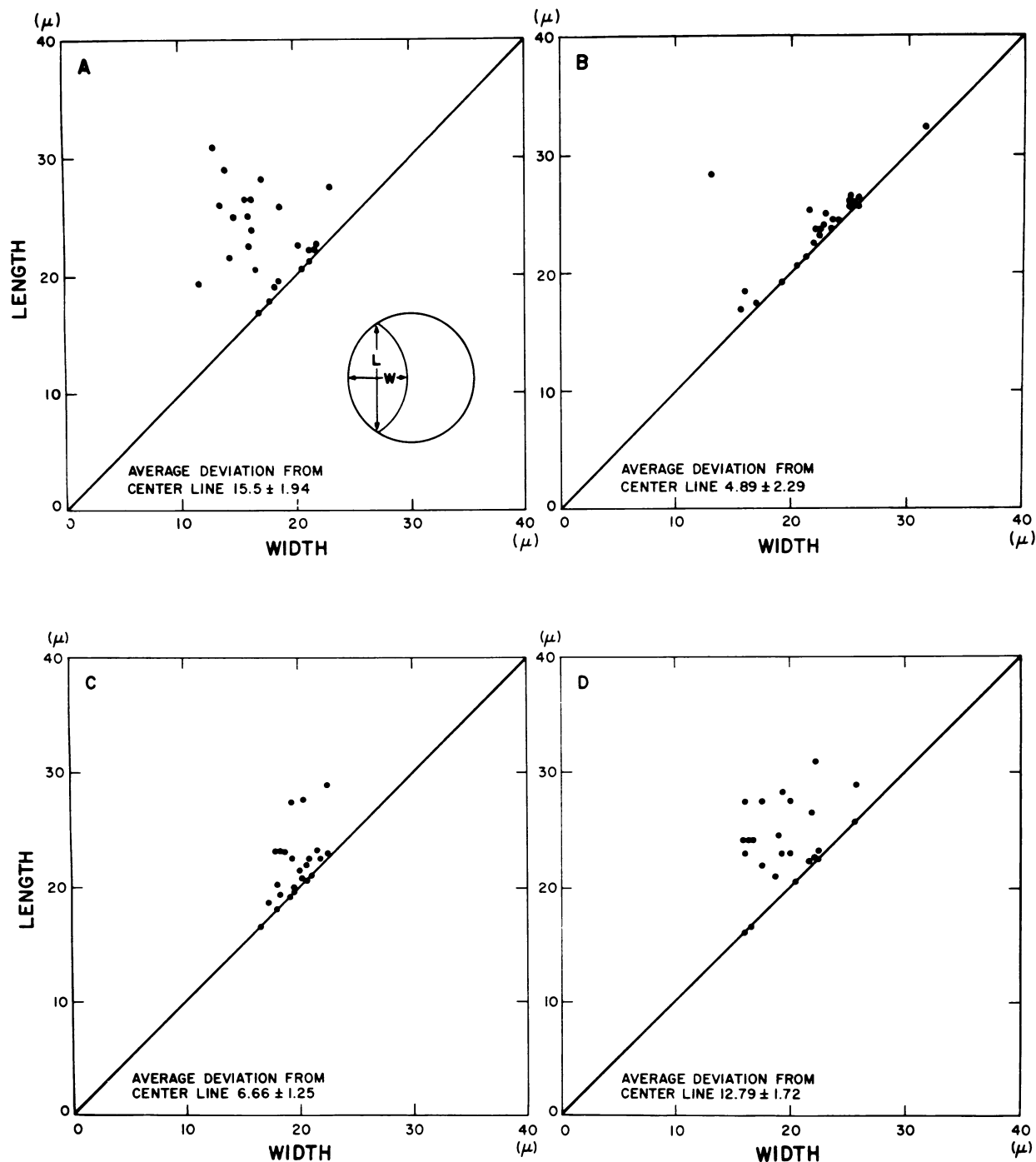


FIG. 8. The length and width of tannin vacuoles in the abaxial *Mimosa* motor cell during recovery. In each case, the diagonal line indicates the dimensions of a perfectly circular outline. A: Cells soaked in 10 mM LaCl_3 for 20 min.; B: cells soaked in distilled water for 20 min.; C: cells soaked in 10 mM CaCl_2 for 20 min.; D: cells soaked in 10 mM CaCl_2 + 10 mM LaCl_3 for 20 min.

expands slightly, but the abaxial half contracts. At some time during the bending process, a small amount of Ca^{2+} probably moves rapidly out of the adaxial half of the pulvinus, where it migrates from the tannin vacuole and from the cell wall. Considerable evidence has accumulated that the presence of Ca^{2+} increases the rigidity of the cell wall (6, 26, 29). It is probably advantageous that the Ca^{2+} leave the thick cell wall of the adaxial half of the pulvinus in order to decrease its rigidity and facilitate the bending movement of the primary pulvinus.

The degree of wall binding of this Ca^{2+} is unknown, but it is remarkable that it is induced to move so quickly.

A schematic illustration of the events in the abaxial motor cell are summarized in Figure 10, d and e. Upon stimulation, most of the Ca^{2+} leaves the tannin vacuole membrane and moves to the central vacuole of the motor cell, while the tannin vacuole assumes a nonspherical form (Fig. 4b) (25, 35, 36). The change of the fine fibrillar contents of the central vacuole was observed by electron microscopy as summarized in Figure

10, f and g. Since the fine fibrils in the central vacuole can be dissolved with Weber-Edsall solution (41), a preparation that removes contractile proteins, and since the binding of Ca^{2+} with proteins has been demonstrated to alter their conformation (4, 12), it may be that the fibrils contain a contractile protein. These fibrils were seen to be connected to the tonoplast, and the Ca^{2+} which leaves the tannin vacuole may form a complex with them (shown schematically in Fig. 11). Thus, their contraction might reasonably be expected to affect permeability changes in the membrane of the central vacuole. Several authors have mentioned that Ca^{2+} is essential for the integrity of boundary membranes, such as the plasmalemma (25, 27). We have observed that Ca^{2+} accelerates the resumption of a spherical form by the tannin vacuole. In the abaxial motor cell, we proposed that Ca^{2+} is necessary for maintaining the membrane of the tannin vacuole in its normal resting state.

The recovery phase of the bending movement of the *Mimosa* pulvinus may be brought about by the recovery of tannin vacuoles which fix the Ca^{2+} from the central vacuole on their surface membranes. In muscle, the sarcoplasmic reticulum retains the Ca^{2+} which leaves the contractile protein during relaxation of the muscle fiber (9, 10, 14, 22), and the tannin vacuole may play an analogous role in the *Mimosa* abaxial motor cells. LaCl_3 has been shown to inhibit Ca^{2+} transport

in mitochondria (21), in smooth muscle cells (43), and across artificial membranes (21). Recently, it was shown to have a competitive effect on Ca^{2+} in the growth of oat coleoptiles (24). Our experiments *in vivo* using LaCl_3 and/or CaCl_2 support the probable physiological importance of the migration of Ca^{2+} between the surface of the tannin vacuole and other cellular elements, such as the central vacuole. It is probable that the LaCl_3 inhibits the migration of Ca^{2+} back to the surface of the tannin vacuole and, therefore, the recovery of the tannin vacuole to the spherical shape. The recovery of the bending movement probably is brought about by the recovery of turgor of the abaxial cells which is caused by permeation of an osmotic agent back into the motor cells (31). The movement of K^+ out of motor cells in the primary pulvinus during the bending movement has been demonstrated by the use of isotopically labeled K^+ (2), microashing techniques (31), and supported by electron microprobe evidence on pulvinal sections of *Albizia julibrissin* during nyctinasty (28). In these studies, K^+ has been implicated as the osmotic agent. Under certain conditions, Ca^{2+} enhances the uptake of K^+ by excised roots and other tissues (13, 15, 42). Thus, in the *Mimosa* motor tissue, Ca^{2+} may enhance the uptake of the osmotic agent (K^+) from the intercellular spaces during the recovery process. Because the tannin vacuole is semipermeable (37) and is spherical in the resting state, it seems probable that during recovery it may enlarge as an osmotically active agent (K^+) moves back into it, drawing water in. Although evidence for a coordinated turgor mechanism has not been demonstrated in plants heretofore, it is plentiful in experiments with animal material. Thus, in both nerves and muscle the release of Ca^{2+} has been shown to trigger the efflux of monovalent cations (e.g., H^+ , K^+), which results in volume changes in the cell or organelle. In animal systems, the release of Ca^{2+} is effected by the neurohumor, acetylcholine. A system which utilizes acetylcholine to affect the efflux of H^+ , has recently been demonstrated in bean roots (16), and may also function in the abaxial motor cells of *Mimosa pudica*.

In order to integrate our data with other observations in a causal sequence that makes physiological sense, we propose the following model of seismoreception and response in the primary pulvinus of *Mimosa pudica*. Stimulus energy is absorbed at an as yet unidentified receptor site. Transduction of the en-

Table III. Effect of LaCl_3 on Recovery of the Tannin Vacuoles in Abaxial Motor Cells of the Primary Pulvinus

Thin, living sections of pulvinal tissue were tactically stimulated to contract and then were placed in the test solution. The dimensions of the tannin vacuoles were measured then and 15 min later, and the change computed. Each datum is the average of 25 or more measurements and is followed by its standard error.

	Average Change in Length of Tannin Vacuole	Average Change in Width of Tannin Vacuole
Distilled water	23.79 ± 0.78	22.33 ± 0.82
10 mM CaCl_2	21.83 ± 0.65	19.67 ± 0.31
10 mM LaCl_3	23.87 ± 0.63	17.73 ± 0.60
10 mM CaCl_2 + 10 mM LaCl_3	23.94 ± 0.77	19.61 ± 0.50

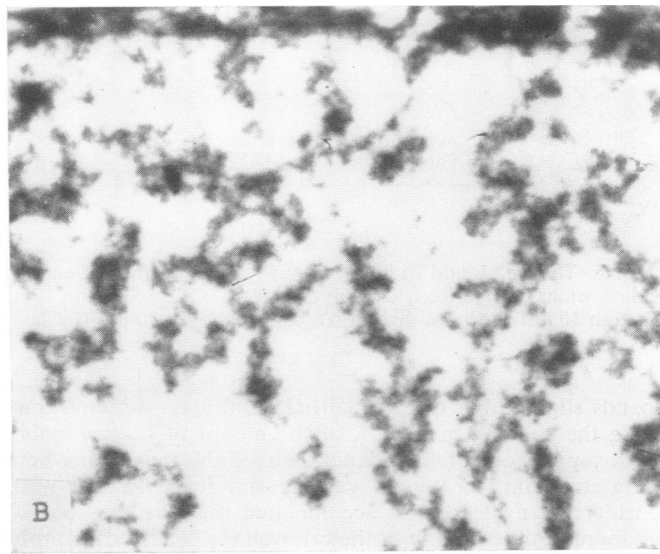
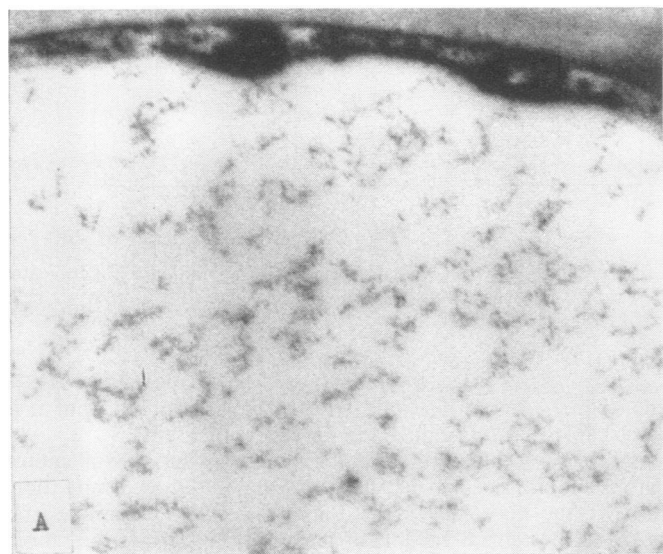


FIG. 9. A part of the central vacuole of *Mimosa* motor cell. A: Before stimulation; B: after stimulation. $\times 18,000$. Both microphotographs were produced from cell materials fixed with OsO_4 (37).

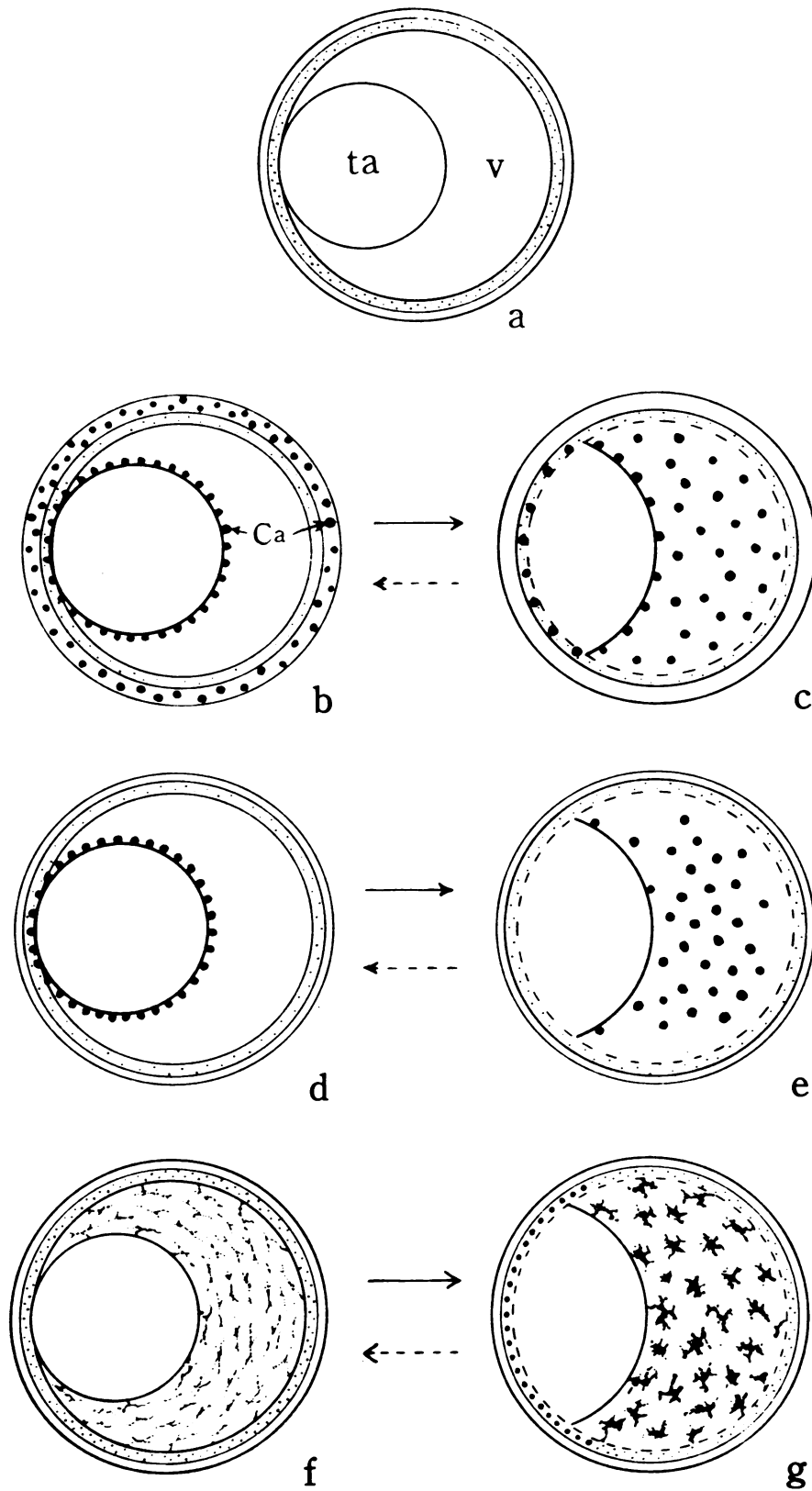


FIG. 10. Scheme of the motor cell of the primary pulvinus of *Mimosa pudica*. a: Fundamental structure of a motor cell; ta: tannin vacuole; v: central vacuole; Ca: indicates calcium; b: before stimulation, adaxial half; c: after stimulation, adaxial half; d: before stimulation, abaxial half; e: after stimulation, abaxial half (above four schemes show the migration of calcium); f: before stimulation; g: after stimulation (above two schemes show the change of central vacuole). →: indicates bending movement; -→: indicates recovery phase of bending movement.

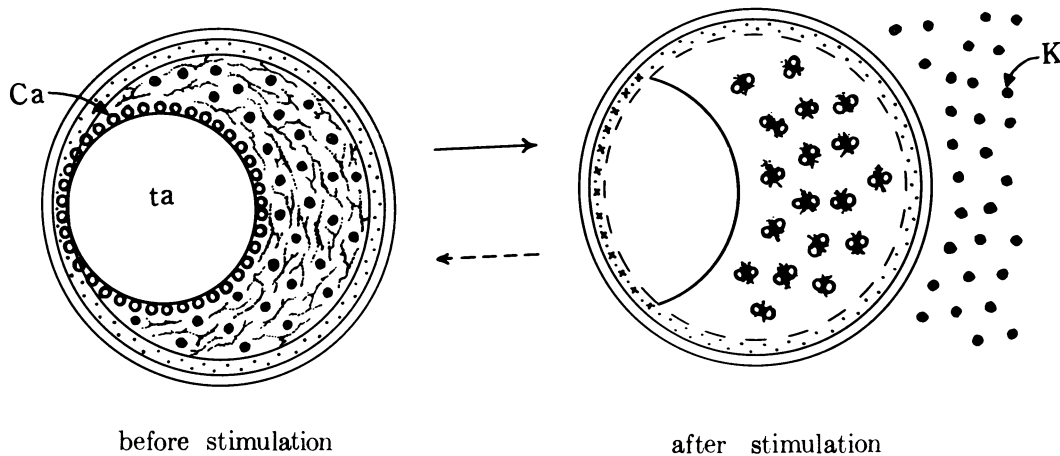


FIG. 11. Integrated scheme of the function of the motor cell of abaxial half of *Mimosa* primary pulvinus. —→: indicates bending movement; - - -→: indicates recovery phase of bending movement.

ergy occurs with the result that calcium ions are released from the tannin vacuole membranes and from the walls of the abaxial motor cells, probably making them more flexible. Of all this released Ca^{2+} , some leaves the cells and motor tissue and can be detected in the bathing solution of a pulvinus-petiole preparation. In the abaxial motor cells, most of the Ca^{2+} which leaves the tannin vacuole can be detected inside the central vacuole. At the same time, the fine fibrillar contents of that vacuole, which are continuous with its tonoplast, can be seen to radically alter their conformation. Since these fibrils have functional characteristics in common with contractile proteins, their conformation may be changed by complexing with the Ca^{2+} . The conformation of certain protein components of the tonoplast may themselves be altered as well, so that the tonoplast and plasmalemma change their permeability characteristics. Evidence for the latter can be found in the rapid efflux of K^+ observed by both cytochemical and tracer techniques (2, 31). This effluxing K^+ probably acts as an osmotic agent to draw water out of the motor cells and probably into the stem, resulting in a rapid abaxial turgor loss and a drooping of the leaf.

The recovery of the leaf and of the tannin vacuole probably represents the reversal of the process described above. The calcium ions are gradually released from the central vacuole and return to the membrane of the tannin vacuole. Potassium returns to the vacuoles which swell up with water, lifting the leaf.

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