Potassium Flux and Leaf Movement in *Samanea saman*

I. *Rhythmic Movement*

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ABSTRACT *Samanea* leaflets usually open in white light and fold together when darkened, but also open and close with a circadian rhythm during prolonged darkness. Leaflet movement results from differential changes in the turgor and shape of motor cells on opposite sides of the pulvinus; extensor cells expand during opening and shrink during closure, while flexor cells shrink during opening and expand during closure but change shape more than size. Potassium in both open and closed pulvini is about 0.4 N. Flame photometric and electron microprobe analyses reveal that rhythmic and light-regulated postassium flux is the basis for pulvinar turgor movements. Rhythmic potassium flux during darkness in motor cells in the extensor region involves alternating predominance of inwardly directed ion pumps and leakage outward through diffusion channels, each lasting ca 12 h. White light affects the system by activating outwardly directed K^+ pumps in motor cells in the flexor region.

INTRODUCTION

Virtually all organisms contain temperature-compensated oscillators that enable them to measure the passage of time in the absence of environmental cues. Because of their accurate timekeeping, these oscillators are referred to as biological clocks, and they manifest themselves in a variety of overt, measurable rhythms. During the past few decades, it has become clear that the proper functioning of these biological clocks is a prerequisite to the orderly completion of the life cycle in both plants and animals (4, 14, 51). Many overt rhythms are circadian and such rhythms have common features, whether they occur in plants or animals, in unicells or in highly complex multicellular creatures (4). Despite numerous investigations, the physical and chemical nature of the oscillator(s) underlying these rhythms remains obscure.

It is probable that observations on biological rhythms started with plants. More than two millennia ago, Androsthenes noted that certain plants (described as nyctinastic) have leaves whose blades are horizontal during the day and folded together at night (4). In 1729, de Mairan (9) reported that rhythmic leaflet movement persists in darkness or dim light of constant intensity, and suggested that leaf movement is regulated endogenously as well as by light dark (LD) cycles. Subsequent studies confirmed this view.

Leaflet movement results from turgor changes in motor cells of the pulvinus, a small organ at the base of the leaflet blade. Kinetic correlations between leaflet movement and K^+ flux in pulvinal motor cells have been demonstrated in two nyctinastic species, *Albizzia julibrissin* (17, 36, 38, 39, 40, 42, 43) and *Mimosa pudica* (2, 49), and in view of the rapidity and magnitude of this K^+ flux, it seems likely it is the physiological basis for the turgor changes that lead to leaflet movement.

Pulvini of *Mimosa pudica* and *Albizzia julibrissin* are too small for convenient biochemical analysis, but *Samanea saman*, with larger secondary pulvini, is a logical choice for such experiments. The doubly compound leaves of *Samanea* (Figs. 1 and 2) are divided into paired pinnae, in turn subdivided

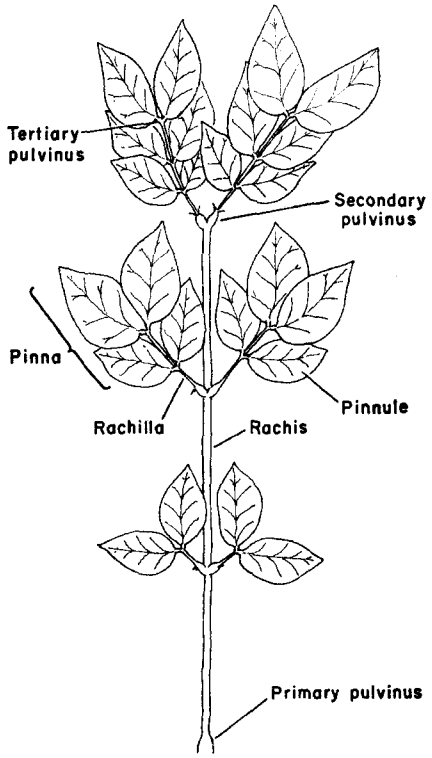


FIGURE 1.

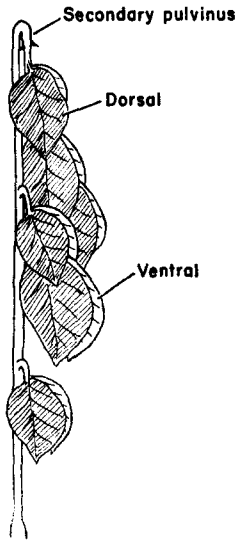


FIGURE 2.

FIGURES 1, 2. *Samanea saman* leaf in the open and closed condition.

into paired pinnules. Each pinna is subtended by a secondary pulvinus that weighs 30–50 mg (500 × as much as the tertiary pulvinus and 25 × as much as the secondary pulvinus of *Albizzia* [44]). In its natural habitat in the tropics, where day length is approximately 12 h throughout the year, *Samanea* leaflets (pinnae and pinnules) begin to open a few hours before sunrise and close at sunset. Leaflet movement displays an endogenous circadian rhythm during prolonged darkness; open and closed periods each last ca. 12 h, with phase determined by the previous light-dark cycle (29). Both nyctinastic and rhythmic movements are also affected by the state of the chromoprotein, phytochrome (47).

In the experiments described below, we monitored changes in pinna movement and the distribution of K^+ in the secondary pulvinus of *Samanea* during a light-dark cycle and during prolonged darkness. The results, which bear on the nature of the rhythmic oscillator, are discussed. Interactions between phytochrome and rhythmic control are reported in another paper (41).

MATERIALS AND METHODS

Plant Material

Samanea saman (Jacq) Merrill plants were grown from seed (courtesy of Agricultural Research Service, Mayaguez, Puerto Rico and D. Friend, University of Hawaii) in controlled chambers with a 16-h photoperiod (ca. 1,500-foot candles cool white fluorescent) at 24°C ± 2°, relative humidity = 60 ± 10%. Pinnae on the third to seventh youngest fully expanded leaves were taken from plants that ranged from 2 mo to 2 yr old. Young plants were analyzed with the electron microprobe, while older plants with larger pulvini were analyzed by flame photometry.

Experimental Conditions

Experiments on rhythmic movement were conducted during a long dark period representing an extension of the usual 8-h dark period. All measurements and experimental manipulations with darkened plants were conducted under a morphogenetically inactive green safelight (42).

Samanea leaves are divided into beautifully matched pairs of pinnae well suited for use as experimental and control partners (Figs. 1, 2); the behavior of the two paired members was compared in all experiments except that represented in Fig. 9. In that experiment, the three pulvini analyzed were chosen to be of similar size, age, and physiological behavior.

Electron Microprobe Analysis

Pulvini to be analyzed with the electron microprobe (Acton Laboratories, Inc., Acton, Mass.) were frozen in modified Tissue-tek (42) at −30° immediately after excision from the plant, and 40-μm sections were cut in a cryostat microtome. Sections were lyophilized, pressed between glass slides, mounted on carbon rods, and coated with a thin layer of carbon. Operating conditions were 12.5 KV, 55-nA beam current, and 12-μm beam diameter (42).

Atomic Absorption Spectrophotometric Analysis

Tissues were excised, washed in deionized water, dried at 85° for 2 h, weighed, homogenized with a Potter-Elvehjem glass-on-glass tissue grinder (Potter Instrument Co., Inc., Melville, N.Y.), centrifuged at 1,200 *g* for 5 min, and the supernatant analyzed. All glassware was acid rinsed. The model 303 flame photometer was manufactured by Perkin-Elmer Corp., Instrument Div., Norwalk, Conn.

Experiments with Excised Pinnae

Excised terminal pinnae were used in experiments testing the effect of temperature and chemical treatments on pinna movement. The rachis was cut to separate the paired pinnae from the plant, and was then split along its longitudinal axis to separate the two pulvini (Fig. 3). The apical ends of the pulvini were put into individual 2-ml vials containing water or test solutions. Five pairs of pulvini were used for each treatment and all experiments were repeated at least three times.

Pinna movement was monitored at 30-min intervals by measuring the angle between the pinna and the rachis on shadowgraphs. These were made by laying the pinnae on Kodabromide photographic paper (Eastman Kodak Co., Rochester, N.Y.), exposing them to bright green safelight for 5 s, and developing in Dektol. Angles were also measured by laying the material directly on an angle chart or protractor.

RESULTS

Anatomy of the Pulvinus

The secondary pulvinus of *Samanea* has a central vascular core surrounded by 20–30 layers of cells. Motor cells, defined as cells that change shape and/or size during leaflet movement, are in the outer layers (13, 29). Ridges in the outer region of the accordion-like pulvinus are deep when motor cells are compressed and shallow when they are turgid. The diameter of a mature pulvinus varies from 0.5 mm (2-mo old plant) to 3 mm (2-yr old plant).

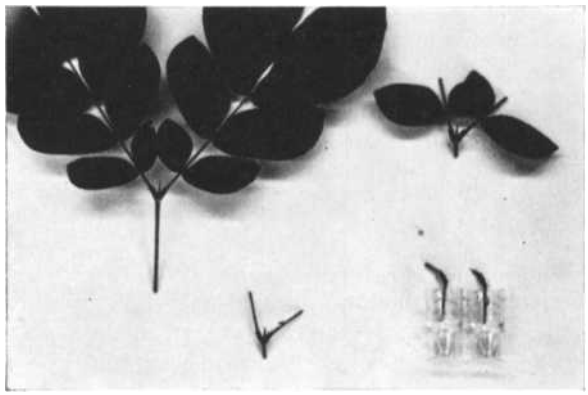


FIGURE 3. Preparative techniques for experiments with excised paired pinnae × ⅓.

The cross-sectional area of dorsal[1] motor cells in dorsi-ventral longitudinal sections is ca twice as large when pinnae are horizontal (open) as when they are vertical (closed). The ventral motor cells become longer and thinner during pinna closure, but their cross-sectional areas remain relatively constant.

In transverse sections, motor cells appear more regularly shaped, and their areas do not change very much during pinna movement. A narrow band of densely chlorophyllous tissue surrounds the vascular core in *Samanea* as in *Albizzia* (44). We hope to publish a detailed study of pulvinar anatomy in the near future.

The gross anatomy of this pulvinus is similar to that of the tertiary pulvini of *Albizzia* (44) and *Mimosa* (50) although cells in the *Samanea* pulvinus are both larger and more numerous. Also, the dorsal and ventral motor cells in secondary and tertiary pulvini have reverse roles, i.e., *dorsal* motor cells of secondary and *ventral* motor cells of tertiary pulvini are turgid when pulvini are open and flaccid when they are closed. These differences are responsible for the basipetal movement of pinnae and the acropetal movement of pinnules during closure. To facilitate comparison of experiments with secondary pulvini of *Samanea* and tertiary pulvini of *Albizzia*, we use the terms *extensor*[2] to designate motor cells that expand during pinna or pinnule opening and contract during closure, and *flexor*[2] to designate motor cells that contract slightly during opening and expand during closure but change shape more than size. The extensor and flexor regions (Fig. 5) are not identical with the dorsal and ventral regions, since *Samanea* pinnae move in two planes, i.e., inward toward each other and abaxially toward the rachis (Figs. 1, 2) during closure, and in the reverse direction during opening.

We used the atomic absorption spectrophotometer to measure the concentration of several cations in pulvinal extracts. One g (dry wt) of tissue contained: 50 mg K^+, 0.5 mg Na^+, 5 mg Ca^{2+}, and 5 mg Mg^{2+}. The K^+ concentration is 0.4 N, and K^+ is the only cation sufficiently concentrated so that flux could have a significant osmotic effect. We also measured K^+ in other tissues; the tertiary pulvinus has 62 mg K^+, the lamina 25 mg K^+, and the rachilla 30 mg K^+/g dry wt. The gross distribution of cations in *Samanea* is very similar to that reported for *Albizzia* (42).

White-Light Promoted Turgor Changes and K^+ Flux

When *Samanea* plants are grown with daily 16 h light - 8 h dark cycles, the pinnae are open during the first 12 h of the light period, then slowly close

[1] The dorsal region is abaxial and the ventral region adaxial. Botanists and zoologists tend to define these terms differently.

[2] These terms usually have a different meaning when used by animal physiologists. We are indebted to George Gardner for suggesting their use here.

and remain closed until an hour or two before the end of the dark period. They begin to open slowly in the dark and more rapidly when the light period begins.

To determine whether these movements are correlated with K^+ flux, paired secondary pulvini were excised at the fifth hours of the dark and light periods, when the angle between pinna and rachis had minimum (0°) and maximum (140°) values, respectively (Figs. 1 and 2). Flame photometric analysis showed that the K^+ content of the entire secondary pulvinus remains constant during pinna movement. However, localized analyses of open and closed pulvinar sections with the electron microprobe revealed that pinna movement is accompanied by a major redistribution of K^+ ions. The K^+ distribution in median dorsi-ventral longitudinal sections is shown in Fig. 4. These figures can be taken as representative of the entire pulvinus, since there was no evidence of a longitudinal gradient in any of the measured regions. It is clear that the K^+ content of the ventral motor region is 4.7 × that of the dorsal motor region when pinnae are closed in the dark, and this ratio drops to 0.5 when pinnae open in the light. The K^+ content of each group of motor cells changes threefold during pinna movement. Turgid cells always have a high K^+ content and flaccid cells have relatively little K^+.

We also used flame photometry to measure K^+ in extracts of motor cell tissue from open and closed pulvini. Careful excision of the outer few layers of cells revealed large changes in the K^+ content of both extensor and flexor cells. Average values for K^+/g dry wt were: extensor (open) 16 mg, (closed) 4 mg; flexor (open) 19 mg, (closed) 26 mg. Values for the flexor cells should not be directly compared to those for extensor cells, since each sample of flexor tissue weighed approximately 2.8 mg, while extensor tissue samples weighed only one-third as much. Thus the latter probably contained a higher percent of damaged cells than did the former. Damaged cells contribute to the weight of the extracts but their K^+ is probably lost during washing.

The magnitude of the flux in the flexor region is much smaller when measured by flame photometry than by the electron microprobe, although both methods give qualitatively similar results. One possible reason for the discrepancy is that microprobe measurements include both extracellular and intracellular ions, while extracellular ions are probably washed out of tissue prepared for flame photometric analysis. Microprobe analysis of thin sections using a high resolution technique (26) might differentiate intracellular from extracellular K^+.

The K^+ measurements of the midcortical region are much higher than those of the motor region (Fig. 4). Although there is a difference in the K^+ content of the midcortex on the dorsal compared to the ventral side of the pulvinus, the dorsi-ventral gradient in the midcortex is much smaller than that in the motor region. Thus cells showing the maximum change in size

or shape during pinna movement also undergo the largest K^+ fluxes. It is likely that the inner cortex functions as a reservoir where K^+ ions are stored when pinnae are stationary (see Discussion).

The K^+ content in pulvinar transverse sections was also analyzed with the electron microprobe (Fig. 5). Measurements revealed a gradual gradient in K^+ in both the motor tissue and the midcortex of closed, darkened pulvini (Fig. 6), with maximum values in the central portion of the flexor region and minimum values in the central portion of the extensor region. The ratio of K^+ in the midflexor region to K^+ in the midextensor region approximates 6.

K^+ ions are distributed in an entirely different pattern in pulvini that open in white light (Fig. 7). Instead of a gradual gradient, there are two regions of relatively uniform K^+ values; the K^+ content is consistently low throughout the flexor region, and high throughout the extensor region [K^+ (flexor)/K^+ (extensor) = 0.3]. The small peak in the flexor region contrasts with the very large peak in this region of closed pulvini. The transition between low

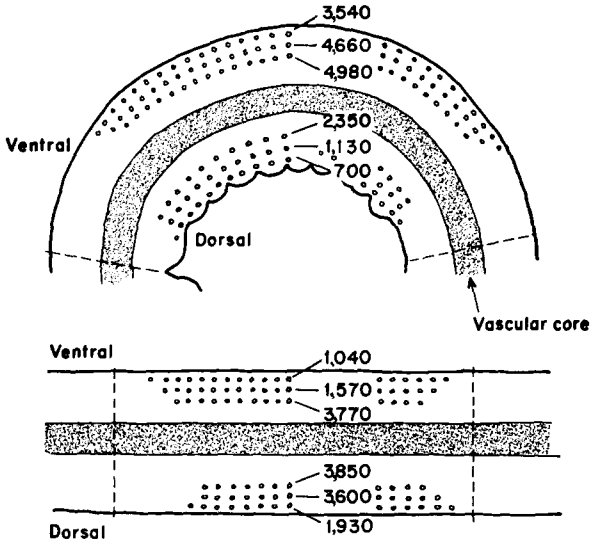


FIGURE 4.

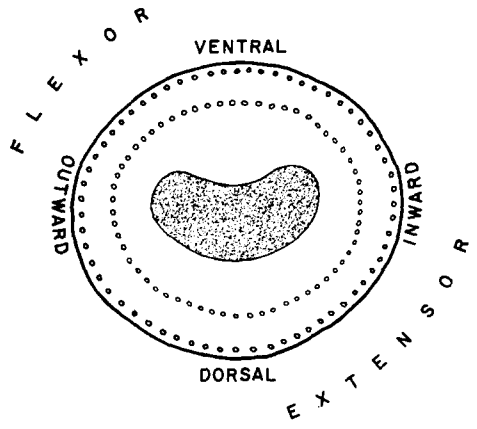
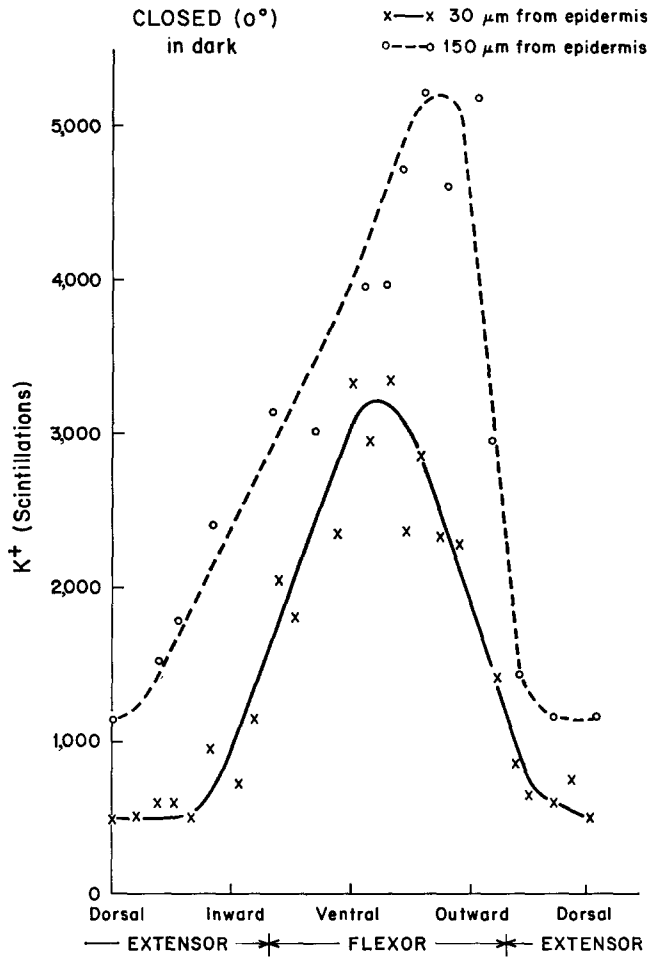


FIGURE 5.

FIGURE 4. K^+ distribution in longitudinal, dorsi-ventral sections of a closed pulvinus at hour 5 of the dark period (top) and an open pulvinus at hour 5 of the white light period (bottom). K^+ was analyzed in the circled regions with an Acton electron microprobe. The measured regions were in the motor tissue (outer cortex, 30 μm from the epidermis), and midcortex (100 and 225 μm from the epidermis). Measured values are scintillations during 15 s. Each datum is an average of 24 measurements. The average standard deviation was ca 25%. See (42) for additional details.

FIGURE 5. Transverse section of an open or closed pulvinus. K^+ was analyzed in the circled regions, in the motor tissue (30 μm from the epidermis) and the midcortex (150 μm from the epidermis).



FIGURES 6–9. Electron microprobe analysis of K^+ (scintillations/15 s) in the motor tissue (30 μm from the epidermis) and in the midcortex (150 μm from the epidermis). Measurements were made in areas 100 μm apart. The abscissa indicates distance from middorsal (see Fig. 5).

FIGURE 6. Closed (0°) pulvinus at hour 5 of the dark period.

and high K^+ regions is very sharp and includes only a few cells. These strikingly different patterns of K^+ distribution in open and closed pulvini (Fig. 9), imply the operation of different mechanisms, e.g. diffusion during the closed phase, and active transport during the open phase. Experiments testing the effect of temperature alteration support this view, as will be shown below. Both open and closed pulvini have more K^+ in the midcortex than outer cortex (motor region), but the K^+ distribution patterns within both outer and midcortex are similar (Figs. 6, 7).

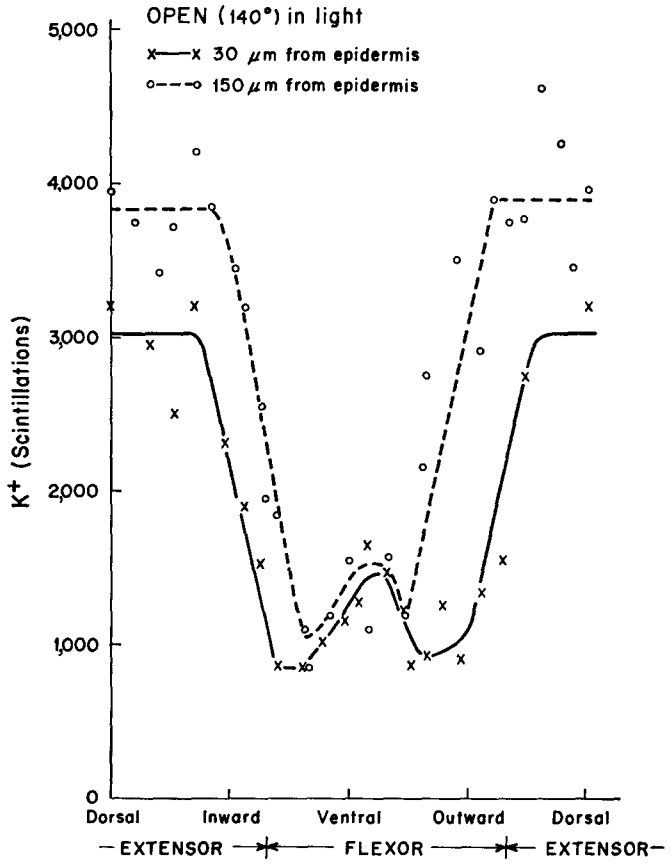


FIGURE 7. Open (140°) pulvinus at hour 5 of the white light period.

Endogenously Rhythmic Turgor Changes and K^+ Flux

The usual 8-h dark period was extended several hours for experiments on rhythmic pinna movement and K^+ flux. Pinnae begin to open after 7–8 h of darkness and open approximately half as wide as they do in white light. They remain open for approximately 12 h, then close and remain closed for another 12 h. Rhythmic opening and closure continues for several days in constant darkness (29).

To study the K^+ fluxes that accompany rhythmic opening, pulvini that had opened to 75° at hour 15 of the dark period were excised and prepared for microprobe analysis. The K^+ distribution in cross sections was analyzed (Fig. 8) and compared to that of cross sections of closed pulvini excised at hour 5 of the dark period (Fig. 9). The most marked change in K^+ occurs in the extensor region; K^+ increased fourfold during rhythmic opening. The K^+ content of the flexor region, by contrast, remained relatively constant,

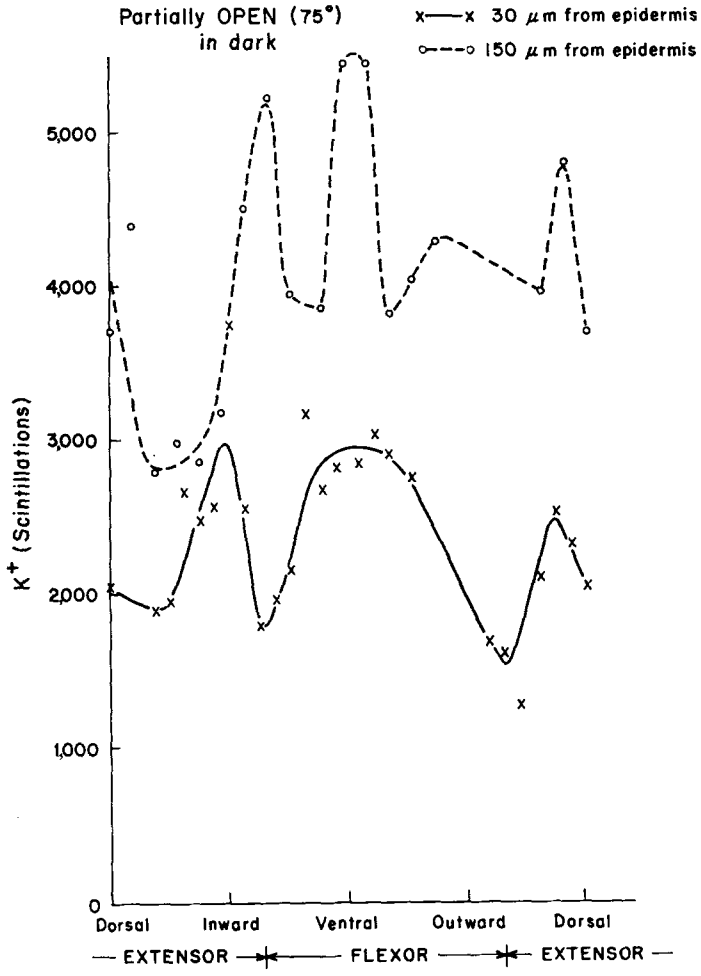


FIGURE 8. Rhythmically open (75°) pulvinus at hour 15 of the dark period.

although it decreased to one-third its former value during full opening (140°) in white light (Figs. 8, 9). Even though flexor cells do not show large changes in size during opening, they change shape, and K^+ efflux from these cells is associated with these changes.

Effect of Temperature Alteration on Rhythmic Movement of Excised Pinnae

In the experiments described above, pulvini opened and closed on the intact plant and were prepared for microprobe analysis immediately after excision. Excised pinnae with cut ends in H_2O, as shown in Fig. 3, also open and close in response to white light and rhythmic control, and removal of laminar tissue does not alter such movement (Fig. 10). This indicates that the photoreceptor for opening, the rhythmic oscillator, a reservoir of K^+ ions, and

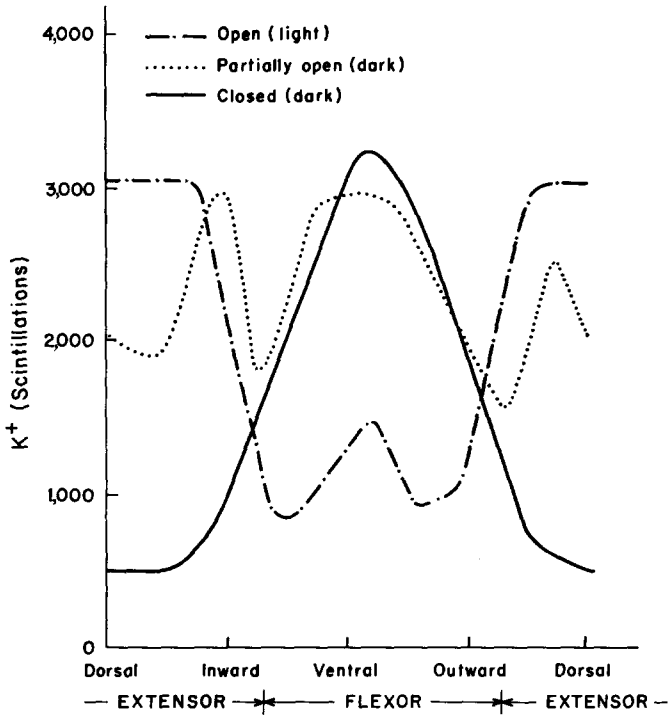


FIGURE 9. Combined data from Figs. 6–8. Only motor tissue data are included.

energy substrate for ATP synthesis, are all localized in this pulvinus, as in the tertiary pulvinus of *Albizzia* (39). However, excised pinnae open less than intact ones, and delaminated pinnae do not respond to white light or rhythmic control for more than one or two movement phases; therefore, we excised pinnae no more than an hour or two before experimental incubation.

Pinnae were incubated at different temperatures during rhythmic opening and closure to determine Q_{10} values for each type of movement. A Q_{10} of 2.0 or higher was taken to imply K^+ flux by an active transport process, while a low Q_{10} is consistent with the operation of a physical process such as diffusion. Closed pinnae excised at the beginning of the dark period remain closed during 2-h incubation at temperatures between 5 and 37°. When excised at hour 7 of the dark period (just before rhythmic opening) they opened more rapidly and completely at 37° than at 24°, and pinnae that had opened at 37° closed when the temperature was lowered (Fig. 11 *a*). Open pinnae excised at hour 18.5 of the dark period (just before rhythmic closure) closed more slowly at 37° than at 24° (Fig. 11 *b*). Q_{10} values for rhythmic opening and closure are 2.5 and 0.8, respectively. These experiments suggest that ion pumps are responsible for increase in the K^+ content of the extensor cells during rhythmic opening, and that K^+ diffusion out of these cells predominates over active uptake during rhythmic closure. In *Albizzia* pulvini, both

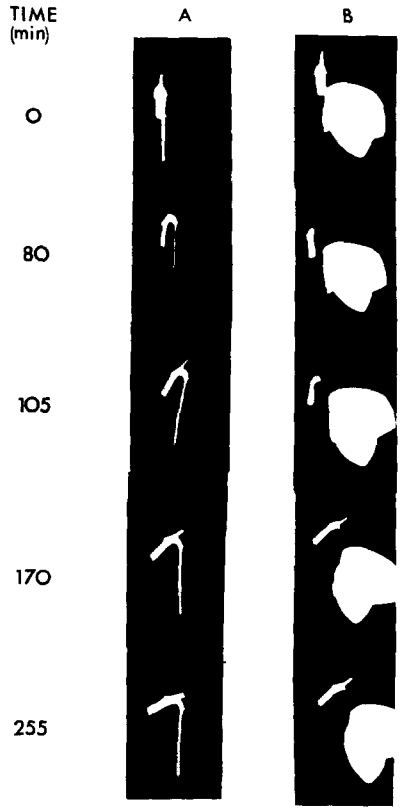


FIGURE 10. Rhythmic opening of darker paired pinnae with and without laminar tissue.

low temperature and sodium azide reduced the K^+ content of extensor cells, thereby inhibiting rhythmic opening and promoting rhythmic closure (36).

DISCUSSION

Evidence that K^+ Flux Is the Basis for Leaflet Movement

The $[K^+]$ of pulvinal tissue from nyctinastic plants ranges from 0.4 to 0.75 N (this investigation and 2, 42, 49). K^+ fluxes have been monitored by experiments with ^{42}K (2), histochemical methods using K^+ cobaltinitrite (49), flame photometry (this investigation), and electron microprobe analysis (Figs. 4, 6–9, and 36, 38–43). These several studies have revealed consistent correspondence between the K^+ content and the turgor of pulvinal motor cells in *Samanea*, *Albizzia*, and *Mimosa*, whether movement is stimulated by endogenous rhythm (38, 39), light-dark transition (38, 42, 49), change in the far-red phytochrome (P_{fr}) level (38, 39, 42), temperature alteration (36), mechanical stimulation (2), or chemical treatment (36, 43). The K^+ content and

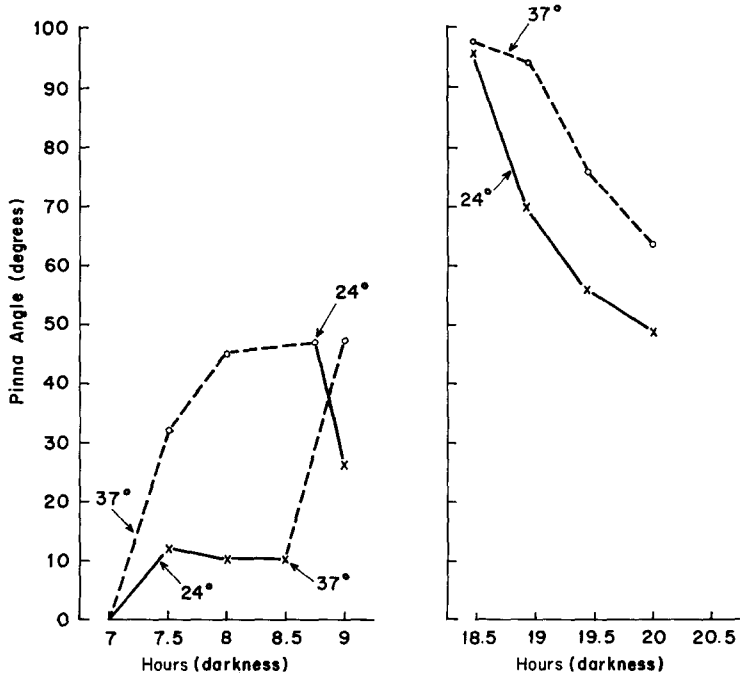


FIGURE 11. The effect of temperature alteration on pinna opening and closure. Paired pinnae were excised at hours 7 and 18.5 of the dark period, and were incubated at 24°, X–X–X, or 37°, —O—O—O—.

K^+ fluxes of the guard cells whose turgor changes control stomatal movement are similar to those of pulvinal motor cells (16, 23, 45). Hsiao's recent demonstration (22) that K^+ flux is an essential prerequisite for turgor changes in guard cells strongly supports the view that K^+ fluxes are the basis for, rather than a consequence of, leaflet movement in nyctinastic plants.

Chloride and malate ions move with K^+ ions through guard cell membranes (1, 28, 34). Anions that accompany K^+ ions through motor cell membranes have not yet been identified, to the best of our knowledge. We intend to study this problem and also the possibility of H^+/K^+ exchange.

Evidence that Active Transport Promotes Opening and That Leakage Causes Closure

Incubation at low temperature or on azide inhibits the K^+ fluxes that lead to rhythmic opening and promotes the fluxes that lead to closure in *Albizzia* (36, 37, 40). We proposed (40) that K^+ ions are actively transported into extensor motor cells during the open period and diffuse out of these cells during the closed period, i.e., rhythmic leaflet movement is due to rhythmic change in active transport, membrane permeability, or both. *Samanea* experiments indicating that Q_{10} = 2.5 during opening and 0.8 during closure

imply that changes in membrane properties are similarly responsible for rhythmic K^+ flux and pinna movement in *Samanea*. The K^+ distribution patterns in transverse sections of open and closed pulvini (Figs. 6–9), provide strong evidence supporting this view. The gradual circumferential gradient in K^+ values in closed pulvini suggests a symmetrical movement of K^+ ions from a peak area in the middle of the flexor region by a physical process such as diffusion, although the mechanism for generating high K^+ in the midflexor region remains to be explained. By contrast, the sharp transition between regions of uniformly high and uniformly low K^+ values in open pulvini suggests that ion pumps in flexor cell membranes transport K^+ outward while those in extensor cell membranes transport K^+ to the cellular interior. Alternatively, another ion such as H^+ might be actively transported through motor cell membranes, thereby promoting K^+ flux by exchange (31, 46).

The pattern of K^+ distribution in pulvini that have opened rhythmically during prolonged darkness (Figs. 8, 9) incorporates features characteristic of the patterns in both closed pulvini and in those that have opened in white light. Absolute values and the gradient of K^+ in the flexor region are similar in rhythmically opened and closed pulvini, while the fourfold increase in K^+ values in the extensor region during rhythmic opening is similar to that occurring during opening in white light. However, the K^+ distribution pattern in the extensor region when pulvini open rhythmically suggests a dynamic equilibrium between active K^+ transport into and diffusion out of these cells. Experiments indicating that open darkened pinnae close rapidly when the temperature is lowered support this view, since diffusion is favored at low temperature.

In *Albizzia* the K^+ content of both extensor and flexor cells varies rhythmically, but the two rhythms are several hours out of phase (36, 38). Although microprobe analyses at hours 5 and 15 of the dark period did not reveal any change in the K^+ content of the flexor cells of *Samanea*, K^+ flux in these cells might occur at a later time. This possibility is supported by Palmer and Asprey's experiments indicating slight endogenously rhythmic movement in darkened pinnae with extensor cells removed but flexor cells intact (30).

The K^+ Reservoir

The K^+ content of the extensor cells increases during rhythmic opening without concomitant decrease in the K^+ content of the flexor cells (Fig. 9) indicating that K^+ ions do not merely shuttle between contracting and expanding motor cells. *Albizzia* experiments gave similar results (39). Where are K^+ ions stored before opening? There must be a K^+ reservoir in the pulvinus or small attached section of rachilla, since excised, delaminated

pinnae open rhythmically (Fig. 10). It seems likely the reservoir is in the inner cortex, since the flexor-extensor gradient disappears in this high K^+ region which surrounds the vascular core, an obvious passageway for ion interchange between pulvinal and other cells.

Reevaluation of Experiments by Palmer and Asprey

Data on the relationship between K^+ flux and leaflet movement are useful in interpreting the ingenious experiments of Palmer and Asprey (30) who studied the effect of light and darkness on the movement of pinnae with pulvini cut to remove the lower or upper half.

Pinnae with extensor cells intact show pronounced rhythmic movement during prolonged darkness, and our data (Figs. 8, 9) indicate that rhythmic K^+ flux could be responsible. Pinnae dissected in this way also open in the light, but the final angle is no greater than that attained during a long dark period. We similarly found that K^+ flux into extensor cells takes place upon transition from darkness to light or during a long dark period (Fig. 7–9).

Pinnae with flexor cells intact but extensor cells removed, showed, by contrast, only minor rhythmic movement; this is consistent with our experiment showing little rhythmic K^+ flux in flexor cells (Figs. 8, 9). However, these pinnae move vigorously upon transition between white light and darkness, opening when illuminated, and closing when darkened. Since white light decreases the K^+ content of the flexor cells to one-third its former value (Figs. 4, 7, 9 and flame photometric data), K^+ flux appears to be the basis for the turgor changes. Although Palmer and Asprey's experiments and ours are different in design, both lead to the conclusion that turgor changes in extensor cells are most sensitive to rhythmic control while similar changes in flexor cells respond most readily to transitions between white light and darkness.

CONCLUSION

Samanea pinnae kept in darkness for several days open and close with an endogenous circadian rhythm (29). Although our analyses of K^+ distribution were confined to the first cycle of the rhythm, it is reasonable to expect that oscillations in K^+ distribution persist for several cycles and are correlated with rhythmic leaflet movement, as shown for *Albizzia* (38). We thus propose that rhythmic change in membrane permeability and/or active transport in pulvinal motor cells leads to rhythmic pinna movement (40).

Although our *Albizzia* and *Samanea* experiments clearly indicate circadian oscillations in membrane properties, they do not indicate whether these oscillations are part of the clock itself. Such a role for membranes is supported by reports of several investigators. There are diurnal changes in the distance between the two layers of the endoplasmic reticulum and of the nuclear membrane in *Helodea* (5). Ethyl alcohol (12), lithium (10), and D_2O (11) increase

the cycle length and valinomycin (8) induces phase shifts in endogenous rhythms; all these substances probably alter membrane properties. Phytochrome, photoreceptor for changes in the phase (21) and cycle length (6, 7) of endogenous rhythms in plants, binds to membranes (19, 27, 32, 35) and regulates ion flux (24, 25, 33, 42, 48). Blue light also induces phase shifts (21) and changes in the length of rhythmic cycles (18); recent studies indicate that a blue-absorbing pigment, probably a flavin, also binds to a particulate fraction of tissue extracts (Briggs and Marmé, personal communication) and regulates membrane properties in vivo (20). CHI, an inhibitor of protein synthesis, increases the period length of a rhythm in phototaxis in *Euglena* (15). CHI prevents rhythmic K^+ flux in *Albizzia*, suggesting that rhythmic turnover of membrane proteins is part of the clock mechanism (3, 36). We hope to test this theory directly by an examination of isolated motor cell membranes of *Samanea*.

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