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Physiological Studies on Pea Tendrils. V. Membrane Changes and Water Movement Associated with Contact Coiling

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Abstract. **The coiling of excised pea tendrils in response to mechanical stimulation is accompanied by an increased efflux from their cut bases of electrolytes and label from previously absorbed ^{14}C-acetate and ^{14}C-sucrose. The major excreted cation is H^+; H^+ loss is potentiated by pretreatment with benzoic acid, which also leaves the tendrils during coiling.**

Label from previously absorbed tritiated water is excreted during coiling, mainly from the ventral side of the tendril, which contracts in the initial phase of coiling. Such label does not pass from the ventral to the dorsal side. Similarities between this and other rapidly moving systems in plants are surveyed and a hypothesis to explain turgor movements is advanced.

The rapid movements of plant parts have often been attributed to turgor changes resulting from alteration in cell membrane permeability (17). This view has been based largely on changes in size of motor cells before and after movement (10, 17) and effects on motor cells of reagents such as diethyl ether and ethyl alcohol, known to affect membranes (5, 9, 15, 16).

Recently we were able to implicate membrane changes in the phytochrome-controlled rapid nyctinastic movements of excised pinnae of *Albizzia julibrissin* (8). We reasoned that if contact coiling of tendrils involves the rapid efflux of water from cells of the contracting ventral side (5), the efflux of solutes and of water from such coiling tendrils might be detectable by sensitive techniques. This paper reports evidence for such movement.

Materials and Methods

Unbranched tendrils from the fifth node of 10 to 13 day old Alaska pea plants, grown as previously described (5), were used in 2 ways. In one procedure, previously described (5), 10 excised tendrils were shaken in 10 ml of solution in a petri dish containing 0.1 or 0.01 % Tween-20 as a wetting agent. Where test addenda were included, 30 mM phosphate buffer (pH 6.4) was added, but all conductance measurements were made on effluents subsequently collected in distilled water.

In a second procedure, tendrils were excised under water containing 0.01 % Tween-20, usually with about 1 cm of subjacent petiole attached, and placed upright in 2 ml of solution in a plastic vial. The occasionally flaccid tendrils were allowed to regain turgor by an overnight incubation in distilled water or in 100 μM benzoic acid. Three tendrils per vial were used for the electrolyte efflux experiments and 1 per vial for the experiments with tritiated water.

The efflux of label from previously absorbed ^{14}C-labeled sucrose and sodium acetate was measured as follows. Tendrils were floated in solutions of the labeled material for 2 hours, rinsed, and then either shaken or allowed to remain at rest for 30 minutes in fresh buffer. The radioactivity of the buffer was then determined by mixing an aliquot with scintillation fluid and counting in an Ansitron liquid scintillation spectrometer. Since both acetate and sucrose were partially metabolized, the results were expressed as cpm per mg final fresh weight of the 10 tendrils rather than in molar terms. Electrolyte efflux was determined on tendrils incubated in distilled water containing 0.01 % Tween-20 for 2 hours. Conductance measurements of the bathing solution were made at the start and end of the incubation period with a previously described apparatus (3).

The efflux of electrolytes from the cut base of the tendril was determined as follows. Leaf No. 5 was severed from the plant at the base of the petiole and the parts below the tendril immersed in water containing 0.01 % Tween-20. The leaflets and all but 1 cm of petiole base were then excised under the water leaving the tendril attached to the petiolar stump, which remained immersed. All subsequent handling was gentle, and limited to the petiolar stump. The system was allowed to recover overnight, usually in the presence of 100 μM benzoic acid, after which the solution was removed, and after some rinsing replaced by distilled water whose conductance was measured at time zero and again after 30 minutes. Some of the tendrils were then stimulated by stroking their ventral surfaces with

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a glass rod, and others remained unstimulated as controls. The conductance of the basal bathing solution was then measured at various intervals. Measurements of specific ions in the effluent solution were made with a Perkin-Elmer Model 300 Atomic Absorption Spectrometer. Absorbance measurements and pH determinations were made on effluents of 5 tendrils using a Perkin-Elmer Model 350 recording spectrophotometer and a Leeds and Northrup pH meter fitted with microelectrodes.

The efflux of tritium label and the label in the dorsal and ventral sides of coiled or uncoiled single tendrils were measured as follows. The cut petiolar stumps of excised tendrils were immersed in tritiated water for 2 hours, then carefully rinsed and transferred to ordinary distilled water in a plastic vial. A zero time aliquot of this distilled water was removed for radioactivity determination and the tendril either stimulated with a glass rod or allowed to remain as an unstimulated control. After 15 minutes, aliquots of the water were again removed for counting. The tendril was now rinsed 3 × with distilled water, the petiolar stump removed, the tendril wetted with 0.01 % Tween-20 and placed on a glass slide. A small quantity of warm 10 % gelatin was applied along the ventral side of the tendril with a pipet. Now the tendril was fixed to the slide and frozen by transfer of the slide to the surface of a dry ice block covered with aluminum foil. The frozen tendril, still resting on the cold block, was then carefully sliced down the middle with a razor blade under a dissecting microscope. The radioactivity in each half of the tendril was measured by scintillation counting. Fresh and dry weights were measured on other tendril halves derived by the same procedure.

The effect of pH transitions on coiling was studied by holding unstimulated tendrils in petri dishes first for 30 minutes at pH 5.0, then for 30 minutes at pH 8.5 and finally shaking all treatments for 2 hours at pH 6.4. Controls in petri dishes were held at pH 6.4 in the light or in the dark.

Results

If alteration of membrane permeability is involved in coiling, changes in solute efflux should be detectable during coiling. Table I shows that such changes do occur. Coiling tendrils lose electrolytes and label from previously absorbed ^{14}C-sodium acetate and sucrose more rapidly than tendrils at rest. That this increase in electrolyte efflux is due to coiling rather than to shaking is

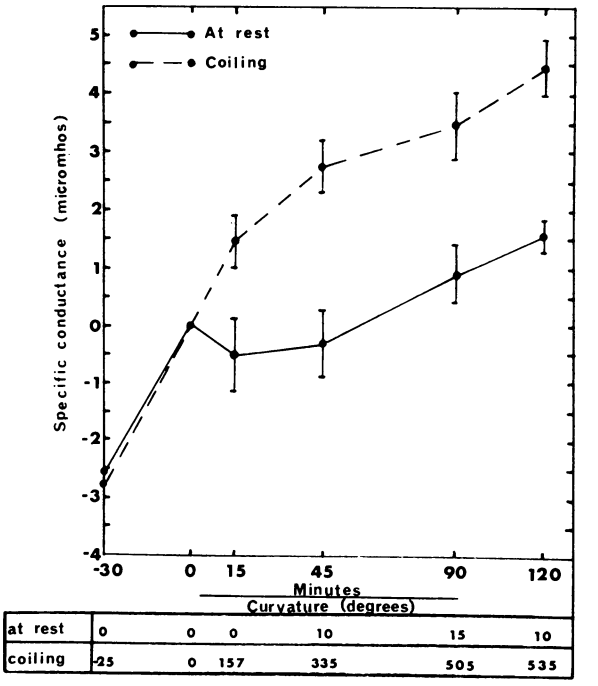

FIG. 1. Kinetics of coiling and of electrolyte efflux through the cut subjacent petiole fragment of excised tendrils. These excised tendrils were not pretreated with benzoic acid. The vertical bars represent the standard errors.

Table I. *Comparative Solute Loss from Unstimulated and Coiled Tendrils into the Bathing Solution*

Degrees of curvature Unstimulated	Coiled	Measurement	Unstimulated	Coiled
39	113	Label from fed ^{14}C-sodium acetate (cpm per g fr wt) N = 2[1], 30 min incubation	917 ± 141[2]	1187 ± 96
25	113	Label from fed ^{14}C-sucrose (cpm per g fr wt) N = 5, 30 min incubation	282 ± 95	445 ± 84
75	366	Electrolytes (Specific conductance, as micromhos) N = 6, 2 hr incubation	12.0 ± 0.0	15.4 ± 1.3

[1] "N" is the number of times the experiment was replicated.
[2] Standard error.

Table II. *The Effect of Prior Stimulation on the Release of Electrolytes from the Cut End of Excised, Upright Stationary Tendrils*

Incubation time was 2 hours.

Treatment		Curvature	Conductance
Petiole	Stimulated		
		deg	*micromhos*
Absent	no	20 ± 10[1]	1.8 ± 0.6
Absent	yes	330 ± 21	4.2 ± 0.3
Present	no	0 ± 0	0.9 ± 0.6
Present	yes	740 ± 114	4.7 ± 1.1

[1] Standard error.

indicated in the data of table II, where coiling was stimulated prior to immersion of the tendril base in the measured solution. The increased electrolyte efflux was apparent as early as 15 minutes after stimulation. The presence of a subjacent centimeter of petiole significantly increased the rate of coiling but was without significant effect on the rate of electrolyte efflux. This may indicate that the petiole normally acts as a sink for the materials lost from the tendril during coiling.

The increased electrolyte efflux from coiling tendrils continues for about 45 minutes, (fig 1), after which efflux is the same as in the unstimulated controls. The early increase is shown more clearly when the tendrils are pretreated overnight with 100 μM benzoic acid (fig 2). After such treatment, the increased efflux is discernible within 1 minute after stimulation and in some instances

Table III. *Effect of 24 Hour Pretreatment with Benzoic Acid on Electrolyte Efflux from Tendril Bases 15 Minutes after Stimulation*

Treatment		Curvature	Conductance
Stimulated	Benzoic acid		
	μM	*deg*	*micromhos*
No	0	30	2.4
Yes	0	120	3.0
No	100	0	0.9
Yes	100	90	12.9

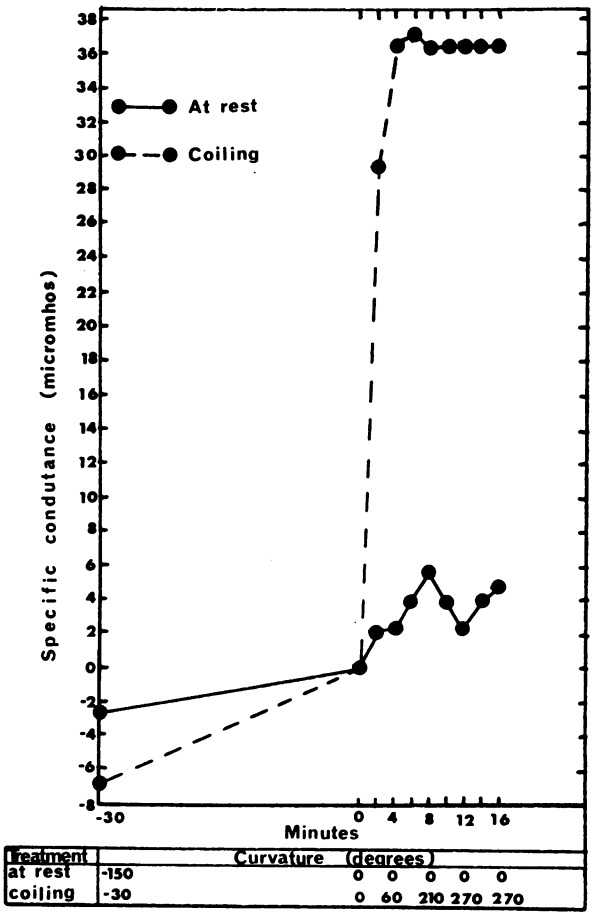

FIG. 2. Typical kinetics of coiling and electrolyte efflux from 3 excised tendrils. The tendrils were pretreated overnight with 100 μM benzoic acid.

Table IV. *The Composition of the Effluent Material from Resting and Coiled Tendrils after 15 Minutes*

Tendrils were pretreated overnight with 100 μM benzoic acid.

No. tendrils per vial	Measurement	Resting tendrils (unstimulated)	Coiling tendrils (stimulated)
3	Curvature (°)	5 ± 7[1]	258 ± 13
3	Fr wt per tendril (mg)	9.1 ± 0.7	8.6 ± 0.7
3	Electrolyte efflux (μMhos)	0.6 ± 0.3	5.7 ± 1.8
3	K^+ efflux (nanomoles)	33 ± 4	24 ± 4
3	Na^+ efflux (nanomoles)	94 ± 11	78 ± 35
3	Mg^{2+} efflux (nanomoles)	60 ± 4	63 ± 3
5	Curvature (°)	50 ± 14	292 ± 14
5	H^+ efflux (nanomoles)	−2.7 ± 3.5	9.3 ± 3.8
5	Benzoic acid efflux (nanomoles)[2]	21 ± 4	44 ± 7

[1] Standard error.

[2] Based on absorption at 226 nm.

almost half of the ultimate increase is detectable after 30 seconds. This was especially interesting since coiling itself is not normally detectable until about 2 minutes after stimulation (5). Thus, benzoic acid appears to influence directly the rapid electrolyte efflux and only indirectly the slower movement of the organ. Approximately 16 hours of pretreatment with benzoic acid were necessary to obtain this effect; sample data from a 24-hour pretreatment are given in table III. The increased electrolyte efflux during coiling could not be accounted for by such cations as K^+, Na^+ or Mg^{2+} determined by flame photometry (table IV). There was, however, a greater excretion of H^+ ions and benzoate in the effluent from coiled tendrils than from unstimulated tendrils (table IV, bottom). It appears that we may consider H^+ as the main cation lost during coiling, and benzoate as a convenient anion accompanying it during outward passage. The inequity of H^+ and benzoate could be due either to excretion of other non acidic substances absorbing at 226 nm or to partial substitution of other cations for H^+.

Because of its potentiating effect on solute efflux, the benzoic acid pretreatment was used in the experiments with tritiated water. In almost all trials, there was a greater efflux of tritium label from coiling tendrils than from those at rest (table V), much of this coming from the ventral side of the coiling tendril (table VI). There was no apparent change in the label on the dorsal side, indicating that water leaving the ventral side was not translocated to the dorsal side.

Tendrils subjected to a pH transition from 5.0 to 8.5 coiled an average of 27 % more than dark controls (table VII). Although this increase was considerably lower than that caused by light, it occurred in every experiment. Such pH shifts have been held capable of generating ATP at membranes (11), and evidence for such action has been obtained with chloroplast fragments (4). In experiments with organelles and fragments thereof, the treatment times were much shorter than those used here; since whole organs were employed in our experiments, the penetration of addenda must certainly have been slower. If ATP is being generated as a result of pH shifts, its concentration appears to be less than 100 μM, based on the response of excised tendrils to exogenous ATP (6).

Table V. *The Effect of Coiling on the Release of Tritium Label from Previously Absorbed Tritiated Water*
Excised tendrils were pretreated overnight with 100 μM benzoic acid.

Treatment	Curvature	Radioactivity per tendril in effluent
	deg	*cpm*
Unstimulated	45	3510
Coiled	342	4370
Least significant difference at 5 % level (13) for N = 14	...	376

Table VI. *The Effect of Coiling on Label Efflux in 15 Minutes from Dorsal and Ventral Halves of Tendrils Previously Fed Tritiated Water for 2 Hours*

Expt	Unstimulated tendrils		Coiled tendrils	
	Dorsal side	Ventral side	Dorsal side	Ventral side
	cpm	*cpm*	*cpm*	*cpm*
1	3839	2775	3433	2626
2	3398	2679	3085	2503
3	3384	3384	3985	2853
4	829	1070	1115	1180
5	1001	1365	846	800
6	601	933	449	333
Avg	2180	2035	2160	1715
% Change in label associated with coiling			—1	—16
% Net change in fr wt associated with coiling			0	—12

Table VII. *The Effect of pH Transition on the Coiling of Excised Tendrils*
Each figure represents the mean of 10 tendrils.

Expt	Curvature		
	Dark control	Dark pH transition	Light control
	deg	*deg*	*deg*
1	171	270	270
2	207	243	507
3	261	297	567
4	90	108	135
5	108	140	325
6	240	291	351
Avg	167	212	361

Discussion

When excised pea tendrils are made to coil, they release more electrolytes and previously absorbed organic solutes than do tendrils at rest. Most if not all of the increased electrolyte efflux is from the cut base of the tendril or subjacent petiole fragment. This parallels a similar phenomenon recently demonstrated in *Albizzia julibrissin* (8), where we found a correlation between the rate of electrolyte efflux from the rachis and the nyctinastic closure of the pinnule pairs on the excised pinna. In studying electrolyte efflux during movement of the primary pulvinus of *Mimosa pudica*, Blackman and Paine found only a slight relation between the two (1). However, an examination of their data leads us to the view that there is an increase in electrolyte efflux immediately after each movement of the pulvinus (see their table I and fig 2).

There are a number of other similarities between these different movements. In both *Albizzia* and *Mimosa*, blue light causes opening of the pinnule pairs after a dark period (2, 8) and contact coiling of excised pea tendrils following a dark period is also increased by blue light (6). Movement in both pea tendrils (6) and *Mimosa* (9) is accompanied by a reduction in the endogenous ATP level, and a contractile ATPase has been found in both systems (7, and T. Sibaoka, personal communication). Treatment with IAA affects both tendril movement (5) and *Mimosa* pinnule movement (18) in similar ways.

The effect of benzoic acid may be due to its facilitation of the efflux of H^+ ions by a cation pump during coiling. Higher concentrations (10^{-3} and 10^{-2} M) have been shown to increase membrane permeability, promote sugar loss and decrease respiration in etiolated barley leaves (12). At the concentrations used here (100 μM) no such effects appear to be produced. Benzoate has little effect on coiling or on the rate of electrolyte efflux from resting tendrils. Pretreatment with benzoic acid must be at least 16 hours long; hence we assume that absorption precedes the response and that benzoate is retained in the tendril and released only during coiling. Since increased electrolyte efflux after benzoic acid pretreatment can be measured even before coiling is visible, it may be an early link in the chain of events between reception of the mechanical stimulus and ultimate response. Benzoate is not unique in producing this effect; in general more rapidly metabolized acids, such as malate, were less effective.

We can now hypothesize a series of events starting with mechanical stimulation and leading to coiling. A) A mechanical stimulus is sensed by an unknown receptor and B) transduced into activation of ATPase at a membrane (6, 7). This results C) either in release of H^+ ions from the cell, accompanied by any available anions, or the release of anions, accompanied by a cation, preferentially H^+. This release of osmotically active solute results D) in a loss of water, especially from the ventral cells, probably into the ventral vascular bundle and out of the tendril. This leads E) to a loss of turgor in the ventral cells and F) a contraction of the ventral surface noted in the earliest stages of contact coiling (5). G) The subsequent coiling, due to differential elongation of dorsal and ventral cells of the tendril, results from uptake of water via the petiole and vascular bundles by mechanisms normally involved in growth.

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