PHYSIOLOGY OF RAPID MOVEMENTS IN HIGHER PLANTS

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Among the various behaviors in higher plants, a peculiar one, that a part of the leaf or flower in certain plants shows very rapid movement upon stimulus, has invited the attention of a number of investigators since the beginning of botanical research. However, in spite of this long history of study, numerous fundamental aspects of the phenomena remain to be clarified. Since the definition of the rapid movement is still unclear, discussion in this review will be restricted to three types of the movements: (*a*) rapid response in the pulvini of the mimosas; (*b*) shutting movement in the traps of two carnivores, *Dionaea* and *Aldrovanda* (an aquatic plant); and (*c*) visible movements in the stamen and pistil of some plants upon stimulus. This review is not intended to be a comprehensive résumé of all published works on the movements. I intend to restrict my discussion to a consideration of various results in an attempt to discover a general mechanism underlying these movements, and will stress the relation between electrical response and the movement. Several reviews and monographs have been published that approach this problem from various points of view (18, 19, 24, 42, 101, 103), including the author's own works (11, 71). In this review I will return to some of the important older literature which provides a basis for understanding the mechanism.

Perception of Stimulus and Response Behavior

Dionaea and Aldrovanda.—Features of perception of stimulus and the process of response in these two carnivorous plants (3) are essentially similar to each other, though their form, size, and habitat are quite different. Normally, six (three on each lobe) sensory hairs in *Dionaea muscipula* and 30 to 40 in *Aldrovanda vesiculosa* (3) are found on the upper surface of the trap-lobes. When a small animal touches the distal lever above the joint of a sensory hair, or when the lever is pushed slightly with a fine rod, the hair bends at the joint consisting of morphologically special cells. In *Dionaea,* the mechanical stimulus is received in the remaining part of the hair after removal of the distal lever (14). Deformation of the thin-walled joint cells in *Aldrovanda* by pinching with two fine glass rods results in shutting of the trap (4). The special cells at the joint (42) seem therefore to be mechanical receptors, but in *Dionaea* no direct evidence for this conclusion has yet been obtained.

In *Dionaea,* at moderate temperature a shutting of the trap usually follows two stimuli (22, 27), disturbing either the same hair twice or two dif-

ferent hairs at an interval of less than 20 sec (14, 71). At higher temperatures (35 to 40° C), however, the shutting frequently follows only one stimulus (14). The absolute refractory period for perception of the stimulus is less than 1 sec (14, 70). Latent time between the second stimulus and onset of the shutting, as well as the process of the movement, are independent of the intervals of two stimuli less than 20 sec (71), so that the response seems to obey an all-or-none principle. However, slow and partial closures are caused with each of successive stimuli at longer intervals than 20 sec, ultimately the trap making complete closure; the number of stimuli required for complete closure depends upon the time intervals between the stimuli (13, 14, 22). Also, the trap of *Aldrovanda* commonly closes after the sensory hair has been bent twice (4). There are few quantitative data about the intervals of stimuli and number of bendings required because of experimental difficulties in *Aldrovanda*.

Jacobson (51) has found that when a sensory hair of *Dionaea* is bent, an electrical potential change that is somewhat similar to the receptor potentials in mechano-receptor of animals appears in the hair stimulated. When this potential goes over a certain magnitude, it generates a propagated action potential. The magnitude of receptor potentials roughly depends upon velocity of hair bending, but fast bending is rather ineffective. The propagated action potential starts from the base of the hair stimulated and spreads in all directions over the entire surface of the trap-lobes; a second action potential elicited within 20 sec after the first one is followed by the shutting of the trap (71). In the trap of *Aldrovanda,* one would expect that the receptor potential and action potential should be generated prior to the shutting, but no attempt has been made as yet to prove this.

In *Aldrovanda,* deformation of a few cells in the three-cell layered region which is the central part of the trap, and the zone where the cells active in closure are located, results in the shutting movement (4). When some part of the trap-lobes of *Dionaea* is scratched or pricked with a needle, a propagated action potential (101) occurs, followed by shutting (14). From the above results, it appears that mechanical stimuli are also received directly by the cells of the trap-lobes.

An electrical stimulus is also effective in the trap of both *Dionaea* and *Aldrovanda*. Ashida (4) has proved in *Aldrovanda* that the sensory hairs are not the chief receptor of the electrical stimulus, while cells in the motor zone are more sensitive than any other cells of the trap. A strong induction shock is always sufficient to cause the mechanical response in *Aldrovanda* (4) and in *Dionaea* (14). The strong current seems to stimulate more than two points of the trap at the same time. Since in these experiments one of a pair of stimulating electrodes is normally placed on the trap and the other one on the petiole in the case of *Dionaea* (14), or relatively large stimulating electrodes are placed apart from each other into the water with the trap of *Aldrovanda* positioned between them (4) the electric current must pass through a substantial portion of the trap in the both cases. On the other hand, when a pair of stimulating electrodes are positioned close to each

other on a part of the trap of *Dionaea,* each electric pulse above threshold strength elicits a propagated action potential, and the second action potential is followed by the shutting of the trap (71). Thus, two electrical stimuli, each of which elicits a propagated action potential, are required for the shutting of the trap, as has already been shown with mechanical stimuli.

A sudden rise or fall of temperature results in the shutting of the trap in *Aldrovanda* (4). The threshold temperature differences are smaller for a sudden fall than for a rise, the thresholds depending upon leaf age and initial temperature (5). Reaction time (2 to 50 sec) for the shutting with thermal shock is much longer than that with mechanical or electrical stimulus (about 0.1 sec) (5). It appears that when the trap is exposed to a new temperature, more than two excitations (action potential?) are elicited at certain intervals which depend on temperature differences, and then the shutting occurs.

It seems clear from the above results that in the *Dionaea* trap-lobes, and probably also in those of *Aldrovanda,* mechanical response (shutting movement) follows after a definite amount of accumulated effect. The mechanism of accumulation or memory is still unknown. There is no indication that the memory is closely associated with the receptor potential (51). The velocity of propagation of the second action potential is markedly larger than that of the first—within about 30 to 60 sec after propagation for the latter. In other words, the effect of the first propagated action potential remains in the trap for some time (70, 71). Recently, DiPalma et al. (28) found that if the lower surface or marginal ciliated part in the trap of *Dionaea* is stroked with a stiff-bristle brush several times, the number of bendings of the sensory hair required for the shutting of the trap after stroking becomes fewer than the number required without stroking. The investigators concluded that stellate trichomes which are distributed on the region just mentioned act as tactile receptors, and touching the trichomes results in some accumulated effect in the trap. The stroke causes a small nonpropagated potential change in the trap (28).

When several drops of 3 per cent NaCl solution are placed in the trap of *Dionaea,* a series of action potentials appears; a similar effect is obtained with equivalent osmotic quantities of other salts or even glucose, and also with juices from another trap that has captured an insect within 48 hr (8). These effects are followed by the shutting movement. In the process of digesting the captured insect, the trap shows spontaneous action potentials (8). This suggests a possible mechanism for keeping the trap closed during digestion. The closed trap reopens within about 10 hr if there is no insect in it, while the closure lasts for 10 or more days if the trap has captured an insect (58).

Mimosa.—While there has been much investigation of conduction phenomena, relatively little attention has been directed toward the nature and mechanism of stimulus perception in *Mimosa pudica* and its allied species. Among them, *M. pudica* (which is the most sensitive to stimulus) has been well investigated, so in this review our discussion will dwell mainly on *M.*

pudica and, unless specified otherwise, all references to *Mimosa* are to this plant. Shaking a whole *Mimosa* plant results in the almost simultaneous fall of each leaf and closure of each pair of leaflets, but little or no propagation is observable in this case. These responses must be generated through direct perception of mechanical stimulation by each pulvinus, perhaps with deformation of the pulvinus due to swaying of the leaves when the plant is shaken. It is well known that the lower surface of the main pulvinus of *Mimosa* is sensitive to the slightest touch, since the leaf falls immediately after touching, and usually this response is restricted within the stimulated pulvinus so that no propagation is seen. On the other hand, every part of the leaf and stem of *Mimosa* is receptive to many types of stimuli other than shaking, and a series of visible successive responses of pulvini (76, 92) or propagated action potential (45, 67, 93, 94) starts from the site stimulated. This means that excitable cells and their connections or other conduction systems extend throughout the whole plant, and the excitable cells may play the roles of both receptor and conductor.

Visible responses (rapid movements) occur only at the pulvini, whereas electrical responses (potential changes) can be observed not only at the pulvini but at points along the path of propagation such as stem, petiole, pinna-rachis, and leaflet. The propagated responses in the stem and leaf are analysed into three kinds from the patterns of electrical response, the propagation velocities, the type of blocks at which propagation is interrupted, and the variety of stimuli invoking a response (45, 62, 76, 92, 94, 101). Most research conducted earlier than the careful works of Snow (76) and Umrath (92) indicated that there was only one mechanism of conduction in the stem and leaf of *Mimosa,* so in some cases these early results appear conflicting.

The slowest propagation has a negative electrical potential irregular in shape and long in duration, and is produced by a wounding stimulus (cutting or burning) (45, 66, 105) or by tetanic stimuli from an inductor coil (100, 101). Among the three forms, only this wave can generally pass through the pulvini and propagate over a distance (45, 66). The rate of propagation depends upon the velocity of the water movement in vessels (45). This wave may correspond with the conduction mechanism found by Ricca (60) in the stem and leaf of *M. spegazzinii,* and with "normal" conduction in the stem of *M. pudica* proposed by Snow (76). These workers have shown that this conduction depends on a stimulating substance which is set free by wounding and carried along in the transpiration stream in the vessels. The electrical negative variation within this wave may depend on electrical potential changes generated by a moving stimultant in the neighboring living cells of the vessels (66).

The most rapid propagation is occasionally observed in the young leaf of intact plants, especially in damp air (45, 62), and frequently in a submerged "stem and leaf preparation," which is an isolated length of stem carrying a single leaf (76). Only the main pulvinus reacts 1 to 3 sec after cutting the

pinna of the same leaf, and neither further propagations nor further visible responses are seen. This wave can pass through a cooled ($\sim 3°$) zone of the petiole but not a killed zone (45). When cutting the pinna causes this wave, the system exhibits at the same time the slowest and the moderate waves, both of which can be seen as electrical potential changes (45). Electrical changes associated with the most rapid propagation are not clear, since the mechanism of this wave has not been explored. Recently, Umrath (104) reported a small action potential associated with this wave, but this seems to be somewhat questionable.

The moderately rapid conduction, which is by means of an action potential, is produced everywhere in the leaf and the stem by every sort of stimulus (45, 67, 94, 101). The use of electrophysiological investigation with microelectrode technique, which can determine the kind of cells that generate action potentials (69), reveals that elongated parenchyma cells in the protoxylem and phloem of the petiole are excitable and that the same cells are very probably the pathway of propagation. The elongated parenchyma cells found in the pinna-rachis and stem seem to be excitable cells, but no direct evidence of this has yet been obtained. In the petiole of *M. himalayana* and *M. pigra,* the elongated parenchyma cells are found in both the protoxylem and phloem (54). Wound stimulation elicits this mechanism together with the slowest wave, whereas stimulation without wounding, such as rapid cooling or electrical current, produces an action potential alone (45, 66). Under normal conditions, transmission of the action potential is interrupted at the pulvini (45, 62), so that when elicited alone this response occurs solely within the length of the stimulated pinna, petiole, or stem. When the transmission is produced together with the slowest wave by a wounding stimulus, the former stops at the pulvinus, but the latter passes through it a little later and regenerates the transmission of an action potential on the other side of the pulvinus (45, 62). The same reaction can be observed at a killed (71) or cooled (45) region, because the action potential cannot pass through such a region although the slowest wave can pass through it. This transmission may correspond with the "protoplasmic excitation" propounded by Bose (12), the "high speed" conduction in the stem and "rapid phloem mechanism" in the leaf found by Snow (76), and the "rapid conduction" in the submerged stem explored by Ball (7).

When an action potential propagated basipetally through the petiole arrives at the joint between the slender part of the petiole and the main pulvinus, another type of action potential in the main pulvinus is elicited here after a latent period of about 0.2 sec (65). The pulvinar action potential which may be caused as a result of the stimulative effect of the petiolar action potential at the joint has a more sharply rising phase than that in the petiolar action potential (99) and also shows a propagated nature (65); its velocity is about twice the latter. Mechanical response of the main pulvinus (rapid fall of the leaf) begins about 0.1 sec after the occurrence of the pulvinar action potential (32, 65). Thus the mechanical response always fol-

lows the pulvinar action potential, but only the action potential is generated when the second stimulus is applied within about 1 min after the first (95, 99). This means that when the absolute refractory period for the pulvinar action potential is over, the pulvinus does not recover immediately from the mechanical response. What kind of cells generates the action potential in the pulvinus has not yet been determined, but motor cells (cortical parenchyma cells), occupying a large part of the pulvinus, are most likely to be the source of the action potential, because of considerable activity in the motor cells during the movement. In the subpulvinus and the pulvinule the action potential elicited prior to their rapid mechanical responses is not yet clear.

Snow (76) has observed that when the cut distal end of a petiole is dipped into NaCl solution (3.5 per cent or stronger) the main pulvinus responds usually after 3 to 5 sec, but no response is obtained with a concentrated sucrose solution. It seems that such a solution of NaCl can generate the action potential at the cut end because the reaction time of 3 to 5 sec may correspond to the transmission time of the action potential through the petiole. Response in a fresh section of the pulvinule occurs with the addition of more than 0.5 per cent NaCl solution (108); apparently the motor cells directly generate the action potential with such a solution. The effect of NaCl on the cells is not osmotic but seems to be related to an ionic correlation between both sides of the cell membrane.

Rapid response in the main pulvinus also occurs with a light stimulus (38). When plants placed in a dark room are irradiated by 400 to 500 mμ of light at 0.8×10^{-8} Einsteins cm^{-2} sec^{-1}, the petioles fall within 30 to 80 sec after the beginning of exposure; irradiation of 450 mμ is most effective (39). High intensity irradiation 0.5×10^{-6} Einsteins cm^{-2} sec^{-1} of 1 to 10 sec exhibits the same response as continuous exposure of the lower intensities mentioned above (39). Where the light stimuli are perceived is as yet unknown.

Both the perception of stimulus and response behaviors in *M. spegazzinii* (77, 93, 94, 101), *M. invisa* (97), *Neptunia plena* (94, 101), *Biophytum sensitivum* (41, 94, 96), and *Biophytum* sp. (72) are similar to those found in *M. pudica*.

Filaments of stamens.—Only the adaxial surface (that facing the flower center) at the base of the filaments of *Berberis vulgaris, B. thunbergii,* and *Mahonia aquifolium* can directly perceive touch stimulus. A rapid response, bending toward the flower center, occurs locally in the same region that has just received the stimulus: no further response is seen (15, 19). In *Sparmannia africana* and *Helianthemum vulgaris,* a bending movement away from the flower center at the filament base is caused by a touch stimulus only on the abaxial surface (that facing away from the center) of the base or with bending inwardly so that this surface may be stretched (15, 19, 26). In all these plants, both perception of stimuli and responses are restricted within a small portion at the filament base, and the bending response occurs

in a definite direction. In *Portulaca grandiflora,* on the other hand, bending occurs everywhere through the length of the filament at stimulated sites, and the direction of the response is opposite to the bending direction for stimulus (47, 48). Trichomes or papillae distributed on the surface of the filaments of these plants do not seem to be part of the receptor apparatus (15, 49, 50). The perception mechanism of mechanical stimulus is not clear. No propagation of response is observed in the filament stimulated, but in *Sparmannia* some neighbors of the stimulated filament show the same response (15).

In *Sparmannia* and *Berberis,* a nonpropagated action potential (negative potential change) appears only at the base of the filament when stimulated (17). No difference in potential pattern is seen with different sorts of stimuli: mechanical, electrical, chemical, or thermal (17). The beginning of the action potential precedes the movement by 1 to 2 sec in *Sparmannia* (17). It seems that in *Berberis* also the electrical response is prior to the mechanical one. However, latent times for both electrical and mechanical responses are very short (0.03 to 0.07 sec), so that exact determination of time relation between the two responses is difficult (17, 101). Simultaneous records of action potential and movement in *Berberis thunbergii* (101) reveal that neither action potential nor movement appears within a 3-min period after the latest response, while a normal action potential with little or no movement appears within 7 min after the latest response. Thus, different refractory periods for electrical and mechanical responses are observed, as mentioned above for the main pulvinus of *Mimosa.*

Stigmas of pistils.—Bilobate stigmas in some plants are sensitive to mechanical stimulus and the lobe stimulated bends toward the other one. The stigma lobes in *Mimulus luteus* generally respond only to a pressure-stimulus on the inner surface (that facing the other lobe), but a simple touch is ineffective (23). Violent shaking of the pistil or strong bending of the lobe outside is effective (23). Accordingly, distortion of the inner surface cell layers must act as a stimulation. When a small part anywhere on the inner surface is stimulated gently, the response occurs locally at this part only, while with a strong stimulus the response occurs all over the surface of the lobe (23).

Recently, Sinyukhin & Britikov (75) found a propagated action potential in the sensitive bilobate stigmas of *Incarvillea grandiflora* and *I. delavayi.* The action potential caused by mechanical stimulus spread throughout the lobe stimulated but was blocked at the fork of the lobes. About 0.1 sec after the action potential spread throughout the lobe, the mechanical response occurred. If pollen is placed gently on a lobe so that there is no mechanical effect, no electrical potential change and no movement appear within 3 to 17 min after the pollination. An action potential is finally elicited after the generation of a slow potential change in the stigma lobe following the pollination, and hence movement occurs.

Action Potential and Its Transmission

As early as 1873, Burdon-Sanderson (20) found that deflection of a galvanometer connected with the *Dionaea* trap occurred when a fly creeping on the upper surface of the trap reached the sensory hairs and touched them. This was the first known observation of an action potential in plants. As described in the preceding section, the moderately rapid conduction from the stimulated part in the petiole, pinna-rachis, and stem of *Mimosa* is by means of transmission of an action potential (45, 62, 67, 94, 101); and another type of action potential triggered by the petiolar action potential starts at the entrance of the main pulvinus and propagates through it before onset of its mechanical response (65). An action potential starts at the sensory hair just stimulated and spreads over the whole surface of the trap-lobes of *Dionaea* (21, 27, 71, 81). Thus the transmission of the action potential plays an important role in the conduction of response in sensitive plants.

TABLE I

Action Potentials in Higher Plants

Species	Organ	Spike Height (mV)	Transmission (cm/sec)	Key References
Biophytum sensitivum	pinna-rachis	60	0.3–0.5	41, 94
Biophytum sp.	pinna-rachis	65–90	0.15–0.2	72
Biophytum sp.	peduncle	100–120	0.2	72
Clematis zeylanica	stem	—	0.15–0.25	46
Cucumis melo	tendril	30–80	(decrement)	98
Cucurbita pepo	stem	20 (80)[a]	6–1 (decrement)	6, 74
Dionaea muscipula	trap-lobes	40–60 (100)[a]	6–17	71
Incarvilla grandiflora	stigma-lobe	55	1.8	75
Incarvilla grandiflora	style	—	2.9	75
Mimosa pudica	pinna-rachis	80	0.3–0.7	64
Mimosa pudica	petiole	120 (140)[a]	2–3	67, 69
Mimosa pudica	stem	60	4–5	101
Mimosa spegazzinii	petiole	50	0.8	101
Neptunia plena	petiole	60	1	94
Vitis discolor	stem	—	0.2–0.7	45
Vitis gongylodes	tendril	—	0.6	45

[a] The values in the parentheses showed membrane action potential recorded in a single excitable cell.

Table I summarizes the results of experimentation by many authors with action potentials and their transmission in the various higher plants. Some of the plants shown in the table do not exhibit any visible mechanical response; the role of action potential transmission in these plants is not understood. On the other hand, some plants show a propagated electrical po-

tential change (101); but it appears that the change is not a true action potential but is an indication of the movement of a stimulating substance in the organ.

As far as we know, the most rapid transmission of action potential in higher plants is found in the trap of *Dionaea*. Within a few tenths of a second after stimulus an action potential spreads in all directions over the whole surface of the trap which consists of two lobes (71). The velocity of the spread is higher in the direction parallel to the vascular bundles than in the perpendicular direction (71). There is some variation in the form of the action potentials obtained from the upper and lower surfaces of the trap (70). Thus the patterns of action potential recorded from the surface of the trap are somewhat complicated, though the action potential recorded intracellularly from a single cell has a simple form (71). These differences may be the result of some distortion in the form of the action potential recorded from the surface due to dorsiventrality of the structure of the trap. The forms of action potential change successively with repeated excitation in relatively short time intervals (21). This seems to be an indication of the accumulated effects of repeated stimuli. The patterns of the diphasic recorded action potential (27, 81) must be largely modified by both the transmission velocity and the accumulated effects from repeated stimuli. No additional change in electrical potential in the trap can be found when a mechanical response (shutting of the trap) occurs (71). Accordingly, it appears that the mechanical changes occur in the same cells that generate the action potential immediately prior to the change; the relation between them recalls the mechanism of excitation-contraction coupling in muscle cells.

Excitable cells which generate an action potential and serve as the path of transmission in the petiole of *Mimosa* are found in the vascular bundles; the elongated parenchyma cells in protoxylem and phloem are excitable, and they form a number of cell rows along the longitudinal axis (69, 71). The membrane of the excitable cells is polarized; the cell interior is about 160 mV negative to the exterior, which is significantly larger than that in other insensitive cells found in the petiole, and during activity the potential changes transitorily about 140 mV toward the direction of depolarization (69). The latter is the membrane action potential of an excitable cell. This feature in the membrane is essentially similar to that in the axon, muscle fiber, and characeous internodal cell.

An action potential can be propagated over the whole length or the whole area of a certain region of the leaf, as stated previously. An approximate estimation based on the size of excitable cells and the transmission velocity shows that each excitable cell along a certain cell row in the *Mimosa* petiole successively generates the action potential with an interval of 4 to 6 msec (71). The mechanism of the transmission of the action potential from an excitable cell to another one remains unknown, but the results below suggest a possible mechanism.

When the petiole of *Mimosa* is entirely immersed in a large volume of

salt solution having relatively high conductivity, a significant and reversible rise (15 to 60 per cent, depending on the concentration of the solution) in velocity of transmission of the action potential can be obtained (68). Since, under these conditions, the resultant resistance outside the excitable cells is expected to be much reduced by shunting with the low resistance of the solutions, these results strongly suggest not only that the conduction of the action potential along a single excitable cell but also the transmission from cell to cell may be mediated by local current which flows between the just activated part and the adjacent yet unexcited part.

Another piece of evidence about transmission can be obtained from microelectrode experiments (71). The electrode is inserted into an excitable cell along a certain row; the latter is surgically interrupted some distance from the stimulated site. In spite of the disconnection from the stimulation, the excitable cell in question normally generates an action potential. This means that the transmission must take place transversely from the rows connected with the stimulated point to those disconnected from it. Similar experiments reveal that the transverse transmission can also take place not only between the rows located within the large central vascular bundles but from the central bundles to two slender ones which are isolated from the former; the tissues between the slender and large bundles consist of only the unexcitable cortical tissue having some intercellular spaces. From the results above, it may be concluded that the excitable cells may be affected electrotonically by the action potentials in other cells located nearby and may elicit the action potentials by themselves, so that any chemical transmission scarcely needs consideration.

Thus, good evidence for the transmission of action potentials in higher plants may be afforded by the petiole and the pinna-rachis of *Mimosa*. The transmission velocity depends on temperature and maturity of the plant, but it is independent of the strength of stimulus except in the stimulated portion (63). At the point of stimulus, when the stimuli are weak, only local potential changes are observed, the size of which increase with increasing strength of the stimulus. When the strength exceeds a certain value and the local potential reaches a certain size, the transmitted action potential suddenly appears, showing all-or-none characteristics (67). Since size of the local potential change may depend on the number of activated excitable cells in the stimulated part, it appears that generating transmission requires the activation of a certain number of excitable cells, and thereafter excitation spreads transversely until all the excitable cells are suddenly activated.

The velocity of transmission also depends on the size of the petiole and pinna-rachis, that is, the number of excitable cells (63, 64). It seems that the greater the number of excited cells present in the petiole the stronger the electrotonic current that is generated. The latter may result in a shortening of the time interval between reception of the current and occurrence of excitation in each of the excitable cells that are not yet activated, and hence it may increase the velocity of transmission. Accordingly, the transmission in this plant takes place only as a result of the cooperation of many

cells. In fact, the transverse spreading from the slender vascular bundles to the large ones does not occur, and transmission cannot take place very far along the slender bundle alone (63, 71). Decrement transmission of action potential in *Cucumis* and *Cucurbita,* as shown in the table, seems to occur because the number of excitable cells is too small for the transmission to the necessary distance.

Stimulating Substances

In 1916, Ricca (60) found important evidence of the propagation of a stimulating substance in *M. spegazzinii.* His research shows that a substance is released from cells wounded by stimulation, enters the vessels, moves an appreciable distance, and can stimulate the pulvini. The same phenomenon is observed in *M. pudica* (76). This is the second means of propagation of response in *Mimosa.* When the basal cut end of the petiole of a detached leaf is immersed into a water extract of other leaves, the pinnae eventually exhibit successive closures of the leaflet pairs; a 1:5000 dilution of the extract is still effective (36). More than a thousand substances have been tested for the pinna response in the same way: among them DL-alanine, DL-serine, DL-glutamic acid, and some derivatives of anthraquinone are effective to the response, but the threshold concentrations of them are as high as 10^{-3} to 10^{-4} *M* (36).

Several workers (9, 37, 43, 44, 78, 79) have made attempts at purification and determination of chemical structure of the stimulating substance in *Mimosa,* but so far no one has succeeded. An amorphous concentrate gives the pinna response at a dilution 1.5×10^{8}: the substance behaves as an oxy-acid containing nitrogen (4.5 per cent) with an estimated molecular weight between 300 and 450 (78). Supposing the molecular weight is 400, the threshold concentration could be as low as 5×10^{-9} *M*. Other results (43, 44) show that the extracted substances from the *Mimosa* plant are highly sensitive to oxygen and can turn easily into an inactive form, their chemical behavior being that of a reducing agent: meso-inositol is perhaps one of the primary compounds leading to the active substances. The reducing power of the active form seems to have a stimulating effect on the cells. When the extraction is carried out after applying an anesthetic to the *Mimosa* plant, the activity of the substance is reduced to one-fourth to one-tenth of the normal (79). This suggests that the cells contain the substance in an inactive form that may turn into the active form upon wounding stimulus.

A water extract from the stamen or leaf of *Berberis vulgaris* is effective to the response in the filament of this plant (102). Effectiveness of the extract is found in dilutions of more than about one-sixtieth; an extract from the leaf anesthetized previously with ether show about a half effect of that from the normal (102).

Mechanism of Rapid Movements

Most of the literature concerning the mechanism of rapid movements in higher plants concludes that the movements are caused by the diminishing

or sudden loss of turgor in the motor cells or decrease in their volume or both. This seems to explain the rapid movements satisfactorily, but the changes in turgor and volume of the motor cells must be brought about passively as a result of an action that is initiated in the cells when the response starts. This action is not precisely understood. Various facts, reported by many authors, of morphological and physiological changes in the motor cells during the movement will be considered below from such a point of view.

Excretion of water or cell sap.—There are several observations on the excretion of water or cell sap from the motor cells during response in plants exhibiting rapid movements. This phenomenon seems to be important for explaining the mechanism. In the stamen of *Sparmannia,* swelling of cell wall materials and formation of droplets over the wall surface are observed on the outer wall of the irritable cells after the movement occurs (16). Extrusion of liquid from the cells into the intercellular spaces of the tissue in the stigma-lobes of *Mimulus* is observable under the microscope when the lobe responds to stimulus (23).

Various observations of liquid movement in the pulvini of *Mimosa* during the rapid movements have been conducted. Expulsion of water from the main pulvinus during the movement, and reabsorption of water during recovery, can be seen by a micropotometer: the quantities of the water reabsorbed are always greater than those expelled (31). Long ago, Blackman & Paine (10) demonstrated that electrical conductivity of a small quantity of water which surrounds an excised main pulvinus is increased after its mechanical response. This seems to be a result of excretion of electrolytes from the cells in the pulvinus during the movement. They concluded that the amount of electrolytes exosmosed was far too small to account for the sudden decrease of turgor of the motor cells. However, the amount of electrolytes actually exosmosed during the movement must be larger than they reported, because it seems that the electrolytes just secreted from the excited cells could not move out quickly from the inside of the tissue to the surrounding water; thus only a fraction of those secreted from the cells near the cut surface contribute to changes in conductivity of the water. Electrical resistance measured longitudinally between both ends of the intact main pulvinus is decreased with the mechanical response: the amplitude of the response closely corresponds to the decrease of resistance (61). The electrical resistance of the intact pulvinus must depend mainly on the amount of electrolyte present in the liquid of the intercellular spaces rather than that in the cell interiors. Microscopic observation of the main pulvinus reveals that there are many air bubbles in the well-developed intercellular spaces which are localized at a certain zone in the motor tissue, but almost no bubbles are found after the pulvinus receives a stimulus (83). A juice containing tannins, potassium, and other substances issues from the cut surface of the main pulvinus under stimulus (83). Observation of the incinerated sections of the main pulvinus reveals that ashes are distributed homo-

genously over the cytoplasmic layer before a stimulus, but these ashes almost disappear in the cytoplasm and masses of ashes are located about the cell walls after a stimulus (86). Histochemically detectable potassium salts are found in the motor cells before response, but the large crystals of the salts appear in the intercellular spaces after response (84, 86). A liquid containing potassium salts is found to be present in the intercellular spaces in the section of the fresh main pulvinus upon stimulation: before stimulation and after recovery from the response such a liquid is not found (30). Tannin substances seen in a particular vacuole (tannin vacuole) in the motor cells of the pulvinus are also found in the peripheral cytoplasmic layer or the intercellular spaces after a stimulus (82, 83).

In the trap-lobes of *Aldrovanda,* a capillary-active substance, such as alcohol or acetone, rapidly enters the intercellular spaces at the motor zone only and drives out the air which filled them; and if the lobes are dipped in a solution of osmic acid, the cell contents in the motor zone becomes dark brown in color, although no other part shows this change in color (3). These facts indicate that the outer walls in the motor zone are particularly permeable to the liquids even during the rest period.

All of the above results clearly indicate that some of the cell sap escapes to the exterior during movement, and the excape may result in decrease of turgor in the motor cells. Explaining what causes the motor cells to move their cell sap out to the exterior should be a most important step toward understanding the mechanism of the rapid movements.

Increase of permeability.—Several authors (3, 16, 23, 61, 90) have proposed that the mechanical response in the motor cells, seen in the rapid movement, is caused by an increase in permeability of the cell membrane. There is, however, no conclusive evidence that a rise in permeability actually does occur when the mechanical response starts in the motor cells. Since a transient increase of membrane conductance which depends on the permeability to ions is seen to occur concomitantly with the action potential in the excitable cells of animals and characeous internodes, it may also occur in the motor cells of sensitive plants. However, this does not mean that the increase of permeability to ions during the action potential results directly in the changes in turgor, because movement of ions through the membrane during the action potential seems to be in insufficient quantity to account for the decrease of turgor and, as already described, there exists a certain time interval between the occurrence of the action potential and the mechanical response seen in the filament of *Sparmannia* (17), in the trap of *Dionaea* (71), and in the main pulvinus of *Mimosa* (32, 65). If the decrease in turgor of the motor cells were brought about directly by an increase in permeability to water or other liquid, the latter should be superimposed on the rise of permeability during the action potential. The actual existence of such a change in permeability has not yet been demonstrated.

Microscopically visible changes in the motor cells and tissues.—The attention of many investigators has been directed to the vacuoles in the motor

cells and their changes during the response. Additional observations have been made concerning the difference in structure of the motor cells before and after the response and about morphological characteristics of the motor cells.

Weintraub (108) studied sections of the fresh pulvinule of *Mimosa*. In the motor cells, he found numerous small vacuoles (about 1 μ in diameter), which are not usually evident in the fixed material; these were in addition to the central and tannin vacuoles. Behavior of the small vacuoles is of great interest and suggests an explanation for the mechanism of the movement. During the response, the small vacuoles already present disappeared, but at the same time, rapid formation of new vacuoles of the same type and their subsequent disappearance were observed under the microscope. The number of small vacuoles present after the response was greatly diminished, and the sizes of both the cell and the central vacuole decreased during the response. After recovery, the number of small vacuoles and the size of the cells became nearly as great as the initial ones. When the section was immersed in a dilute solution of neutral red, the small vacuoles as well as the large central vacuole were stained; during the response, the newly formed small vacuoles contained none of the dye, unlike those already present, and the color of the stained central vacuole, which decreased in size, deepened. Weintraub concluded from these results that active contraction of the small vacuoles, as well as the formation of new vacuoles and their subsequent contraction, caused a sufficient decrease in water content in the motor cells to result in the lessening of their turgor.

In the main pulvinus of *Mimosa*, small contractile vacuoles are also observed in the motor cells in fresh sections; these are fewer in number and smaller in size than those found in the pulvinule (30). On stimulus these vacuoles disappear completely, the tannin vacuole becomes smaller in size, and a slight contraction of the cell wall occurs (30). During recovery, on the other hand, the small vacuoles reappear, and the tannin vacuole returns to its initial size (30). Upon electrical stimulation, the vacuole (probably the tannin vacuole) of the motor cells of the main pulvinus rapidly contracts, and at the same time deformation of the cell and decrease in cell volume also occur; no difference in this behavior is seen between the lower half—seemingly more sensitive than the upper—and the upper half of the main pulvinus (2). In one experiment, the large central vacuole of the motor cells in a freshly hand-sectioned main pulvinus was tinted violet with brilliant cresyl blue (25). When an extract from cut pulvini, which has a stimulating effect, was added on the medium in which the section was located, the violet color of the vacuole deepened in shade (25). Upon treatment with a glycerine solution, the vacuolar membrane crumpled, but after a while it recovered partially, and when water or pulvinar sap was added to the outside medium the recovery of the membrane was promoted (25).

All of the above results clearly indicate that the motor cells in *Mimosa* have contractile vacuoles whose activity causes liquid (probably cell sap) to

be expelled from the motor cells, just like the contractile vacuoles seen in the protozoa; this activity may result in a decrease in turgor or volume of the motor cells.

Toriyama (82, 87, 89, 90) observed the variously fixed and stained sections of the main pulvinus of *Mimosa* and recognized some differences in structure and content in the motor cells before and after receiving a stimulus; he stressed changes of the tannin vacuole. Affinity of the tannin vacuole for basic dyes is higher during the rest period than after response (89, 90): the outline of this vacuole is clear before stimulus and somewhat obscure after response (89). In the sections fixed by Müller's fluid and stained by Ehrlich's haematoxylin, the peripheral cytoplasm is seen as a thin layer before the response, while it shrinks and becomes somewhat granular after the response (89). When the section is treated with chromium salts the granular contents are seen in the central vacuole during rest, but little is found after the section receives a stimulus (87). These observations indicate that the nature of the motor cells seems to change during the response, but its relationship with the mechanism of movement is not clear. There is no direct relationship between the presence of tannin vacuole and the occurrence of rapid movement, because no tannin vacuole is found in the motor cells of the very young plants in which the movement takes place normally (85). Recently Toriyama found a membrane structure surrounding the tannin vacuole (91).

Microscopic observations of the fresh motor cells of the filament of *Berberis vulgaris* reveal that the cell form changes greatly during response, but without a change in cell volume; the size of the central vacuole greatly decreases, while the peripheral cytoplasm thickens; and both granulation of cytoplasm and change in shape of the nucleus from spherical to spindly are seen (24).

Force of the movement.—The motor zone in the trap of *Aldrovanda* consists of three cell layers: both inner and outer epidermis and the middle layer. Ashida (3) proposed the following mechanism of rapid shutting movement and inward bending of the lobes in *Aldrovanda,* based on his own and other workers' results. Upon stimulus, an increase in the permeability of the inner epidermal cells occurs as the response, and then sap from these cells is pressed out by their own wall pressure and by the water deficit of the outer two layers, the latter not being irritable and remaining quite turgid. In other words, the motive force of this movement originates from inherent tissue water tension in the outer layers of the trap when the turgor (or volume) of the irritable cells in the inner epidermis suddenly decreases upon receiving a stimulus. The same mechanism of movement in *Dionaea* was suggested by him. Stuhlman (80) demonstrated the shutting process of the trap in *Dionaea* with a movie camera. The results reveal that the shutting is a typical dynamical action exhibiting a decrease in angular displacement proportional to the square of the time, namely $t^2 = a - b\theta$, where θ is angular separation of the lobes at t, and a and b are constant.

From this fact, it follows that the increase in angular speed is proportional to the time elapsed since the start of the movement and that the angular acceleration is constant during the movement. This acceleration is attributed to the restoring forces such as tissue tension of the outer layer of the lobes, which comes into action when a pressure maintaining the lobes in open state is abruptly removed, causing a loss of turgor of the inner layer. Shutting forces of the traps in these plants are measured with a small silica dynamometer in *Aldrovanda* (3) and with a strain gauge transducer in *Dionaea* (27): the forces are 22 to 55 mg, depending on the leaf age, and about 7 dyn, respectively.

As shown in well-known classical experiments, fall of the petiole in *Mimosa* occurs weakly but otherwise normally when the upper half of the main pulvinus is surgically removed, while no movement occurs if the lower half is removed. Accordingly, it is widely accepted that the loss of turgor is caused only in the cells of the lower half, which are sensitive to stimulus, and the movement itself is caused by an extension of the opposite half (107). In the pulvinule (the pulvinus of leaflet), in fact, significant differences in shape, content, activity, and especially in nature of the cell walls are seen between upper and lower halves (108), and only the cells in the upper half seem to be irritable in this organ. In the main pulvinus, however, this simple explanation for the rapid movement seems to be somewhat questionable on the basis of the following results: (*a*) no differences in the affinity change for dyes and the change in size of the tannin vacuole during response are found in the cells of the two halves (83); (*b*) there is no difference in the threshold values of electrical stimulus for the contraction of the vacuole in both halves (2). By an improved recording apparatus, Aimi (1) proved that sensitive motor cells exist even in the upper half as well as the lower half of the main pulvinus. When the lower half is removed and the plant is inverted, the petiole bends downwardly (i.e., upwardly in normal position) on stimulus: the magnitude of bending in this case is almost the same as when the upper half is removed. Aimi (2) concluded that magnitude and direction of the petiolar movement in *Mimosa* is expressed by two forces antagonizing each other, each of which consists of bending force due to the contraction of one half and the tissue tension in the other half; normally, the force in a downward direction would be much greater than that the upward force. Some differences between the halves can be actually observed in the morphology and activity of the cells. The wall of cells in the upper half is thicker than that in the lower (83). The magnitude of the extracellular action potential in the main pulvinus, which occurs prior to the movement and the size of which may depend on the number of cells excited, is about fivefold greater in the lower half than that in the upper half (65).

Role of ATP and ATPase.—Recent results suggest that the rapid movement in *Mimosa* is caused by a reaction of the ATP-ATPase system. Poglazov (59) found first that ATPase activity in the fresh leaf of *Mimosa*

is markedly stronger than that in the senile insensitive leaf as in the leaves of other plants which do not exhibit the rapid movement. No activation with addition of Mg^{++} and Ca^{++} but an inhibition with ethylenediaminetetraacetic acid (EDTA) are observed. The maximum activity is shown at pH 5 to 6. However, Lyubimova et al. (57) also found an ATPase in homogenates of the various tissues in *Mimosa* which is activated by Mn^{++} with the optimum pH at 6.5. This is widely distributed in the insensitive parts, such as leaflet, pinna-rachis, and petiole, but scarcely in the sensitive pulvini. They pointed out, therefore, that the ATPase seems not to be related to the mechanism of rapid movement, and this may be identical with results obtained by Poglazov (59). Lyubimova and associates extracted another ATPase found largely in the puvini (56), which is activated with Mg^{++} and Ca^{++} with an optimum pH between 8 and 9. The precise chemical nature and physiological function of the ATPase found in the pulvini are still unknown.

The ATPase activity can be demonstrated histochemically in the main pulvinus and the pulvinule of *Mimosa* (88). The activity is found homogenously through the peripheral cytoplasmic layer in the motor cells when Mg^{++} is present: sodium fluoride inhibits this activity. A section of the main pulvinus which is treated with 50 per cent glycerin for 2 hr exhibits contractions of the motor cells with addition of a solution containing ATP and Mg^{++} (56). The number of yellow crystals of ammonium phosphomolybdate which occur in the motor cells of the glycerol-treated main pulvinus section treated previously with ammonium molybdate is significantly larger with addition of ATP than those without ATP or with ATP and PCMB (106). This shows a liberation of inorganic phosphate from the added ATP caused by ATP-hydrolysing activity which is present in the glycerinated pulvinus.

The ATP contents, assayed by the firefly tail system, in the motor organs such as main pulvinus, subpulvinus, and pulvinule of *Mimosa* are three to four times greater than those in the pinna-rachis and petiole (55). After receiving a stimulus, the contents decrease to less than half their original value, and increase again during recovery (55). A marked decrease in ATP content in higher plant tissues is also demonstrated in the tendril of *Pisum sativum* during its coiling movement; at the same time, a remarkable increase in the endogenous inorganic phosphate is observed (52).

No direct evidence of the existence of contractile proteins in the motor cells of *Mimosa* and other plants exhibiting the rapid movements has yet been obtained. However, extracts with the Weber-Edsall solution of the tendril of *Pisum sativum* (53), the vascular bundles of *Cucurbita moschata* and *Nicotiana tabacum,* and the leaf blade of *Hydrilla* sp. (109) exhibit a transient reduction in their viscosity when ATP is added to them. The same extracts also show a liberation of inorganic phosphate from added ATP (53, 109). Driessche (29) showed an implication of contractile proteins in the movement of *Mimosa,* based on the following results. When the basal cut end of the petiole is immersed in the solutions of mersalyl and protamine sulfate (with a wetting agent), the pinnulae do not maintain the open

position. Treatments with EDTA by the same method show no effect on maintaining the open position of the pinnulae, but the pulvinules of the treated leaf lose their motor response to shock stimulus. Driessche concluded from these results that maintenance of the open position in the pulvinar cells is dependent on the energy set free by ATP-hydrolysis in the pulvinule, which is blocked with mersalyl; this position corresponds to the elongated state of the contractile proteins, a state no longer possible in the presence of protamine sulfate, and divalent cations such as Ca^{++} and Mg^{++} play a role in the mechanical response in the pulvinule.

Concluding remarks.—The central problem concerning the mechanism of the rapid movements, that is, what does occur as the first mechanical change in the motor cells, is still far from a solution. From the preceding discussion, the liquid extrusion from the motor cell which has just received the stimulus seems to occur due to the activity of the contractile vacuoles, but not due to increase in permeability in the plasma membrane. It appears that the activity of the contractile vacuoles depends on a mechano-chemical reaction caused with an ATP-ATPase system.

It is well known that secondary response in the protoplasm, such as muscle contraction, is triggered by the electrical potential change (action potential) of the membrane. The same correlations are also seen in bioluminescent flash and tentacle movement in the dinoflagellate *Noctiluca miliaris* (33, 34), and in the shock stoppage of protoplasmic streaming in characeous internodal cells (73), etc. All of the motor organs which have been studied elecrophysiologically and discussed in this review exhibit the generation of an action potential prior to the rapid movement. It is therefore to be expected that an action potential elicited in the motor cell triggers mechanical or chemical changes or both in its protoplasm as a secondary response: the triggering seems to occur through ionic changes about the membrane. A direct effect of stimulus on the motor cells, or an effect of the propagated action potential which reaches them from the stimulated site, should cause the motor cell to generate a depolarization of the membrane and then an action potential. This may be the first response in the motor cells to stimulus.

How the liquid, which has been expelled into intercellular spaces during response, re-enters the cell interior is still unknown. Potassium and other salts are found in the intercellular liquid of *Mimosa* (84). Recovery of contracted central vacuole in the motor cell of the main pulvinus of *Mimosa* is promoted with addition of the pressed sap from cells to the external medium (25). Recently, it was found that the stomatal opening, involving an increase in volume of the guard cells, depends on an effect of active accumulation of potassium (35, 40), and ATP in the guard cells is involved in the uptake of potassium (40). An active accumulation of the salts or ions may take part in the recovery process of the motor cells.

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