Observational and Experimental Studies of Sensitive Plants

VI. The migration of potassium in the primary pulvinus¹

Hideo Toriyama

Biological Section, Tokyo Woman's Christian College, Tokyo

Received August 25, 1955

Referring to the literature, some attempts have been made to see the physiological mechanism of the bending movement of the pulvinus of *Mimosa pudica* from the chemical aspect. And in several others the motor tissue is dealt with from the electrophysiological point of view. But their data were not enough to find the key point in the mechanism. In the preceding papers the present author has reported the morphological and histochemical changes in the pulvinus and in the petioles before and after the stimuli. By employing narcotics the present author was able to find the migration of potassium in the motor tissue of *Mimosa*, which was preliminarily reported in 1954. The purpose of the present paper is to complete the work and to describe this interesting phenomenon.

Material and method

The material used in this work was the primary pulvinus of *Mimosa pudica* L. The plants were grown in pots exposed to the open air in the field. The experiments were carried out in the summer of 1953 and 1954. In order to examine the motor tissue before movement, the plants were exposed to ether vapour, after which the plants were not responsive to the stimuli after 20 to 25 minutes. Still further, as indicated in Fig. 2, a stem with a petiole was anesthetized by setting in a bottle containing 0.2 M ethyl urethane solution for 2 to 10 hours. The primary pulvinus thus treated became completely non-reacting to the stimuli. Such anesthetized condition was also proved from observations of the motor organs.

In order to disclose the mineral elements in the protoplast by microincineration, the pieces of the pulvinus were fixed with Scott's solution². The tissue was imbedded in paraffin and cut at 4 to 6 micra thick by means of the microtome. Sections for the incineration were placed in a small quartz tube electric furnace similar to that devised by Policard and Okkels (1930). For the observation of the incinerated section, the dark field illumination was indispensable.

For the microscopic observation of the localization of potassium in the

¹ Contribution No. 19, from the Biological Section, Tokyo Woman's Christian College.

² Scott's solution is composed of 9 volumes of absolute alcohol to 1 volume of neutral commercial formalin.

motor tissue, the material was fixed with Macallum's modified solution¹ at 0°C for 24 hours. Then the material was washed thoroughly with distilled water. Next, the material was directly soaked in 70 per cent alcohol, and then the ordinary paraffin technique was followed there after. Paraffin blocks were cut 10 micra in thickness. The sections were stained with Mallory's triple staining method. The present technique not only gave excellent results in demonstrating the potassium, but also was outstanding in the fixation of the tannin substance.

Results of experiments

Experiment 1. Wallace (1931) has shown that the concentration of ether vapour from 13 to 15 per cent to volume of air prevented the movement of leaflets and petiole after 10 to 45 minutes exposure. As in the previous paper the present author used the same method in this work to anesthetize the material. In this experiment the effect of the ether vapour upon the distribution of salts in the cells was confirmed.

Seedlings of *Pisum sativum* and pulvinus of *Robinia pseud-Acacia* were exposed to the ether vapour following the same procedure as in *Mimosa*. These anesthetized materials and their control were fixed with Scott's solution. The preparations after the staining technique were compared with those of spodogram. In this experiment a change in the distribution of salts in the protoplast of the anesthetized material was not observed. From this result, it may be deduced that this narcotic gives no remarkable effect upon the protoplast.

Figs. 1-2. 1, the apparatus for setting the petiole with its leaflets. 2, the apparatus for setting stem with its petiole and leaflets.

Experiment 2. Usami (1937), in the study of physiology in the water moss², reported that 0.1 M ethyl urethane inhibit the respiration of these plants. As shown in Fig. 1, *Mimosa* petiole with leaves was set in a bottle containing 0.1 M ethyl urethane solution for several hours. It was realized that this concentration did not give an inhibiting effect upon the bending movement. Next the effect of 0.2 M urethane solution, as in the preceding experiment, upon the seismonasty of primary pulvinus was tested. Table 1 shows the mode of effect as the time elapsed. As seen in the

¹ It is convenient to prepare Macallum's modified solution after the following formula: solution A, 5g. of cobalt nitrate in a mixture of 10 ml. of distilled water and 2.5 ml. of acetic acid; solution B, 15g. of sodium nitrite in 25 ml. of distilled water. For use, pour solution A and B together, shake for a few minutes until the bulk of the nitrous fumes which develop on mixing has escaped (see G. Gomori, 1952).

² *Fontinalis antipyretica* L., *Chiloscyphus fragilis* SCHIFFN, etc..

Table 1 after 70 to 80 minutes, the leaflets did not respond to the stimulus. These anesthetized materials were then removed into a bottle containing distilled water for 30 to 35 minutes. The leaflets of these plants thus treated again reacted to stimulus. This apparently indicates that 0.2 M concentration of ethyl urethane causes the reversible anesthetic effect on the motor organ.

Table 1

Time in min.		0	20	45	70	80	100	120	140
Urethane solution	No. 1	—	+	+	+	—	—	—	—
	2	—	+	+	±	—	—	—	—
	3	—	+	+	±	±	—	—	—
	4	—	+	+	+	—	—	—	—
Distilled water (control)	5	—	+	+	+	+	+	+	+
	6	—	+	+	+	+	+	+	+
	7	—	+	+	+	+	+	+	+
	8	—	+	+	+	+	+	+	+

+ indicates the seismonasty.

Table 2

Time in min.		15	30	60	90	120	150	180	210	240	270
Urethane solution	No. 1	+	+	+	+	+	+	+	±	—	—
	2	+	+	+	+	+	+	+	±	—	—
	3	+	+	+	+	+	+	+	±	—	—
	4	+	+	+	+	+	+	+	±	—	—
Distilled water (control)	5	+	+	+	+	+	+	+	+	+	+
	6	+	+	+	+	+	+	+	+	+	+
	7	+	+	+	+	+	+	+	+	+	+
	8	+	+	+	+	+	+	+	+	+	+

+ indicates the seismonasty

Experiment 3. As shown in Fig. 2, a petiole with leaves was set in a bottle containing 0.2 M urethane solution for 3 hours. By this treatment the primary pulvinus became non reacting to the stimulus. The time needed for anesthetization is shown in Table 2. Both this material and the pulvinus which was anesthetized with ether vapour, were all fixed with Kaiser's solution and the sections were stained with Mallory's method. As shown in Figs. 3 and 4, there are no differences between the two. Furthermore, compared with the spodiogram of both materials, there is no discrepancy between the two.

Experiment 4. Primary pulvinus of *Mimosa*, before and after receiving stimuli, are both fixed with Scott's solution. In the incinerated sections

Figs. 3, 4. 3, the motor tissue of the primary pulvinus of ether anesthetized materials. 4, the motor tissue of the primary pulvinus of urethane anesthetized material. The above two figures are taken from the materials before receiving stimulus. Fixed with Kaiser's solution and stained with Mallory's method. $\times 800$.

Figs. 5-10. Incinerated sections of the motor tissue of *Mimosa*, photographed by dark-field illumination. 5 ($\times 320$) and 8 ($\times 800$) are before receiving stimulus. 6 ($\times 320$) and 9, 10 ($\times 800$) are after receiving stimulus. 7, photographed by direct illumination of a control section of a motor tissue, stained with Malloy's method, after fixation Kaiser's solution. Showing before receiving stimulus.

before receiving a stimulus, the ash of pale blue color seems to be homogeneously distributed through the cytoplasm (Figs. 5, 8). But, after receiving a stimulus, such kind of ash disappears from the cytoplasm (Figs. 6, 9 and 10). Upon receiving a stimulus, the ash glittering white on the cell membrane gathers

itself into masses and increases in volume. The pale ash throughout the cytoplasm may be displaced toward the intercellular spaces by the flow of water, which is brought about by the bending movement. In the next place, the incinerated tissue of a longitudinal section through the phloem in the vascular bundle was examined. In the sections before the stimuli the pale

Figs. 11, 12. Incinerated material of longitudinal section of phloem, photographed by dark-field illumination. 11, before receiving stimulus. 12, after receiving stimulus. $\times 800$.

ash and white ash were observed to be in fibriform and gave homogeneous spodogram (Fig. 11). Due to the stimuli, these ashes gather into granules as shown in Fig. 12. Thus when comparing the ash images of these two conditions, we can observe a notable difference. This series of experiments, then, shows clearly the migration of salts in the motor tissue cells at the bending movement.

Experiment

5. Both materials, before and after receiving the stimulus,

Figs. 13-16. The motor tissue of under side of primary pulvinus. 13, before receiving stimulus. 14, after receiving stimulus. Both materials treated with Macallum's modified solution, stained with Mallory's staining. $\times 320$. Arrows indicate the crystals. 15, schematic figure of Fig. 13. t, tannin vacuole; c, crystals. 16, schematic figure of Fig. 14.

were fixed with Macallum's modified solution to detect the

potassium salts, and were stained by means of Mallory's triple staining method. The results are illustrated in the Figs. 13 to 16. By this technique, crystals of potassium salts in the cells before receiving a stimuli were found clumping around the tannin vacuoles (Figs. 13 and 15). The crystals are formed roughly equidimensional around the tannin vacuole in each motor cells. The tannin vacuoles are fixed clearly in the cobaltinatrium nitrate material in brownish color. But by this technique the cytoplasm is not markedly differentiated from the other cellular elements. After receiving the stimulus, large crystals appear in the large intercellular spaces. These crystals are larger than those in the protoplast anesthetized motor cells. This phenomenon appeared in the under side of the primary pulvinus, especially in the zone which includes numerous intercellular spaces¹. From this phenomenon it can be supposed that potassium salts in the motor cells pass into intercellular spaces

Figs. 17-20. 17, schematic figure of primary pulvinus of *Mimosa pudica*. Blacked area shows vascular bundle, greyed area shows zone intercellular spaces. Arrows indicate the direction of migration of potassium solution in transitional tissues. pul, pulvinus; tr, transitional tissue. 18, 19, transitional tissue between stem and primary pulvinus, highly magnified figures taken from the square region of Fig. 17b and c. pul, pulvinus. 20, transitional tissue between primary pulvinus and petiole, highly magnified figure taken from the square region of Fig. 17d. Arrows indicate the direction of migration of potassium solution. pul, pulvinus.

¹ These intercellular spaces are included in "P₂-zone" which was named by the author in a previous paper (1954a).

at the bending movement. The recovery from the bending movement may be brought about by the recovery of turgor which is probably caused by the permeation of the potassium solution into the motor cells.

Experiment 6. Before proceeding further it seems to be convenient for the reader's understanding to give a schematic figure of the primary pulvinus (Fig. 17), in which the data obtained in the following experiment are summarized. Above these structures, the transitional zones also, between the pulvinus and the stem, and between the pulvinus and the petiole, must be particularly examined. These transitional tissue are shown in Figs. 18, 19 and 20. The large parenchymatous cells and small intercellular spaces are arranged lengthwise. The directions here is indicated by arrows. These parenchymatous cells have tannin vacuoles which are found in the watery liquid of the central vacuole. The central vacuole is always surrounded by a delicate protoplasmic membrane which attaches to the periphery of the cell. The fresh section of anesthetized tissues of transitional zone between stem and pulvinus, was soaked in a sodium cobaltinitrite solution for 5 minutes. This technique made it possible to demonstrate the potassium by the microcrystalline precipitates¹ with orange color. As shown in Figs. 21 and 23, a few microcrystallines before receiving a stimulus appear in the protoplast of the transitional parenchymatous cells. On the contrary, lumping microcrystalline appears in the tissue after the bending movement (Figs. 22 and 24). It shows that potassium solution passes through the transitional tissue.

Figs. 21-24. 21, 22, transitional tissue between stem and primary pulvinus. 21, before receiving stimulus. 22, after receiving stimulus. $\times 200$. pul, pulvinus. tr, transitional tissue. 23, 24, transitional tissue of the stem parenchyma. 23, before receiving stimulus; 24, after receiving stimulus. Four figures are treated with sodium cobaltinitrite solution. Microcrystalline precipitates are potassium-sodium cobaltinitrite. $\times 480$.

¹ This precipitates are potassium-sodium cobaltinitrite.

Discussion

In the first experiment of this work, the effect of the anesthetics upon the distribution of the salts was demonstrated in the cells of the plants by means of microincineration. From the available data of exp. 1, it was concluded that the ether has no effect upon the distribution of the salts in these cells. It is probable that the fat-solvent anesthetics do not interfere with the release of calcium. This was also clearly shown by the experiment of Mazia and Clark (1937) on *Elodea* cells. In general, the anesthetics may not alter the distribution of the salts in living cells. Since Warburg's work (1910, 1911) and those of others¹ it has often been assumed that the respiration of animal and plant cells are interrupted by the carbamates. Sledmann (1931) reported that the esterase is interrupted reversibly by the treatment with urethane solution. As indicated in the exp. 2, seismonasty of the primary pulvinus is inhibited reversibly by urethane solution. Perhaps, respiratory enzymes or some esterase system in the motor tissue may be interfered with by the carbamates. Furthermore we could not recognize any decided difference between the both materials which were anesthetized with the ether and the urethane. This difference, if any, is so slight as to be almost negligible.

The chemistry of muscular contraction has been thoroughly studied (Szent-Györgyi 1941 to 1950 *et al*). On the contrary, the phenomenon of the bending movement of *Mimosa* from the physicochemical side has not carried out enough. Pfeffer (1874, 1877), Ricca (1916), Blacmann and Paine (1918) *et al* have been the main investigations on this subject². Blackman and Paine, and Sen (1923) have made clear that the fundamental mechanism of the bending movement is based on the sudden decrease in the membrane permeability, a consequent of which is an effective decrease in osmotic concentration and a loss of water. But we have never seen a report concerning the chemical mechanism of the bending movement of the *Mimosa* Pulvinus. Scheer (1953) said "In *Mimosa*, leaflets, leaves, or whole branches appear to wilt, in the sundew, a trap closes on the insect responsible for the stimulation. These change in turgor have electrical components, which however have not been analyzed in detail." Being specially concerned with the motor mechanism of the primary pulvinus, the present author studied especially the change of tannin vacuoles and other cellular elements on receiving stimulus (1953, 1954a). But in the previous paper (1955), it was confirmed that the presence of tannin vacuoles in the motor cells is dispensable in the mechanism of seismonasty. And then, the finding of the migration of salts in this work, especially that of potassium in and out of the motor cell, would contribute much toward the elucidation of this problem cytophysiologically. It is the

¹ For further references on urethane upon the cell, readers are referred to Cornman (1954).

² For further references on the physiology of *Mimosa* pulvinus, readers are referred to Weintraub (1952) and Toriyama (1953).

present author's view (exp. 3, 4 and 5) that the permeation of the potassium through the protoplasmic membrane is probable. Similar observations with the *Mimosa* petiole have been reported briefly by the present author (1954b).

As Aimi (1947) and the author (1953) have reported that the osmotic concentration of the motor cell of *Mimosa* is 0.7 M KCl. This fact means that a thick potassium solution is in the central vacuole of the motor cell. Probably, at the bending movement, the potassium solution may permeate rapidly through the protoplasmic membrane more than in the form of the potassium ions. Weidlich (1930) explained that the exuded liquid is relatively pure water, and that the contraction is not a result of changes of in permeability which would enable the whole cell sap to flow out. But, there is seem to be no evidence to support this assumption. It is the present author's opinion that the recovery of the bending movement may be brought about by the recovery of turgor which is caused by the permeation of the potassium solution into the motor cells. During the recovery process, which takes a considerably longer time than the initial response, the cells in the lower part of the pulvinus apparently reabsorb liquid and regain the normal turgor. The potassium migration was especially well observed in the motor tissue of the under side of the pulvinus. Figure 25 is the schema which is summerized from the data from the experiment concerning potassium migration. The literature indicates a similar discovery of the movement of potassium in the guard cell of *Zeblina* etc. has been carried out by Imamura (1943). The study of migration of salts in the motor cells in the leaves of Carnivore, and in the motive zone (Ashida 1934) of the leaves of *Aldrovanda* is to be the subject of further research. The present author described already in the preceeding part, as shown in Fig. 17, the migration of potassium to the transitional tissue (Figs. 21 to 24). When this preparation is compared with that of the control it may be realized that it is not an artificial image. Perhaps, the transitional tissue to the stem or to the petiole may be the storage places for the potassium solution which shift from the large intercellular spaces of the motor tissue. Thus, the migration of potassium solution can be summarized as follows: Motor cells → intercellular spaces → transitional tissue. A still more interesting fact is that the protoplasmic connections between the neighboring cells are seen in the transitional tissue of the motor organ. The detailed nature or these protoplasmic connections are expected to be the subject of future research.

Fig. 25. Schematic figure of primary pulvinus of *Mimosa pudica*. Arrows indicate the direction of migration of potassium solution. Greyed aear shows zone intercellular spaces.

Summary

The available information concerning the effect of the anesthetics, and migration of potassium in the primary pulvinus of *Mimosa pudica* may be summarized as follows.

1) Ether and urethane may be of no efficacy on the salts distribution of the living cells. And it may be deduced that these narcotics give no remarkable visible effect upon the protoplast of the motor cells.

2) 0.2 M concentration of ethyl urethane causes the reversible anesthetic effects in the motor organ.

3) The plants were anesthetized in ether vapour or by absorbing the urethane solution through the stem. In both materials there are no differences in the sponogram.

4) When the ash image of two conditions, before and after receiving stimuli, are examined, the migration of salts from the motor tissue-cells to the phloem is clearly observed.

5) Parallel to the microincineration, the micro chemical technique for potassium were undertaken. The materials before receiving stimuli are subjected to Macallum's modified solution. By this technique crystals of potassium salts are found in the motor cells. After receiving stimuli, large crystals appear in the large intercellular spaces. From this data it can be supposed that the potassium solution in the motor cells permeates into intercellular spaces at the bending movement. This phenomenon suggests that the permeability of the cytoplasm against potassium increased at the bending movement and its recovery.

6) On the transitional zone, between the pulvinus and the stem and between the pulvinus and the petiole, the large parenchymatous cells and intercellular spaces are arranged lengthwise. In this transitional tissue, potassium solution passes through at the bending movement.

7) The migration of potassium solution may be summarized as follows ; Motor cell → intercellular spaces → transitional tissues.

Acknowledgements

The present study was made under the direction of Professor Sirô Tarao, to whom thanks are due for his help and his criticism during this work. This research has been supported in part by a grant in aid from the Fund of Ministry of Education for Development of Scientific Research.

Literature cited

- Aimi, R. 1947. Bot. Mag. Tokyo 60: 95-96.
Ashida, J. 1934. Mem. coll. Sci. Kyoto Imp. Ser. B. 9: 141-244.
Blackman, V. H. and Paine, S. G. 1918. Ann. Bot. 32: 69-85.
Cornman, I. 1954. Internat. Rev. Cyto. III: 113-130.
Gomori, G. 1952. Microscopic histochemistry. Chicago.

- Imamura, S. 1943. Jap. Jour. Bot. 12: 351-564.
- Pfeffer, W. 1874. Jahrb. wiss. Bot. 9: 308-326.
- 1877. Osmotische Untersuchungen. Leipzig.
- Policard, A. and Okkels, H. 1930. Anat. Rec., 44: 349-361.
- Ricca, U. 1916. Archiv. ital. Biol. 65: 219-232.
- Scheer, B. T. 1953. General physiology. New York, London.
- Sen, B. 1923. Proc. Roy. Soc. (London) B 94: 26-31.
- Sledmanni, ECG. 1931. Biochem. J. 25: 1147.
- Szent-Györgyi, A. 1951. Chemistry of muscular contraction. New York.
- 1954. Physiol. Scand. 9, Suppl. 25, 1-115.
- Toriyama, H. 1953. Cytologia, 19: 29-40.
- 1954a. Ibid. 19: 283-292.
- 1954b. Bot. Mag. Tokyo 67: 104.
- 1955. Ibid. 68: 203-208.
- Usami, S. 1937. Acta. Phytochemica 9: 287-297.
- Wallace, R. H. 1931. Amer. Jour. Bot. 18: 215-235.
- Warburg, O. 1910. Z. physiol. Chem. 66: 305.
- 1911. Ibid. 70: 413.
- Weidlich, H. 1930. Bot. Archiv. 28: 219-254.
- Weintraub, M. 1952. New phytol. 50: 357-382.
-