Observational and Experimental Studies of Sensitive Plants

III. On the epidermal system of the pulvinus and leaflet¹

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Only a few investigations have been made concerning the epidermal system of sensitive plants and nyctinastic Leguminous plants. In the present study some new findings of the hydathodes and the stomata are reported as below. These findings on the morphology of the above organs are significant with respect to the mechanism of the motion of these plants.

Material and method

Mimosa pudica L. formed the material for most of the present experiments. Some observations were also performed on other species, namely *Albizia Julibrissin* Durazz, and *Cassia mimosoides* L.. These plants were brought up in pots exposed to the open air in the field. The anthocyan was found to be formed in the epidermis cells of the pulvinus, the petiole and the stem of *Mimosa* which had been brought up as indicated above. For the practical method of observations on the epidermal system, the author used the "Sump method"² and the ultropaque microscope.

The technique of fixing and staining of materials employed in the present study was essentially the same as described in the foregoing paper (1954a), i.e. Kaiser's solution-toluidin blue method. The material in the present paper consisted chiefly those of plants which had already received a stimulus.

Observation

a. Primary pulvinus of *Mimosa*

The transitional zone between the stem and the primary pulvinus is distinctly observed with the naked eye. There is a wide difference in anatomical structure between the stem and the pulvinus as shown in the report of Sibuya (1936) *et al.* The epidermis of the stem consists of flat cells which are arranged lengthwise.

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² "Sump method" is a technique to get a negative image of the surface of a body by covering the film of a solution of celluloid. After drying up the solute, the amyl acetate, a solid celluloid film upon which the negative image is impressed can be obtained.

Further, the cylindrical primary pulvinus is covered with the epidermal cells arranged in circular and contain anthocyan in their upper half. The primary pulvinus transfers into the petiole gradually, but on careful examinations it can be seen that their transitional zone will be found inflated a little. The anthocyan is not formed in the epidermis of this zone¹ (Fig. 1 *tr*). On the upper half of the epidermis, many hydathodes are found evenly distributed.

Figs. 1-3. 1. Side view of primary pulvinus of *Mimosa*. *tr*, transitional zone. 2. Surface view of hydathode on the upper half of the primary pulvinus. $\times 800$. 3. Tannin issued into canaliculated intercellular spaces which are in the middle part of the upper side of the motor tissue, tannin being indicated by arrows. *a*1, *a*2, apertures of hydathodes. $\times 320$.

The author has found every aperture of hydathode to be bounded by several guard cells. The vertical view of the structure of these hydathodes on the primary pulvinus is indicated in Figs. 4 to 6. In the fixed material, cells with a considerable amount of cytoplasm at their cell peripheries are seen underlining the epidermis (Fig. 4). In two or three cells especially which are situated just under the aperture of the hydathode, the cytoplasm seems to be dense in the appearance after fixation with Kaiser's solution. These cells may therefore be called 'internal guard cells' (Fig. 4 *ig*). It is noteworthy that the tannin substance from the parenchymatous motor cells is discharged into the canaliculated intercellular spaces (Fig. 3 arrows), which lead to the hydathodes as narrow canalicules (Fig. 3 *a*₁, *a*₂). As shown in Fig. 3 the

¹ This zone is called 'transitional zone' or '*tr*-zone' in this paper.

tannin is differentiated by staining with toluidin blue in the fixed material. And the issued substance containing tannin is observed most frequently in the middle part of the upper surface of the pulvinus, *i.e.* on the upper side of the motor tissue. On the other hand, another type of hydathode from which a non-tannin substance¹ issued can be found (Fig. 5). These two kinds of hydathodes are usually situated on the tops of the projected parts of the creases of the motor tissue (Fig. 3). At the bottom of the creases, however,

Figs. 4-7. 4-6. Three types of hydathodes on the upper surface of primary pulvinus. *ig*, internal guard cell; *ts*, issued tannin; *s*, issued substance; *se*, special epidermal cells. $\times 540$. 7. The hydathode on the lower surface of primary pulvinus. $\times 540$.

one more type of the hydathode is observed as shown in Fig. 6. The apparatus of this type consists of one or two cells which originate probably from the epidermis. These cells contain a considerable amount of protoplasm (Fig. 6 *se*). Outside of this hydathode, a certain substance which is indifferent to the toluidin blue is observed frequently adhering to the nearby epidermis. (Fig. 6 *s*).

On the surface of the upper half of primary pulvinus, these hydathodes are distributed 14 per mm^2 . On the contrary, considerably fewer hydathodes are counted on the lower half. Moreover, cells having a thick cytoplasmic layer at the periphery of the cell, and also having 'internal guard cells' seem to be lacking completely (Fig. 7). But the structure observed from the surface view is same as that of the hydathodes of the upper half.

¹ The chemical nature of this substance is unknown.

b. 'Transitional zone' of the primary pulvinus and the petiole

As mentioned above, the anthocyan is not formed in the epidermis of this 'transitional zone'¹. The hydathodes described in the primary pulvinus are seen also in this zone, their surface view being shown in Fig. 8. Under the epidermis, and just under the hydathodes or adjacent to them,

Figs. 8-9. 8. Surface view of the hydathode on the upper half of 'transitional zone.' $\times 320$. 9. The lower surface of the petiole adjacent to 'tr-zone.' Tannin substance issuing from the apertures of hydathodes. Two stomatal apparatus are shown. $\times 320$.

Fig. 10. a. Vertical section of a hydathode on the upper side of 'tr-zone.' $\times 800$.
b. Schematic figure of a.

several cells are observed which have a thick cytoplasmic layer at the periphery of the cell. In the fixed material, the cytoplasm of these cells are markedly stained by the toluidin blue. It must be remarked here that the internal guard cells are also found, and that they surround a small cavity as

¹ The histological and physiological investigation on this zone shall be reported in another paper in near future.

shown in Fig. 10a and b. A certain substance¹ stainable with toluidin blue adheres to the epidermis adjacent to the hydathode (Fig. 10a). This indicates, presumably, that the substance is issued through the hydathode from

Fig. 11. The distribution of the hydathodes and the stomata on the 'tr-zone' and a part of the petiole. a. upper half. b. Lower half. $\bullet$ Solid symbols indicate the hydathodes and open symbols indicate the stomata.

the large cells underneath the hydathode. These interior large parenchymatous cells shown in Fig. 10a have thin cytoplasm at their periphery and contain small tannin vacuoles within.

On the upper half surface of 'tr-zone' an average number of 13 hydathodes per mm² is counted (Fig. 11a), on the lower half surface a fewer of apertures are distributed (Fig. 11b). Their structure on the both sides is

almost the same. Observations on the adjacent epidermis of the petiole, especially on the part of the petiole to the pulvinus show that on the upper half, the hydathodes and the stomata are distributed as shown in Fig. 11a, and that the lower half of this part also contains them, but the hydathodes are slightly less than the stomata (Fig. 11b). It deserves to be mentioned that the substance which contains tannin is issued from the apertures of hydathodes. Such a substance, having a yellowish colour, can be seen adhering to the surface of the negative print of the celluloid film as shown in Fig. 9.

c. Secondary pulvinus of *Mimosa*

The secondary pulvinus of *Mimosa* displays the 'sleep-posture' in the nocturnal condition, but does not show the seismonastic movement. In this point, this pulvinus seems to correspond to the pulvinus of the primary leaf of *Phaseolus*. In the epidermal system of this pulvinus, the hydathodes and the stomata were newly discovered as detailed below.

The surface view of the epidermal apparatus is shown in Fig. 12. These apparatus are distributed only on the surface of the upper half, but on the lower half seeming to contain none, especially on the petiole, and more especially on the part adjacent to the secondary pulvinus (Fig. 15). The stomata and the hydathodes are counted ca. 36 per mm². Furthermore, the

¹ The chemical nature of this substance is not exact, but it contains perhaps tannin.

vertical section of these hydathodes reveals quite a different structure as follows. One of the epidermal cells protruds a little from the level of the surface (Fig. 14 *hc*), and two or three specially open symbols indicate the stoma differentiated cells follow in a row vertically to the surface. These cells have very thin cytoplasm at the periphery of the cell, and do not contain tannin vacuoles at all. But the surrounding parenchymatous cells are larger than these special cells, and contain much cytoplasm and small tannin vacuoles.

Figs. 12-15. 12. Surface view of a hydathode on the secondary pulvinus. $\times 540$. 13. The stomata and the hydathodes on the petiole adjacent to the secondary pulvinus. $\times 540$. 14. Vertical structure of a hydathode on the secondary pulvinus. *hc*, hydathodal cell. $\times 540$. 15. The distribution of the hydathodes and the stomata on the secondary pulvinus. Solid symbols indicate the hydathodes.

d. Tertiary pulvinules of *Mimosa*

Tertiary pulvinules are employed as the hinges of leaflets during seismonastic movement in the diurnal condition, and also during nyctinastic movement. A pair of the pulvinules are observed from the surface as shown in Fig. 16. The epidermis of the pulvinule consists of flat cells having the cuticle on their surface. As shown in Fig. 18, the stomata are seen densely scattered in this area. But on the lower surface of the pulvinule the stoma does not exist. Each stomatal apparatus on the upper surface is composed of two guard cells (*g*), and two subsidiary cells (*su*) which contain anthocyan as shown in Fig. 17. In addition, the neighbouring cells of the subsidiary cells occasionally contain anthocyan too. In the vertical section, the guard cells and the subsidiary cells are observed protruding somewhat from the surface of the epidermis. The comparison between the stomatal structures in the opened condition and in the closed condition of the pul-

vinule are shown in Figs. 19 and 20. Directly under these attached cells lack the intercellular air-chamber which is generally present without an exception as the respiratory cavity of the ordinary stomata. These stomata are underlined with cells which have thin cytoplasm at their periphery. By use of the "Sump method" and the ultropaque microscope, these stomata are observed to be in the closed condition irrespective of the closing and the

Figs. 16-18. 16. A pair of leaflets of *Mimosa*. 17. Surface view of a stomatal apparatus on the tertiary pulvinule. *su*, subsidiary cell which contains anthocyan; *g*, guard cell. $\times 800$. 18. Surface view of a tertiary pulvinule by the "Sump method." $\times 80$.

Figs. 19-20. 19. Vertical section of a stomatal apparatus on the pulvinule in opened condition. *su*, subsidiary cell; *g*, guard cell. $\times 800$. 20. The same apparatus on the pulvinule in closed condition. $\times 800$.

opening of the tertiary pulvinules. It is difficult to tear off the epidermis from the mesophyll, therefore, it is difficult to observe the epidermal system cytophysiologically.

e. The leaflet of *Mimosa*

It is a well-known fact that the leaflets close by seismonastic stimulation

and this too, during the nocturnal condition. In the latter case, one used to call it the "sleep-posture". The question whether the stomata of the leaflets are opening or are closing according to their condition remains still quite unknown. The author made an attempt with some experiments on this question. Though much investigation has been made the precise results are not obtained. Among these experiments the morphological and micro-chemical ones are reported as follow.

On the upper and lower surface of the leaflets, the stomatal apparatus are composed both of the small guard cells (*g*), and the latter are again bounded by two large subsidiary cells (*su*) as shown in Fig. 22. Tannin reaction by chromic acid and other reagents appears in the subsidiary cells. The guard cells have no tannin, but they strongly show the reaction to the acetic silver nitrate solution.¹ Beside the guard cells and the subsidiary cells, some other epidermis cells also contain tannin as shown in Fig. 23.

Together with the histochemical studies, cytophysiological studies are made on the components of the epidermis. The epidermis torn off from the mesophyll, is soaked in the distilled water, 0.5 mol and 1 mol KCl solution for 30 to 40 minutes. The condition of the stoma, opening or closing, and the plasmolysis is brought about or not, are summarized in Table 1.

Table 1. + indicates the plasmolysis

		Distilled water	0.5 mol KCl	1 mol KCl
Upper epidermis	Epidermal cells with tannin	—	—	±
	Epidermal cells without tannin	—	+	†
	Subsidiary cells	—	—	±
	Guard cells (Stomata)	(open)	(open)	(closed)
Lower epidermis	Epidermal cells with tannin	—	—	±
	Epidermal cells without tannin	—	+	†
	Subsidiary cells	—	—	±
	Guard cells (Stomata)	(open)	(open)	(closed)

The epidermal cells without tannin are plasmolyzed in 0.5 and 1 mol KCl solutions, while the epidermal cells with tannin are not plasmolyzed before they are soaked in 1 mol KCl solution. This is a marked difference between these epidermal cells. The guard cells, on the other hand, are not plasmolyzed even in 1 mol KCl solution. The stoma opens in the distilled water and 0.5 mol KCl, but does not open in 1 mol solution. The opening of the stoma is shown in Fig. 22. When the epidermis is soaked in 1 mol KCl solution incipient plasmolysis first appears in the subsidiary cells. According to the

¹ The positive reaction to this reagent may indicate the possibility of the presence of ascorbic acid.

results of the above experiment, it may be said that the osmotic concentration of the contents of the subsidiary cell is equivalent to that of 1 mol KCl solution.

f. The epidermis of the pulvinus of some nyctinastic
Leguminous plants

The pinnate leaf of *Cassia mimosoides* and *Albizia Julibrissin* corresponds to the leaflet of *Mimosa pudica*. These plants do not display the seismonastic movement, but their leaflets close in the nocturnal period.

Figs. 21-25. 21. Surface view of a stomatal apparatus treated with chromic acid. The tannin reaction does not appear in the guard cells. $\times 480$. 22. The opening of a stoma on the leaflet. *g*, guard cell; *su*, subsidiary cell. $\times 600$. 23. The subsidiary cells and some epidermal cells which contain tannin on the leaflet of *Mimosa*. Tannin substance is shown by dots. $\times 500$. 24. Stomatal apparatus of *Cassia Mimosoides*. $\times 480$. 25. Surface view of the pulvinule of *Cassia Mimosoides*. $\times 100$.

In the nocturnal period the leaflets close at the hinge-joints which are called pulvinule. A brief report of the observation on the surface of these pulvinules is indicated below. On the upper surface of the pulvinules of *Cassia mimosoides*, the stomatal apparatus (Fig. 24) are many in number and distributed evenly as shown in Fig. 25. The distribution is dense towards the upper surface of the leaflets. On the lower surface, however, they are not found at all. As for *Albizia Julibrissin* the distribution and the features of the stomatal apparatus follow almost the same pattern of *Mimosa* and *Cassia mimosoides*. This coincidence engenders some interest from the view-point of comparative morphology.

Discussion

To analyse the mechanism of the bending movement has attracted the wide attention of botanists for centuries.¹ The present author has attempted microchemical and cytophysiological studies for the past few years (1953, 1954a). In these investigations, the structural alteration in the motor tissue is particularly discussed. The epidermal apparatus, namely the stomata and the hydathodes, ought to have close relation to the metabolism of the motor tissue, and therefore it is by no means useless to observe the epidermal system to analyse the mechanism of the bending movement. Very few investigations on the epidermis of primary pulvinus have been known, and it may be the new finding that this report indicates new findings when it shows the presence of two or three types of hydathodes. It is also worth mentioning that internal guard cells are situated directly under the hydathodes. These internal guard cells have a rich protoplasm which is fixed very well by Kaiser's solution, so that they can be distinguishable from the parenchymatous motor cells. As for the function of these cells, further experiments must be carried.

The present author was able to discover the canaliculated intercellular spaces, in which the tannin solution flows from the surrounding tissue (Fig. 3). The tannin solution issuing into these canaliculated spaces is supposed to come from the lower side of the motor tissue consequent upon the contraction. This tannin solution penetrates the parenchymatous cells to the hydathode of the upper epidermis. Such a phenomenon has never been observed in other plants including *Mimosa*. Substances of other natures are also issued from the aperture (Fig. 5 s); the chemical nature of which is not detailed here. Furthermore, the question can be raised whether the tannin and other substances are issued or not before the bending movement. The investigation of these points must be the subject for future research.

The histological and cytophysiological data of the transitional zone shall be reported by present author in the near future.² The author has observed that many hydathodes are also scattered on the whole epidermis of this zone. The function of these hydathodes is still unknown.

In the next place, we shall have discussion about the epidermis of secondary pulvinus and the petiole. As for the leguminous plant in general, the morphological investigations of the epidermal system of the pulvinus have not

¹ A more extensive history of this research will be found in the investigation by Weintraub (1952).

² The fluid containing potassium and other elements is transferred into this zone at the bending movement. The details shall be reported in another paper in the near future.

yet been described systematically.¹ But Brauner (1932) briefly observed the hydathodes on the pulvinus of *Phaseolus multiflorus*; and the same apparatus and the stomata on the pulvinus of *Ph. vulgaris* were investigated by Arslan (1949). Concerning *Mimosa pudica*, the hydathode and the stomata of the pulvinus have not been investigated before the present work. As shown in Fig. 15, the distribution of the epidermal apparatus and the stomata on the surface of the pulvinus and of the adjacent area raises interest. The former apparatus especially may be called the 'hydathode' from their structure (Fig. 14). But, there, the issued substances are not observed in these apparatus as they are in hydathodes of the primary pulvinus.

In the next place structure of the epidermal apparatus on the tertiary pulvinule must be discussed. Schwendener (1897) wrote about the anatomy of the pulvinus of *Mimosa pudica*, in which, observations on the epidermis of tertiary pulvinules were reported. He observed the stomata on the surface of tertiary pulvinules, but his observation was not perfect. He did not describe the distribution of these stomata and their function. Strange to say, since that time, these stomata have failed to call the botanists' attention. The present author cannot overlook these apparatus in connection with the physiology of the motion of the plants. This apparatus should not simply be called 'stoma'. Because there is no intercellular air-chamber or respiratory cavity right under the guard cells and the subsidiary cells like the ordinary stoma. Furthermore, there are special cells which have thin cytoplasm inside the cell membranes underlined these stomatal apparatus. These cells are supposed to be pressed by the motor cells when the closed condition of tertiary pulvinule is brought about. When these facts are taken into consideration, these epidermal apparatus may be called the hydathode. On the other hand, the surface view of these apparatus would make us identify them as stoma in general. So they may be called stoma at the same time. Perhaps these apparatus are in reality, employed as the hydathode and as the stoma.

In the pulvinules of *Cassia mimosoides* and *Albizia Julibrissin* the apparatus are distributed in a way similar to the distribution of the stoma in *Mimosa pudica*. It is desirable, in future, to make clear if the stomata open or close in both diurnal and in nocturnal condition.

Lastly, the experiments of the epidermal system of the leaflets may be discussed. On the lower surface of leaflets, the stomata are distributed 280 per mm², and also are scattered numerously on the upper surface. The stomatal apparatus is composed of a small guard cell and bounded subsidiary cells which contain tannin, and some other epidermal cells which contain tannin too. The osmotic concentration of the contents of these tannin cells is almost equivalent to that of 1 mol K Cl. While the osmotic concentration of non-

¹ The investigations about the epidermal system of the leaf were reported by Solereder (1908) and Metcalf & Chlark (1950) *et. al.* But epidermal systems of the pulvinus were not described there.

tannin epidermal cells is less than 0.5 mol KCl. This means that the osmotic concentration of the epidermal cells varies according to the different areas. Harmorak (1915) observed the tannin substance in the guard cells of the stomata in *Polygonum sachalinense*, *Oxyria digyna*, *Rheum officinale* and many other plants. On the other Metzner (1930) found that the tannin contained in guard cells and epidermal cells absorbs the long wave part of ultra-violet rays. Perhaps this phenomenon may occur in the epidermal system of *Mimosa* too. The investigation on the relation of this phenomenon to the opening of the stomata offers a subject of further research.

Summary

By means of the "Sump method" and of the ultrapaque microscope, the observations of the epidermal system of the pulvinus and the leaflets were investigated in *Mimosa pudica* L. and other nyctinastic Leguminous plants. Materials which had received a stimulus were mainly observed. The results of our observations and experiments are summarized as follows:

1) On the upper epidermis of the primary pulvinus the hydathodes are distributed 14 per mm². These apertures are bounded by several guard cells. In vertical view, these hydathodes may be roughly classified into two or three categories. In one type of these hydathodes, internal guard cells are situated under the aperture which leads to the canaliculated intercellular spaces. These canaliculated spaces contain tannin solution which is stained by toluidin blue. Other types of hydathodes have no connection with the canaliculated spaces. On the lower surface, the hydathodes are distributed 12 per mm². The vertical structure is different from that of the hydathode of the upper side, but, the surface view is the same in both.

2) The primary pulvinus transfers over to the petiole gradually, this transitional zone being inflated a little. The anthocyan is not formed in the epidermis of this zone which may be called 'transitional zone'. The hydathodes are many in number and are scattered on the surface of this zone. Two or three internal guard cells are situated just under the aperture. A considerable amount of the cytoplasm exists at the periphery of these guard cells.

3) A substance which contains tannin issues from the hydathodes on the lower surface of the petiole adjacent to the 'transitional zone'.

4) On the upper surface of the secondary pulvinus, the hydathodes are distributed 11 per mm². Furthermore, the stomata and the hydathodes are many in number and are scattered on the petiole, especially on the part adjacent to the secondary pulvinus.

5) The stomata are scattered on the upper surface of the tertiary pulvinules, but on the lower surface the stomata are not found. The guard cells and the subsidiary cells are somewhat protruded from the surface of the

epidermis. Perhaps these epidermal apparatus are employed as the stomata and also as the hydathodes too.

6) On the surface of the pulvinules of *Cassia mimosoides*, and *Albizia Julibrissin*, the stomatal apparatus are scattered as in *Mimosa pudica*. But on the lower surface the stomatal apparatus are not found.

7) The stomatal apparatus on the leaflets of *Mimosa* are composed of small guard cells, and the latter again bounded by two large subsidiary cells. The reaction resulting to acetic silver nitrate solution appeared in the guard cells but the tannin reaction did not. Whereas the subsidiary cells and some other epidermal cells contain tannin. According to the kind of these epidermal cells the osmotic concentrations are in various ways.

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