Observational and Experimental Studies
of Sensitive Plants
XIV. On the changes in a new cellular element
of *Mimosa pudica* in diurnal and nocturnal
conditions¹

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Introduction

In the previous papers, the cytophysiological changes in the lacunal parenchyma of the petiole of *Mimosa pudica* in both the diurnal and nocturnal conditions were described (Toriyama 1959, 1960). That is to say, the thread-like apparatus has a tendency to assume bead-like features during the day. On the other hand, at night the thread appears uniformly in a thin slender form. Furthermore, it is concluded that the expansion and contraction of the tannin vacuole is connected with diurnal and nocturnal conditions. In the present investigation, records of observation on the changes in the carotenoid-body² of *Mimosa pudica* in both the diurnal and nocturnal conditions are dealt with.

The material used in the present study is *Mimosa pudica* and was cultivated in the same manner as has been described in the previous papers (*l.c.*). In comparison with this material, the tissue-cells of *Desmodium gyrans*, *Wistaria floribunda* and *Robinia pseudo-Acacia* were also employed. Thus the object of making sure of the presence of a new cellular element in the parenchyma has been attained. The material consisted chiefly of those plants which had already received a stimulus.

Terminology

The present investigation is concerned chiefly with a new cellular element which is changed in both diurnal and nocturnal conditions. It is the question whether the cellular elements is the plastid or one of the metaplasmic elements. Then, if the new cellular element displays the Carr-Price reaction as investigated in the result of experimentations, the positive reaction to it in the cytochemical reaction indicates that the cellular element may contain a certain substance of carotenoids. Therefore, the new cellular element is called caro-

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² see Terminology.

tenoid-body (C-body) in this paper.

Experimental results

a) Results through a new technique of fixing and staining

Experiment 1: For the fixation of the motor tissue-cells, the author employed 10 per cent neutral formalin (commercial formalin neutralized with calcium carbonate, 1 part: distilled water, 5 parts) for at least 96 hours. After completion of the fixation the tissue was thoroughly washed in running water for 10 hours to remove any trace of the reagents. These materials were cut 20 to 50 μ in thickness by means of a hand microtome. The sections were washed in several changes of distilled water. Thereafter the sections were mounted in pure glycerin, without any subsequent staining. This technique is satisfactory in demonstrating the C-body and in fixing the chloroplasts and tannin vacuoles.

The results are illustrated in Figs. 1 and 3. In the adult motor tissue-

Fig. 1 a, b, c. Carotenoid-bodies in motor cells of main pulvinus of *Mimosa pudica*. ch, chloroplast; ta, tannin vacuole; n, nucleus; arrows indicate carotenoid-bodies. $\times 1000$.

cells of the main pulvinus, small granules are fixed in a spherical form which are 2 to 7 μ in diameter, and in the young pulvinus it may be a very small granule in the cytoplasm (Figs. 4, 5), demonstrable too by fixing with neutral formalin. These small granules appear greenish-brown in color.

As shown in Fig. 6, in the features of transverse leaf section of this plant apparently, greenish-brown colored granules are distributed too. Sometimes the C-bodies are attached to the chloroplasts, and sometimes they are developed to such an extent as to form a large globular body. In very young mesophyll of the leaf, it may be very small granules in the cytoplasm demonstrable too by the same technique (Figs. 2, 7, 8).

Experiment 2: The materials were fixed with 10 per cent neutral formalin for 96 hours. After completion of fixation they were thoroughly washed in running water for 10 hours to remove any trace of the fixing reagents. These materials were then cut 20 to 50 μ in thickness by means of a hand microtome as usual. These sections were stained with 0.05 per cent brilliant cresyle blue aqueous solution. The staining time was determined only from experience. For the present materials one hour staining in the

dye solution is quite enough to stain the C-body in the tissue-cells of *Mimosa*. Thereafter, the sections were washed in several changes of distilled water. The sections were mounted in pure glycerin. This technique gave not only

Fig. 2. Transverse section of young leaflet of *Mimosa pudica*. Arrows indicate carotenoid bodies. ep, epidermis; pp, palisade parenchymatous cell; sp, spongy parenchymatous cell. $\times 500$.

excellent results in demonstrating the C-body, but also was outstanding for the fixation of the nucleus, chloroplasts and tannin vacuoles. Generally speaking, brilliant cresyl blue usually stains the C-body a greenish-blue color, and the tannin vacuole yellowish-green, while the chloroplasts and nucleus remain colorless. Staining the tissue-cells with brilliant cresyl blue demonstrates all stages of the C-body formation.

Experiment 3: Both materials, in diurnal and nocturnal conditions, were fixed with 10 per cent neutral formalin to detect the C-body, and were stained with brilliant cresyl blue.

In the diurnal condition (10 a.m.—3 p.m.), C-bodies of motor tissue-cells are fixed in a spherical form 6 to 7 μ in diameter (Fig. 9), and in the nocturnal condition (12 midnight—2 a.m.) they appear in a crushed state (Fig. 10). Brilliant cresyl blue, when applied for the same duration, stains the C-bodies in the diurnal condition more than that in the nocturnal condition in a strong blue tone. The tannin vacuole has a tendency to assume a globe-like shape in the diurnal period. On the other hand, in the nocturnal period, the tannin vacuole appears as crushed spheres. The same phenomenon is observable in the C-body of the lacunar parenchyma of the petiole (Figs. 11, 12).

b) Chemical treatments of the carotenoid-body

Experiment 4: A series of experiments to know the effects of ethanol upon the C-body have been carried out using materials similar to those in the foregoing experiments. Among these experiments it is noteworthy to mention the effects of ethanol upon the C-body which were previously fixed with the 10 per cent neutral formalin. After remaining for 96 hours in this fixative the material was thoroughly washed in running water and then immersed in a series of ethanol solutions. The C-bodies gradually dissolve in this organic solvent, finally being attenuated until no trace of them remained.

Figs. 3-12. Carotenoid-body in the tissue-cell of *Mimosa pudica*. Arrows indicate carotenoid-bodies. 3, adult motor tissue-cell of main pulvinus. $\times 750$. 4, 5, young motor cells of main pulvinus. $\times 1200$. 6, adult mesophyll of leaflet. $\times 1000$. 7, 8, young cells of mesophyll. $\times 1200$. The above six sections fixed with 10% neutral formalin without staining. 9, motor cell in diurnal condition; ta, tannin vacuole. 10, motor cell in nocturnal condition. $\times 1000$. 11, lacunal parenchymatous cell of petiole in diurnal condition. 12, lacunal parenchymatous cells of petiole in nocturnal condition. $\times 1000$. The above four sections fixed with 10% neutral formalin and staining with brilliant cresyl blue.

Figs. 13-24. 13-20, Carotenoid-body in the tissue-cells of *Mimosa pudica*. Arrows indicate carotenoid-bodies. 13, carotenoid-body of motor cell fixed with 10% neutral formalin. 14, 15, the same cell of Fig. 13 in 92% ethanol for 5 to 10 min. $\times 800$. 16, carotenoid-body of lacunal parenchymatous cell of petiole fixed with 10% neutral formalin. 17, the same cell of Fig. 16 in 92% ethanol for 10 min. $\times 1000$. 18, motor cell treated with iodine. $\times 1000$. 19, lacunal parenchymatous cell of petiole treated with Carr-Price's cytochemical test. $\times 1000$, 20, lacunal parenchymatous cell of petiole under phase contrast microscope. $\times 1000$. 21, motor tissue-cells of pulvinule of *Desmodium gyrans*. $\times 1000$. 22, motor cell of main pulvinus of *D. gyrans*. $\times 1000$ 23, motor tissue-cells of pulvinule of *Wistaria floribunda*. $\times 1000$. The above three sections fixed with 10% neutral formalin without staining. 24, a palisade parenchymatous cell of *Robinia pseudo-Acacia* of fresh material in dilute Knop's solution. $\times 1500$.

The color of the C-bodies faded gradually when the dissolution of the cellular elements proceeded. At the place from where the C-bodies disappeared entirely some other cellular elements vanished in the tissue-cells of the main pulvinus and of the petiole (Figs. 13, 14, 15, 16, 17). The speed of this dissolution in the ethonol is shown in the following table (Table 1). It is quite clear that these phenomena are due to the effects of the ethanol.

Table 1

Time in min. %							
	3	5	10	15	20	25	30
100	±	+	+	+	+	+	+
92	±	±	+	+	+	+	+
70	—	—	—	—	—	—	—
50	—	—	—	—	—	—	—

+ indicates dissolution.

Experiment 5 The tissue-cells were fixed with 10 per cent neutral formalin as usual. They were then dehydrated in an ethanol series and tertiary butyl alcohol and finally imbedded in paraffin. The sections were cut transversely at a thickness of 10μ and were stained with brilliant cresyl blue aqueous solution. In this case, the C-bodies are not observable. It is probable that the C-bodies are dissolved due to the ethanol series for the dehydration.

Experiment 6 : Two microchemical methods were attempted on the tissue-cells to see their cytochemical reactions. Further details shall be described in the following paragraph.

The sections were treated with an iodine solution¹. Under this reagent, several granules revealed a positive reaction to the starch test, and the C-bodies appear an indigo-brown color indicated by the arrow (Fig. 18). It is worth mentioning that the C-body is larger than the starch granules in the chloroplasts.

Next, the sections from fresh material were soaked in a solution of Carr-Price reagent². Under this treatment, the new cellular element (carotenoid-body) revealed a bluish-violet color (Fig. 19). This reaction shows that the cellular element contains a carotenoid.

c) Results through non-treated material

Experiment 7 : A fresh main pulvinus or petiole was cut 30 to 50μ in thickness by a hand microtome and by a razor, and then the sections were immersed in tap water. They were freshly prepared at the beginning of each observation. Observations were made under phase contrast optics in conjunc-

¹ It is convenient to prepare iodine reagent after the following formula: iodine 1; potassium iodide, 7; distilled water, 100. (see L. Lison 1953).

² Carr-Price test with antimony chloride (see G. Bourne 1935, A.G.E. Peares 1953, L. Lison 1953 *et al*).

tion with a dark-medium contrast $100\times$ objective with a periplad $10\times$ ocular.

In one of the lacunal parenchymatous cells the C-body is located centrally and is connected to the tannin vacuole which are attached to the chloroplasts (Fig. 20). By this technique, the C-bodies assume a spherical shape. Generally speaking, the case with which the material may be observed in the fresh condition is demonstrated when the pale C-body is capable of being translocated. It is probable that the migration of the C-body is due to protoplasmic streaming. In general, the C-body is almost free from other cellular elements.

d) *Carotenoid-body in several other leguminous plants*

Experiment 8: The pinnate leaf of *Desmodium gyrans*, *Wistaria floribunda* and *Robinia pseudo-Acacia* corresponds to the leaflet of *Mimosa pudica*. These plants do not display the seismonastic movement, but their leaflets close in the nocturnal period. In this condition the pinnate leaf closes at hinge-joints which are called the pulvinule. A brief report of the observation on the C-body in the pulvinus is indicated below.

For the detection of C-body, the materials were fixed by means of the same technique as in the case of *Mimosa*. The results of the fixation proved to be excellent.

In the motor cells of the pulvinules of *Desmodium gyrans*, the C-bodies are distributed evenly as shown in Fig. 21. Furthermore, the C-body in the motor cell of the main pulvinus is observable (Fig. 22). As for *Wistaria floribunda* and *Robinia pseudo-Acacia* the distribution and the features of the C-bodies follow almost the same pattern as *Mimosa* and *Desmodium* (Fig. 23, *Wistaria*). The coincidence engenders some interest from the view-point of comparative cytology.

Next, the fresh leaf of *Robinia pseudo-Acacia* was cut by means of a hand razor and the sections were soaked in tap water or dilute Knop's solution. In this case, the C-body is more easily observed since it has a yellowish-pink color as shown in microphotographs (Fig. 24). In general, the C-bodies of several leguminous plants are observable in both the fixed and the fresh materials.

Discussion

Neutral formalin gave good results on the fixing of C-bodies in the tissue-cells of *Mimosa pudica*. Furthermore, the sections were at the same time stained with aqueous brilliant cresyl blue which was very convenient for differentiating the C-body. Through the same procedure, the original very small granule of a C-body appears clearly in young tissue-cells. That is to say, the cytoplasm of a young cell begins to have an extremely small body of carotenoid nature at a certain stage. Generally speaking, the C-body is imbedded in the cytoplasm through all the growing stages. As shown Fig. 1, the C-body is constructed of spherical shape, deformed to a disk-like or

other shape 2 to 7 μ in diameter.

It may be worth mentioning that unfavourable artificial changes in morphology and location of the C-bodies were never observed in the preparation after the author's technique, even when the treatment of ethanol advanced so excessively that the C-body was entirely dissolved.

In the tissue-cells of *Mimosa* the size of the C-body tends to decrease more in the nocturnal condition than in diurnal. It is probable that the carotenoid in the C-body decreases in quantity in the nocturnal condition. From the available data it is suggested that there occur different physiological conditions in the tissue-cells in diurnal and nocturnal condition. Concerning these problems, it is advisable to refer to several investigations (Toriyama 1954, 1959, 1960, Datta 1959 *et al*).

In the pulvinules and main pulvinus of *Desmodium gyrans*, *Wistaria floribunda* and *Robnia pseudo-Acacia* the C-bodies are distributed in a way similar to that of *Mimosa pudica*. It is desirable, in the future, to make clear if there are C-bodies in other leguminous plants in both diurnal and nocturnal condition.

The main obstacle of the chemical investigations so far has been the difficulty of obtaining a pure sample of the C-bodies. Banerji, Bhattacharya and Bose (1946) have investigated the biochemical nature of carotenoid in the pulvinus of *Mimosa pudica*. Beside Bose (1928) already in his classical work, pointed out the importance of this substance on the movement of the pulvinus. Critical comparison of the carotenoid and the present author's carotenoid-body must be the subject of future research. Recently, Dutt (1957) investigated the small cellular element which is regarded as a small vacuole in living motor cell of the *Mimosa*. The relationship between the author's carotenoid-body and Dutt's small vacuole or other cellular elements, if any, are worthwhile subjects for future investigation.

Summary

In the present investigation there is described the existence of a new cellular element of the tissue-cells of *Mimosa pudica* and that of several other leguminous plants.

1. Carotenoid-bodies in the main pulvinus, petiole and in the mesophyll of the leaf are chiefly dealt with.

2. Fixed with neutral formalin, the carotenoid-body is marked stained with brilliant cresyl blue. It must be remarked here that the carotenoid-body gradually developed in the cytoplasm of young tissue-cells.

3. Carotenoid-bodies are uniformly distributed throughout the tissue-cells of main pulvinus, petiole and of the mesophyll of the leaf.

4. By the author's method, carotenoid-bodies in the diurnal condition are observed to be more inflated than those in the nocturnal condition.

5. Carotenoid-bodies gradually dissolve in 70 to 100 per cent ethanol,

and attenuate, leaving no trace.

6. By treatment in iodine solution, the carotenoid-body appears indigo-brown in color.

7. The carotenoid-body proved reacted positively to Carr-Price reagent, which is probably closely related to its photo-physiological function.

8. The carotenoid-bodies in the fresh material when observed clearly with a phase contrast microscope, are spherical in shape.

9. In the tissue-cells of the pulvinules of *Desmodium gyrans*, *Wistaria floribunda* and *Robinia pseudo-Acacia*, the carotenoid-bodies are distributed as in the *Mimosa*.

10. Judging from the data obtained by these observations, it is concluded that the carotenoid-body is one of the cellular elements, and is by no means a mere artifact.

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