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Polar Vital Staining and Differential Plasmoptysis of *Cladophora* Cells

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With 1 Figure

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It was reported by Prat 1931 a, b and 1932, that the intact *Cladophora* cells were stained differentially, i. e. most remarkably at the apical part. The writer also observed that in some *Cladophora* the vital staining with neutral red, safranin, etc. began to occur at the apex and the polar staining there appeared spread towards the base until the whole was stained uniformly (Nakazawa 1953). The nature of this polar staining is different from the phenomenon that P^{32} or S^{35} are partially accumulated at the base when isolated *Cladophora* cell is immersed in a solution containing those radioactive substances (Taylor 1953, Schoser 1956). That is, the latter does appear one or two days after the material is immersed in the medium, while the polar staining appears instantly after the immersion in the staining medium and that the staining differential gradually diminishes in time to become stained uniformly. The difference indicates that the polar accumulation of the radioactive substance is attributed to the directed transportation of the substance after its permeation into the protoplasm, while the polar staining is due to the partial difference of the permeability of the dye so that it spreads to stain uniformly with the lapse of time. That is to say, it seems that there is a permeability differential for dyes along the longitudinal axis of the *Cladophora* thallus.

This time, the present writer discovered that the staining differential could be attributed to the partial difference of permeability of the cell wall, but not of the plasma membrane, which shall be described here.

In April, 1957, *Cladophora utriculosa* and *C. refracta* were collected at a reef near the Marine Biological Station of Asamushi, Aomori Prefecture, Japan. They were reared in filtered sea water, pH 8.2, for 24 hours at room temperature, about 15°C. Branchlets, about 1 cm from the tip, were cut off, cells were isolated from these with a sharp knife. The isolated cells were immersed in 0.001 per cent neutral red-sea water contained in a Petri dish of 3 cm in diameter, and were observed by use of microscope. To reveal the presumed partial difference of resistence of the cell wall against the turgor pressure in connection with the polar staining, the cells were soaked in distilled water to induce the plasmoptysis. As the control, intact branchlets were also tested likewise.

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In the intact branchlet, staining began from the tip of the apical cell and it spread towards the base, and later the subapical cells were all stained uniformly (Fig. 1a). In the isolated apical cell, the staining began from the apical tip and the base simultaneously spreading towards the middle (Fig. 1b). When, however, some protoplasm of the neighbouring cell was attached to the basal end, staining always began at the apical tip, but the fragment of the neighbouring protoplasm was stained deep instead of the basal end (Fig. 1c). In the isolated subapical cell, the staining broke out from the both ends showing no polarity (Fig. 1d). But when the neighbouring protoplasm was attached to an end, the staining appeared merely from the opposite end regardless of the end was apical or basal

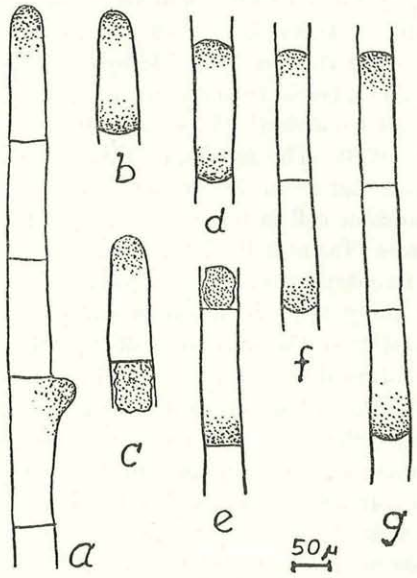

Fig. 1. Vital staining of *Cladophora utriculosa* with neutral red. (Dots represent the staining.) a: Intact branchlet, b: isolated apical cell, c: the same to which plasm of the neighbouring cell is attached, d: isolated subapical cell, e: the same whose one end is sealed with plasm of the neighbouring cell, f: two subapical cells isolated together, g: three subapical cells isolated together.

(Fig. 1e). When two neighbouring cells were isolated together, the staining began at the both ends, but not at the junction of the two cells (Fig. 1f). When three cells were separated together the staining began at the both ends, so that the staining of the middle cell occurred much later than the proximal cells (Fig. 1g).

When the intact branchlet was immersed in distilled water, plasmoptysis broke out at the tip of the apical cell. It, however, occurred at one of the proximal ends in the isolated cell regardless of the end is apical or basal.

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Judging from the experiments, it seems that the polar staining appeared at the tip of the apical cell in intact thallus is attributed not to the polarity of the protoplasm, but to the difference of permeability of the cell wall. The cell wall permeability, however, is not highest at the tip, but it rather seems that there is a difference in permeability merely between the longitudinal cell wall and the transversal cell wall, and that it is much higher in the transversal wall. That is, the dye seems to enter the cell much more promptly through the transversal wall than through the longitudinal wall. In intact thallus, the transversal walls are covered by the neighbouring cells except for the apical tip where the transversal wall is exposed, so that the polar staining appears at the apex. Further, as the plasmoptysis can take place merely at the transversal wall or at the apical tip, the transversal walls seem to be much more breakable than the longitudinal wall. This, probably due to the less deposit of cellulose in the transversal wall, seems to be connected with the difference of permeability between the different walls.

Summary

Intact and isolated cells of *Cladophora utriculosa* and *C. refracta* were stained vitally with neutral red and immersed in distilled water to induce the plasmoptysis. As a result the following was revealed.

1) In intact branchlets, the vital staining begins at the apical tip of the apical cell. But in isolated cells the staining begins at the free ends where the cell was isolated as well as at the apical tip. These indicate that the polar staining appeared in intact branchlet can be attributed to the partial difference of the dye permeability of the cell wall. That is, the transversal wall is much more permeable than the longitudinal wall, so that the apical tip in intact thallus is stained earlier than the other part.

2) Plasmoptysis also breaks out at the apical tip in intact thallus, but it does occur at one of the free ends in the isolated cell.

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