"Avacuolate" Cell in Cortical Tissue of Woody Plant with Special Reference to Permeability of Its Ectoplast to Ions*

By

Akira SAKAI

酒 井 昭

Biology Section, The Institute of Low Temperature Science

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Abstract

Cells without vacuoles have been widely observed in the cortical tissues of the mature twigs of some woody plants. In these cells, neutral red staining only produces a faint, brownish-red coloration throughout the entire cell. These cells have been called "avacuolate" cells by LEVITT and SIMINOVITCH, however, no evidence has so far been presented to show that these cells actually exist in intact plants.

The experiments reported here show that "avacuolate" cells do not originally exist in intact plants, but are merely an abnormal cellular form, in which the cytoplasm coalesces with the vacuolar content as a result of the rupture of the tonoplast during preparation.

Despite the destruction of the tonoplasts, these "avacuolate" cells plasmolyse to nearly the same degree as normal cells in a hypertonic balanced salt solution. In addition, these cells withstand both plasmolysis and deplasmolysis with 2 fold isotonic balanced salt solution and water. From these results, it seems apparent that the ectoplast of the cortical cell is nearly impermeable to ions, at least under experimental conditions.

I. Introduction

A mature plant cell usually has a single, large vacuole. However, cells without such vacuoles have been found in the cortical tissues of the mature twigs of some woody plants. LEVITT and SIMINOVITCH[1,2] reported that there

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is a distinct difference between the appearance of the cortical cells in tissue sections of *Catalpa* and *Cornus*. In the former, the protoplast of every cell consists of a layer of cytoplasm surrounding a single large vacuole which may be intensely stained with neutral red. In the *Cornus*, most of the cells are of this type, but there are others with no distinct vacuole: neutral red staining either fails to stain the vacuole content or at most, produces a faint brownish-red coloration throughout the entire cell. LEVITT and SIMINOVITCH called these unstained cells "avacuolate" cells and the cells with distinct vacuoles, "vacuolate" cells. These "avacuolate" cells have been widely observed in the cortical tissues of some woody plants and have been used in various physiological experiments[3,4,5,6]. No one has determined, however, whether "avacuolate" cells actually exist in the intact plants or are an artifact produced during preparation. If "avacuolate" cells are proven to be abnormal cellular forms, they are unsuitable for experimental use, and it is imperative to clarify this point for experiments making use of the cortical cells of woody plants.

It has long been known that the ectoplast of a plant cell is generally impermeable to ions. Some workers[7,8,9], however, have reported that the semi-permeability of a cell resides only in the tonoplast and that the ectoplast would be essentially freely permeable to ions. If such a concept were true, plasmolysis would not occur when a cell without a vacuole is immersed in a hypertonic salt solution. Because of the difficulty of destroying the tonoplast while retaining cell viability, this problem has still not been resolved, however, "avacuolate" cells make it possible to clarify this point.

II. Material and Method

As experimental materials, cortical parenchyma cells in the winter twigs of a number of woody plants were used. The complete list is as follows:

Willow	*Salix gracilistyla* MIQ.
	Salix sieboldiana BL.
	Salix sachalinensis FR. SCHUM.
Poplar	*Populus nigra* L. var. *italica* MUENCHH.
Alpine rose	*Rosa pendulina* L.
Black locust tree	*Robina pseudo-acacia* L.
Catalpa	*Catalpa ovata G.* DON
Mulberry tree	*Morus bombycis* KOIDZ. var. *takinokawa*
	Morus bombycis KOIDZ. var. *seijyurō*
Cornus	*Cornus controversa* HEMSL.
Red-berried elder	*Sambucus sieboldiana* BLUME

Apple tree *Malus pumila* MILL var. *yamatonishiki*

Thin cortical tissue sections were made with the sharp blade of a hand razer. These sections, with or without vital staining with neutral red, were put into water or into a balanced salt solution and then observed under the microscope.

III. Result and discussion

"Avacuolate" cell

The cortical cells in various woody plants were observed in water or in a balanced salt solution. In the Alpine rose, *Catalpa*, willow (*Salix gracilistyla*), mulberry tree (variety: *seijyurō*) etc., every cell had a layer of cytoplasm surrounding a single large vacuole (Figs. 1, 2 and 3).

On the other hand, in another variety of mulberry tree (variety: *takinokawa*), redberried elder, black locust tree etc., no vacuole was found in any of the cortical cells of these winter twigs, and neutral red staining merely caused a brownish red coloration throughout the entire cell (Figs. 4, 5, 6 and 7).

Every cortical cell in a young shoot of the same variety of mulberry tree had a thin layer of cytoplasm surrounding a single large vacuole[3] (Fig. 8). In the willow (*Salix sachalinensis*, *Salix sieboldiana*), poplar, *cornus*, apple tree etc., some cells in the sections were of the "vacuolate" type, and others had no vacuole (Figs. 9, 10 and 11).

From these facts, it seems likely that an "avacuolate" cell may be an artifact produced by preparation, especially during treatment with water or solutions. To clarify this point, the cortical cells of a variety of mulberry tree and poplar in which no vacuole had been observed in the water or in the balanced salt solution were used as experimental materials. When the tissue sections of these plants were observed directly in liquid paraffin without any pre-treatment, almost all of the cells in these sections had a single large vacuole (Figs. 14 and 16), unlike the cells treated with water or the balanced salt solution (Figs. 13 and 15).

This fact seems to indicate that "avacuolate" cells do not originally exist in an intact plant, but are merely abnormal cellular forms in which the cytoplasm coalesces with the vacuolar content as a result of the rupture of the tonoplast.

From this view point, it seems that these abnormal cells are unsuitable as experimental matarials for physiological studies.

Permeability of ectoplast to ions

As is shown in Figs. 9–12, the "avacuolate" cells also plasmolysed to

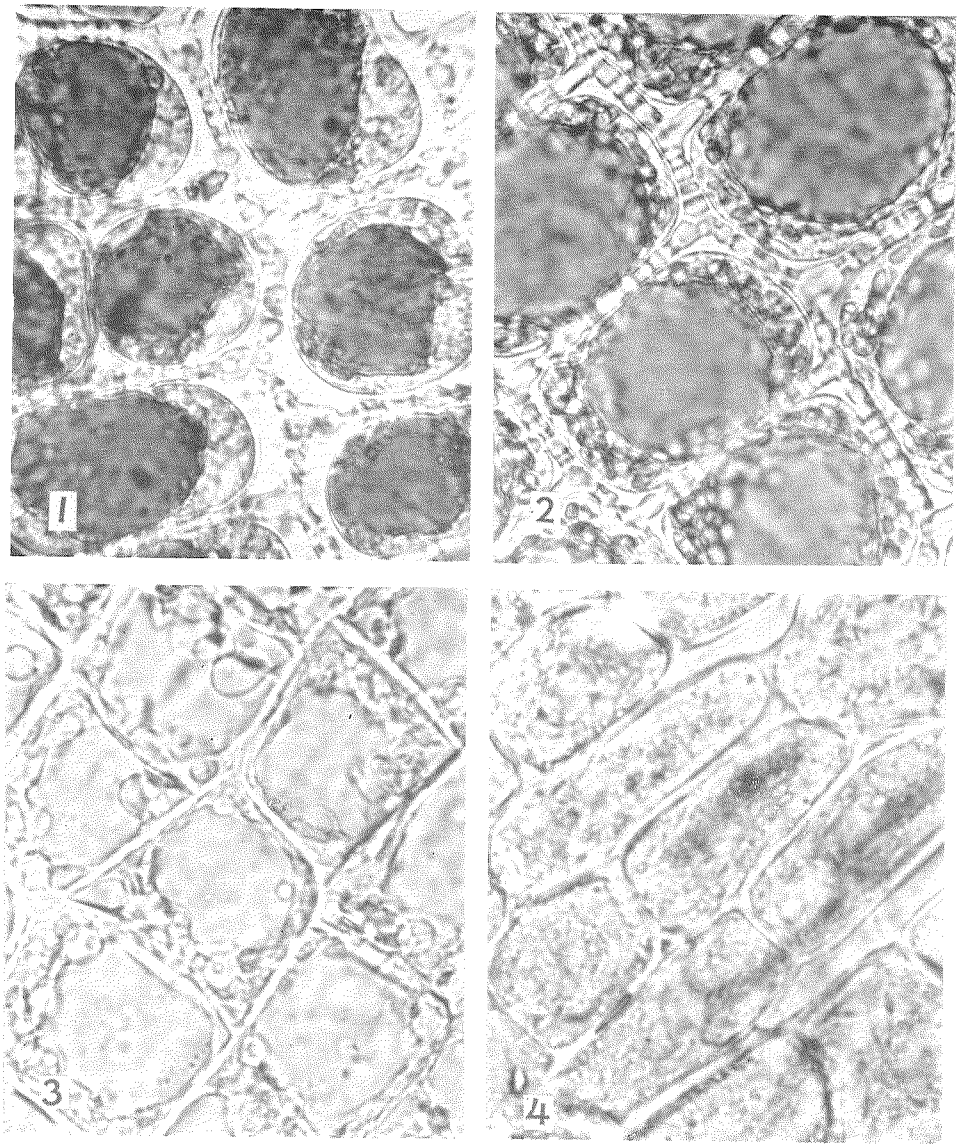


Fig. 1. Plasmolysed cortical cells of Alpine rose immersed in a hypertonic salt solution after vital staining. × 1000

Fig. 2. Plasmolysed cortical cells of willow (*Salix gracilistyla*) immersed in hypertonic balanced salt solution after vital staining. × 1000

Fig. 3. Cortical cells of mulberry tree (vaiety : *seijyuro*) immersed in water. All the cells in this figure are "vacuolate" type. × 1000

Fig. 4. "Avacuolate" cells in the cortical tissue of black locust tree immersed in water. All the cells in this figure are "avacuolate" type. × 1000

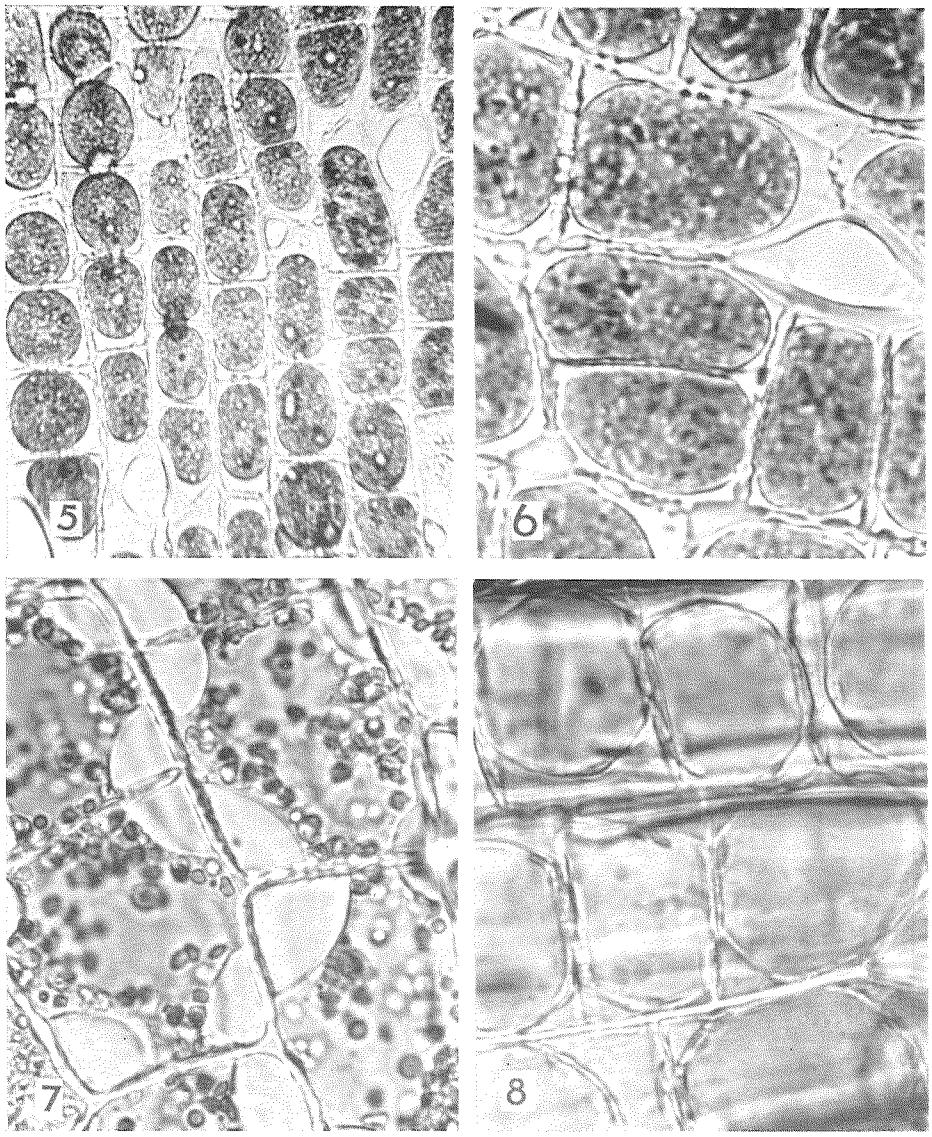


Fig. 5. Plasmolysed cortical cells of mulberry tree (variety: *takinokawa*) in hypertonic balanced salt solution. All the cells in this figure are "avacuolate" type. × 400

Fig. 6. Same as Fig. 5. Details. × 1200

Fig. 7. "Avacuolate" cells of red-berried elder in a hypertonic balanced salt solution after vital staining. × 1000

Fig. 8. Plasmolysed cortical cells of a young shoot of mulberry tree immersed in hypertonic balanced salt solution. Every cell in this figure has a thin layer of cytoplasm surrounding a single large vacuole. × 1000

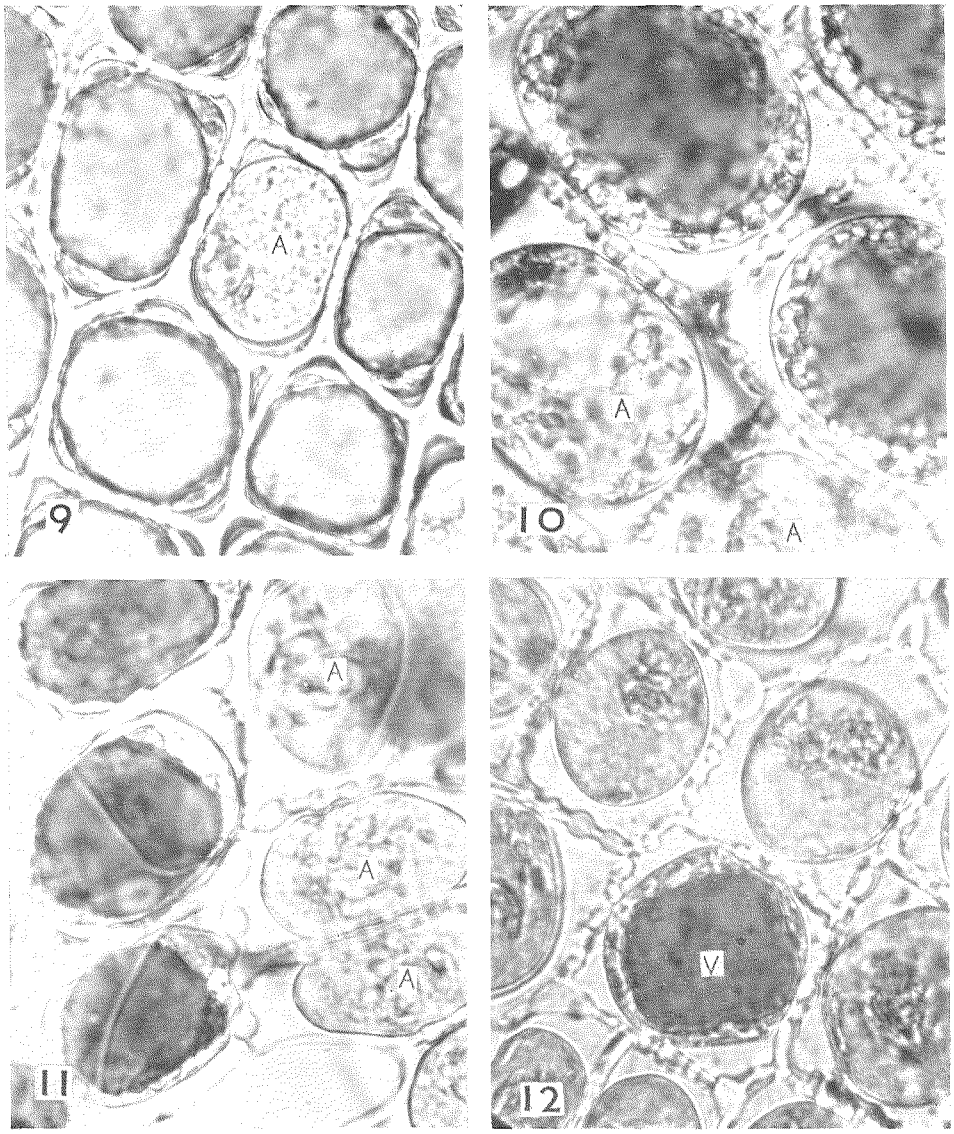
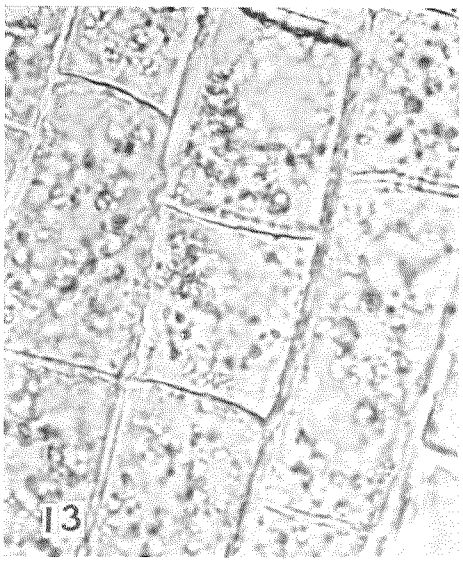


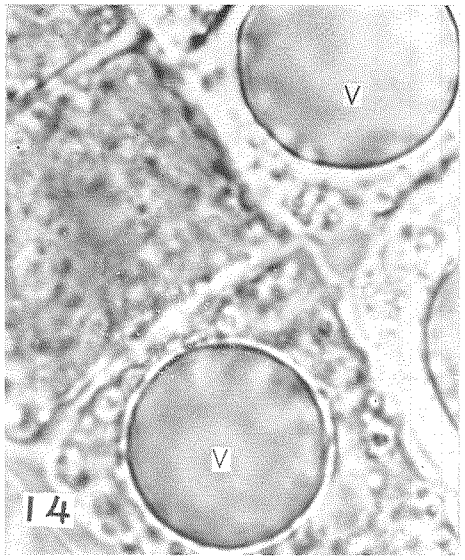
Fig. 9. Plasmolysed cortical cells of willow (*Salix sachalinensis*) immersed in hypertonic balanced salt solution. Cells marked A are "avacuolate" cells. × 1000

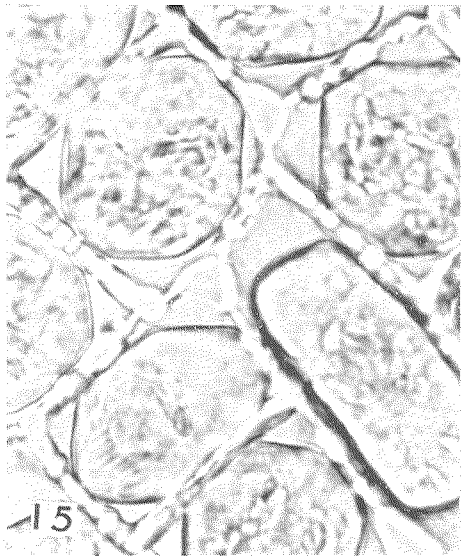
Fig. 10. Plasmolysed cortical cells of willow (*Salix sachalinensis*) in hypertonic balanced salt solution. Cells marked A are "avacuolate" cells. × 1000

Fig. 11. Plasmolysed cells of poplar immersed in hypertonic balanced salt solution after vital staining. Cells marked A are "avacuolate" cells. × 1000

Fig. 12. Plasmolysed cells of poplar in hypertonic balanced salt solution after vital staining. Cells marked V are "vacuolate" cells. × 1000







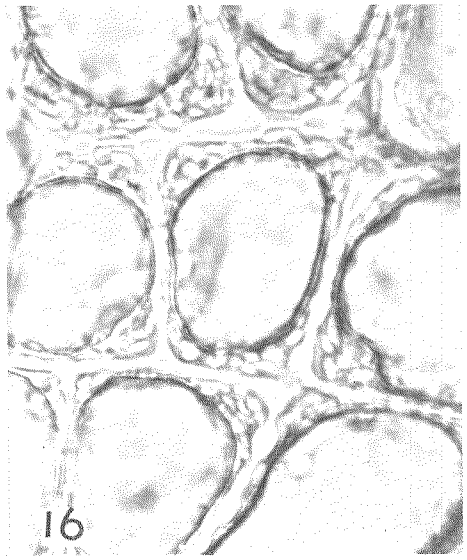


Fig. 13. "Avacuolate" cells in the cortical tissues of mulberry (variety: *takinokawa*) immersed in water. × 1000

Fig. 14. Cortical cells of mulberry tree immersed in liquid paraffin without pre-treatment. Tissue sections were taken from the same twig shown in Fig. 13. Cells marked V are "vacuolate" cells. × 2500

Fig. 15. Plasmolysed "avacuolate" cells of poplar immersed in hypertonic balanced salt solution. × 100

Fig. 16. "Vacuolate" cells of poplar immersed in liquid paraffin without pre-treatment. Tissue sections were taken from the same twig shown in Fig. 15. Every cell in this figure has a single large vacuole. × 1000

nearly the same degree as the normal ones in a hypertonic balanced salt solution, despite of the rupture of their tonoplasts. As to the osmotic concentration of the cell content, there was only a slight difference between the normal and abnormal cells (Figs. 10–12): the osmotic value in normal cells in the cortical tissue of winter poplar is 1.40 M and that of the "avacuolate" cells, 1.35 M–1.36 M. In the mulberry tree, "avacuolate" cells could withstand plasmolysis and deplasmolysis with 2 fold isotonic balanced salt solution and water. In addition, the "avacuolate" cells retained their semi-permeability for at least 1 day, in water at 5°C. These facts seem to show that the ectoplast of "avacuolate" cell is nearly impermeable to ions.

HOPE and STEVENS (1952)[7] reported that reversible diffusion of kalium chloide occurs between an aqueous solution and a young bean root, probably in the protoplasmic phase. Evidence for this view was presented from a study of electric potential difference changes, and changes in an environmental salt concentration. The region of the tissue into which the electrolyte can diffuse reversibly, the "apparent free space", was calculated to be 13 percent for the young bean root. They considered three possible regions in the tissues to be responsible for the "apparent free space":
1) intercellular spaces 2) spaces in the cellulose wall lattice 3) the protoplasm of the cells. The contribution of the cellulose wall lattice to the "apparent free space" was calculated at about 3 percent, and the value of the intercellular space was regarded as zero, since almost all of the intercellular spaces are filled with air and not liquid. They concluded from this that, of the 13 percent of "apparent free space" in young bean root cells, 10 percent probably results from protoplasm from which they assumed that the properties of the boundary between protoplasm and environment do not include high resistance to ion diffusion. This conception has been also accepted by some workers in some plants[8,9]. No direct evidence has been presented, however, to show that the ectoplast is freely permeable to ions. If the semi-permeability of the cell resides only in the tonoplast, and the ectoplast is freely permeable to ions, plasmolysis would not occur in "avacuolate" cells. The present experiments demonstrate that "avacuolate" cells immersed in a hypertonic salt solution plasmolyse to nearly the same degree as normal cells. In addition, these "avacuolate" cells withstand repeated plasmolysis and deplasmolysis. From these facts, it seems apparent that the ectoplast of plant cells is nearly impermeable to ions, at least under experimental conditions.

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