Cell Wall Development of Chambered Crystalliferous Cells in the Secondary Phloem of *Populus euramericana**

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ポプラ二次師部における多室結晶細胞の壁の発達*

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Abstract

Cell wall development of chambered crystalliferous cells in the secondary phloem of *Populus euramericana* is described. These cells were differentiated from parenchyma strands which contacted with phloem fiber groups. A crystal was formed in a vacuole surrounded by tonoplast in each chamber. Then, the secondary wall deposition of chambered crystalliferous cells began only at the part adjacent to phloem fibers forming a protruding fringe which encircled the crystal. When the vacuole disappeared, the crystal had been surrounded by the wall substance on all sides except that away from the fiber. The wall on the crystal surface was not formed by the growth of any protrusion of the secondary wall, but formed independently by the deposition of wall substance on the crystal surface. The secondary wall development was completed after filling up the space between the crystal-surface wall and the thickened side wall adjacent to phloem fiber with newly deposited wall. The crystal-surface wall must be deposited after the tonoplast surrounding the crystal is converted into the plasma membrane.

要 旨

多室結晶細胞は分化中の師部繊維に接する柔細胞ストランドから分化した。結晶は最初液胞中に認められた。師部繊維が二次壁の堆積を始める頃，結晶細胞壁の師部繊維側の部分で二次壁形成が始まり，結晶を取巻くように壁の突出部が形成された。結晶を取囲む液胞が見えなくなると，

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師部繊維と反対側の面を除きすべての結晶表面に壁の堆積が起こった。しかし，この壁は結晶細胞の壁の突出部が張出してきて結晶表面を被ったものではなかった。最後に結晶表面の壁と結晶細胞の師部繊維側に堆積した二次壁との間が，新たに堆積してきた壁物質によって埋められ，結晶は厚い細胞壁中に取込まれた状態となる。結晶表面の壁は，結晶を取囲んでいた液胞膜が原形質膜に置換えられた後に形成されたものと考えられる。

1. Introduction

It is know that true phloem fibers of woody dicotyledons are usually accompanied by crystal-containing sclerified cells.[1] Esau[2] considered that some of those cells are chambered crystalliferous cells as the partitions separating each crystal may be septa in the sense that they subdivide the lumen into compartments but whole series of compartments are enclosed in a common wall.

There are very few reports about the fine structure of those crystalliferous cells except the description of *Acacia* bark by Parameswaran and Schultze.[3] Particularly, the developmental process and the structure of the thickened wall surrounding the crystal have never been observed in detail.

The purpose of the present work is to investigate the cell wall structure and development of those crystalliferous cells in *Populus* as a part of the study on the fine structure of sclerenchyma cells in the secondary phloem of woody dicotyledons.

2. Materials and Methods

The materials used in this study were collected from a trunk of 7-year-old poplar (*Populus euramericana* Guinier) grown in the nursery of Kyoto University Forest. Samples including differentiating phloem were obtained from May to July in 1974.

Tissues used for electron microscopy were fixed in 3% glutaraldehyde and postfixed in 2% OsO_4 or in 3% $KMnO_4$ and then they were embedded in Epon 812 by the method of Luft.[4]

For the study of three-dimensional structure of developing crystalliferous cells, serial cross sections were cut on a Poter-Blum MT-1 ultramicrotome using a diamond knife and they were shadowed with Pt-Pd following removal of the plastic embedding medium.[5]

Other sections were either stained with uranyl acetate and lead citrate or shadowed with Pt-Pd. Some of the sections were lined with carbon for gas discharge etching. They were etched by 5 to 60 minutes exposure to AC glow discharge of 400-600V at a pressure of 0.1 to 0.05 Torr in a bell jar of a JEOL-4B vacuum evaporator and observed after Pt-Pd shadowing. On the other hand, from the fixed materials 100 μm thick radial sections were made and freeze-dried. Direct carbon replicas shadowed with Pt-Pd were made from these sections following the method described by Imamura et al.[6]

Observations were made by a JEM-7 electron microscope. Some of the freeze-dried sections were coated with gold and observed in a JSM-U3 scanning electron microscope.

3. Results and Discussion

Populus euramericana has phloem fibers, according to the definition of HOLDHEIDE[1] and EVERT,[7] and those phloem fiber groups are always surrounded by crystal-containing cells.[1] It was confirmed that crystalliferous cells are differenciated from parenchyma strands.[8] For that reason the present authors use the term "chambered crystalliferous cells" according to ESAU.[2] It is also known that the wall of crystalliferous cells, which contact with phloem fibers are conspicuously thickened and lignified, embedding crystals within them.

3–1 Formation of chambers

Some of the parenchyma strands adjacent to elongating phloem fiber groups had already begun the formation of chambers, 10 to 20μm long, and the accumulation of crystals was found in them. At the stage when the phloem fibers finished their deposition of the outer layer of the secondary wall, most of the parenchyma strands seemed to have finished the formation of chambers.

Formation of chambers did not always occur in all cells of the same parechyma strand, and some of them did not accumulate crystals and not deposit the secondary wall.

On the other hand, some chambers were subdivided by vertical septa giving rise to two chambers side by side (Photo 1), each of which accumulated a crystal.

3–2 Cell wall development of chambered crystalliferous cells

Various developmental stages of crystalliferous cells were observed at the periphery of phloem fibers which have finished deposition of the outer layer.

The secondary wall deposition of crystalliferous cells began only at the part adjacent to phloem fibers forming a conspicuously protruding fringe (Photo 2) which encircled the crystal (Photo 3 and Fig. 1). Microfibrils were deposited along the fringe (Photo 4).

At the next stage, the crystal was surrounded by wall-like substance on all sides except that away from the fiber (Photo 5–A) The wall-like substance when viewed between crossed nicols showed strong birefringence (Photo 6–A, B). With the electron microscope, sliced sections of this substance displayed an conspicuous fibriller structure with some lamellae (Photo 6–C). For those reasons, this substance must be the cell wall which consists of cellulose microfibrils. The present authors call this wall as "crystal-surface wall" tentatively.

It was often found that the crystal-surface wall had not any continuity with the cell wall surrounding it (Photo 5–A). The present authors examined serial cross sections so as to know the three-dimensional shape of crystal-surface wall and the relation between the crystal-surface wall and the wall of the crystalliferous cell (Photo 5). It was confirmed that the crystal-surface wall covered most part of the crystal surface except that away from the fiber. Generally, the crystal-surface wall had only a partial connection with the fringe or other part of the thickened secondary wall of the crystalliferous cell. According to the observation on serial cross sections, the crystal-surface wall did not seem to be formed by the growth of any protrusion of the secondary wall, but formed independently by the

deposition of wall substance on the crystal surface.

Figure 1 is a diagrammatic representation of the developing chambered crystalliferous cell at the stage described above.

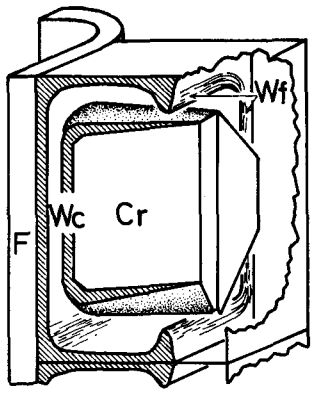

Fig. 1 Diagrammatic representation of the developing chambered crystalliferous cell. Wc: crystal-surface wall, Wf: protruding fringe, Cr: crystal, F: developing phloem fiber.

Photos 7 and 8 are cross and radial sections of mature crystalliferous cells, respectively. They show that secondary wall development was completed after filling up the space between the crystal-surface wall and the thickened wall of the pholem fiber side with newly deposited wall.

The crystal surface away from the fiber was covered with cellulose microfibrils in most cases (Photo 9), but somtimes it remained exposed.

Photo 10 is the scanning electron micrograph of mature crystalliferous cells. Most of the crystals are monohydrate calcium oxalate of the twin crystal type[9),1)] just the same as those of *Acacia senegal*.[3)]

As PARAMESWARAN and SCHULTZE[3)] suggested in *Acacia*, the thickened part of the wall of crystalliferous cells on the phloem fiber side was apparently lignified, but the unthickened part of the wall on the opposit side of the phloem fiber did not deposit the secondary wall. The septum between chambers was lignified only at the part which is sandwiched with thickened walls.

Polarity of thickening and lignification within crystalliferous cells may be caused by some influences of developing phloem fibers adjacent to them. CHATTAWAY[12)] suggested similar polarity about the crystalliferous cells adjacent to fibers in xylem.

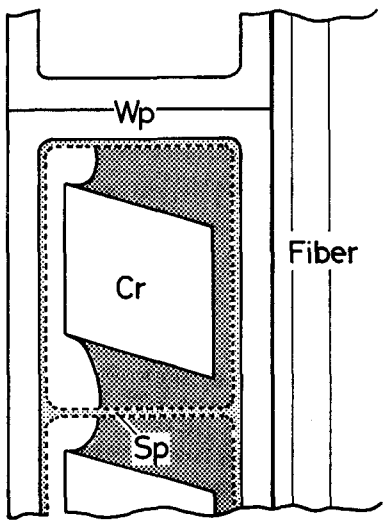

Fig. 2 Diagrammatic representation of the wall structure of compartments. Sp: septum, Wp: parenchyma wall, Cr: crystal.

Cell wall structure of chambered crystalliferous cells in the phloem of *Populus* is similar to that of crystalliferous cells occuring in xylem parenchyma of *Robinia*.[10)] Such similarity between *Acacia* and *Robinia* was also pointed out by PARAMESWARAN and SCHULTZE.[3)]

The crystal-surface wall in *Populus* may be equivalent to the thickened and lignified "sheath" in *Robinia*.[10)] "Pédoncule" found in *Robinia*[10)] could not be observed in *Populus*, but the fringe of the wall must have the same role as "pédoncule" to fix the crystal in the cell lumen.

The nature of the "sheath" covering crystals seems to be different by tree species. For example, *Shorea almon* has the non-cellulosic "crystal-sheath"[12)], while *Robinia*[10)] and *Populus* have the "crystal-sheath" apparently cellulosic in its nature. In the latter

case, "crystal-surface wall" will be more adequate to term the "sheath" for expressing its cellulosic nature.

The present authors also observed the wall structure of compartments which were partitioned by septa. The septa were thinner than the end walls of parenchyma cells (Photo 11). The inner surface of parenchyma cell was covered with a thin layer which was continuous with the septa (Photos 11 and 12, Fig. 2). HOLDHEIDE[1] regard that his "Kristallkammerfasern" are derived from fusiform cells. But from the present observations, it is clear that those crystalliferous cells are differenciated from parenchyma strands and that those are chambered crystalliferous cells as being pointed out by ESAU.[2]

3–3 Membrane system surrounding a crystal

One of the most interesting problems in the secondary wall development of the chambered crystalliferous cells is the mechanisum of crystal-surface wall deposition. Therefore, membrane system surrounding crystals was observed in order to understand the mechanism of the crystal-surface wall deposition.

When the fringe formation of the secondary wall was still not conspicuous, a crystal was formed in a vacuole surrounded by tonoplast (Photos 13 and 14). As the crystal in a vacuole was smaller than a mature one, it must be growing at this stage. Ribosomes were very rich in the protoplast (Photo 14), and this was a characteristic feature of developing crystal-containing cells in comparison with other parenchyma cells or developing phloem fibers.

At the stage when the fringe of the secondary wall was formed, the vacuole surrounding a crystal disappeared (Photo 2), but it could not be confirmed whether the tonoplast was surrounding the crystal surface or not. Meanwhile, small vacuoles which contained multi-vesicular bodies appeared.

When the deposition of the crystal-surface wall began (Photo 15), the unit membrane was observed at the surface of the wall (Photo 16). This unit membrane must be plasma membrane as microtubules were frequently observed inside of it.

The process in which the tonoplast surrounding a crystal at the earlier stage was transposed with the plasma membrane could not be observed.

It was often observed that the crystal made a contact with the primary wall of the crystalliferous cell preceding the crystal-surface wall deposition (Photo 17). The present authors could not confirm whether it was the common case or not, but the crystal will be apt to contact with the wall of the crystalliferous cell as the volume of the crystal within the cell increases. If the crystal contacts with the wall, the tonoplast surrounding the crystal must be pressed against the plasma membrane and may fuse with it, resulting finally in the conversion

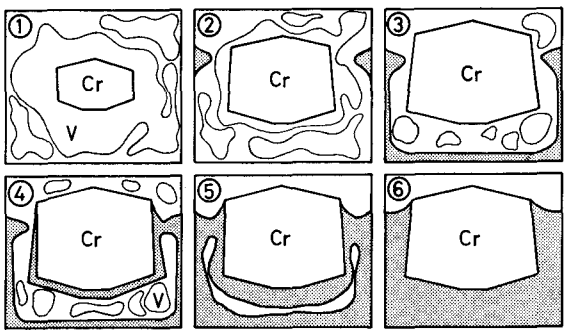

Fig. 3 Diagrammatic representation of the developing process in chambered crystalliferous cells.

of the tonoplast into the plasma membrane. There is another possibility that the tonoplast may fuse with plasma membrane through the reverse pinocytosis to cast the crystal away to the outside of the plasma membrane. Anyway, the crystal-surface wall must be deposited after the tonoplast surrounding the crystal is converted into the plasma membrane.

As a conclusion of this report, developing process of chambered crystalliferous cells is diagrammatically shown in Fig. 3.

It is necessary, in the future, to investigate whether the developing process and the structure of chambered crystalliferous cells shown in Fig. 3 are characteristic to *Populus* or more common for other species.

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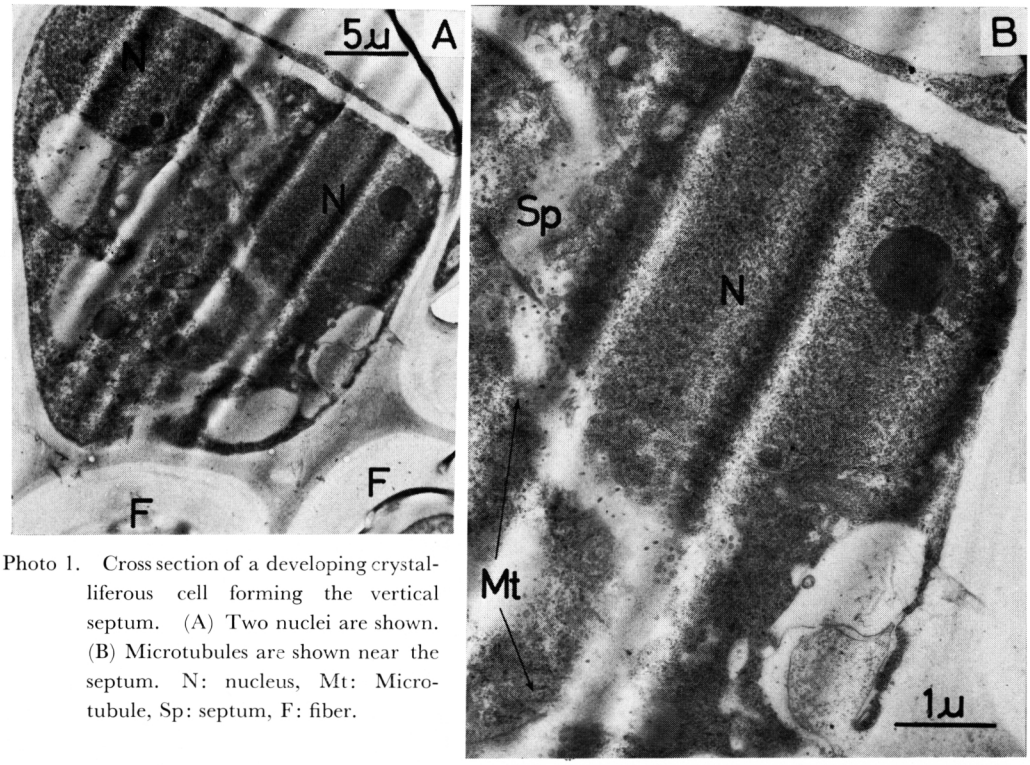

Photo 1. Cross section of a developing crystalliferous cell forming the vertical septum. (A) Two nuclei are shown. (B) Microtubules are shown near the septum. N: nucleus, Mt: Microtubule, Sp: septum, F: fiber.

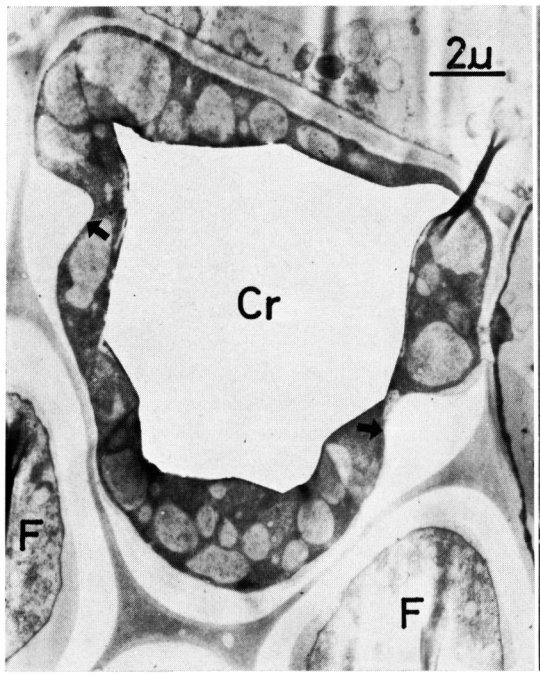

Photo 2. Cross section of a crystalliferous cell. The protrusion of the secondary wall (arows) is obvious. Cr: space occupied by the crystal, F: fiber.

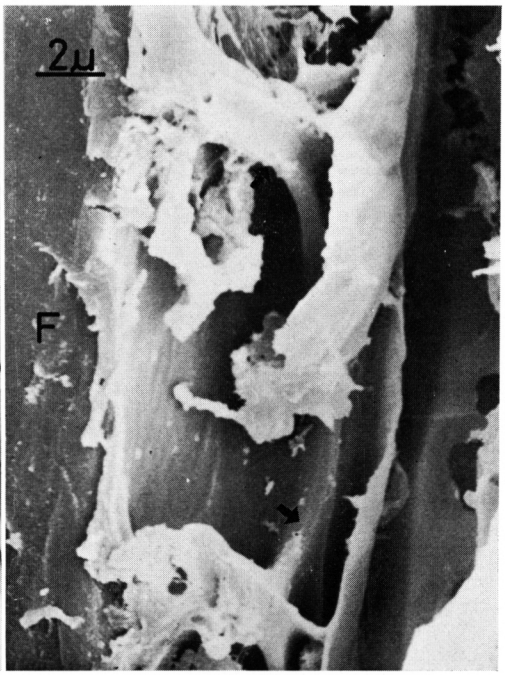

Photo 3. Scanning electron micrograph of differentiating crystalliferous cell radially cut at the center. The protruding fringe (arrow) is shown. The crystal is removed with 1% HCl. F: fiber.

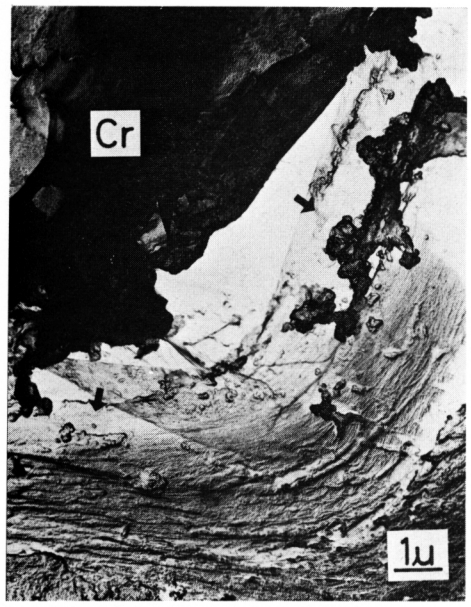

Photo 4. Direct carbon replica showing the surface of the protruding fringe (arrow). Microfibrils are oriented along the fringe. Cr: crystal.

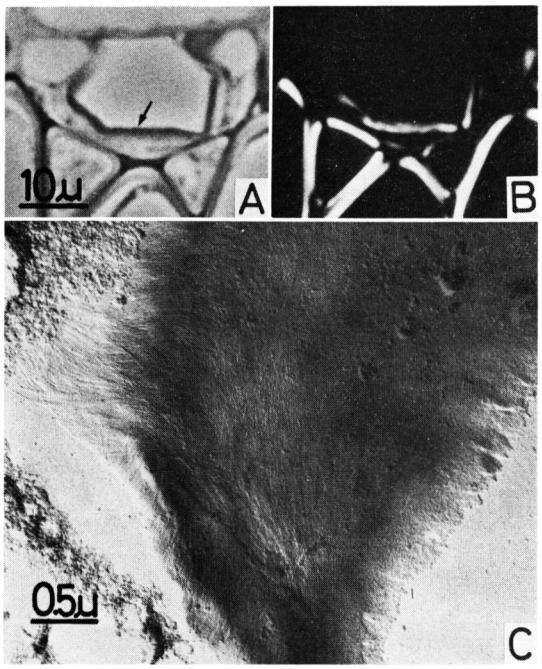

Photo 6. Crystal-surface wall deposition. (A) and (B) are phasecontrast and polarizing micrograph of the same cross section, respectively. Arrows show the crystal-surface wall. (C) Sliced section of the crystal-surface wall.

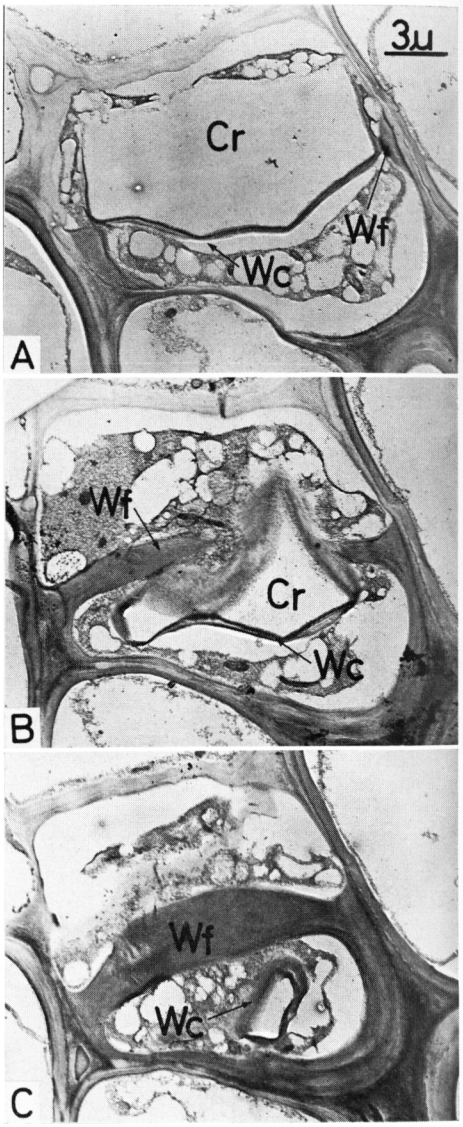

Photo 5. Serial cross sections of a developing crystalliferous cell showing the state of the crystal-surface wall deposition. Shadowed with Pt–Pd following removal of epoxy resin. (A) The section cut at the center of the chamber. (B) The section between (A) and (C). (C) The section cut near the end of the chamber. Wc: crystal-surface wall, Wf: fringe, Cr: space occupied by the crystal.

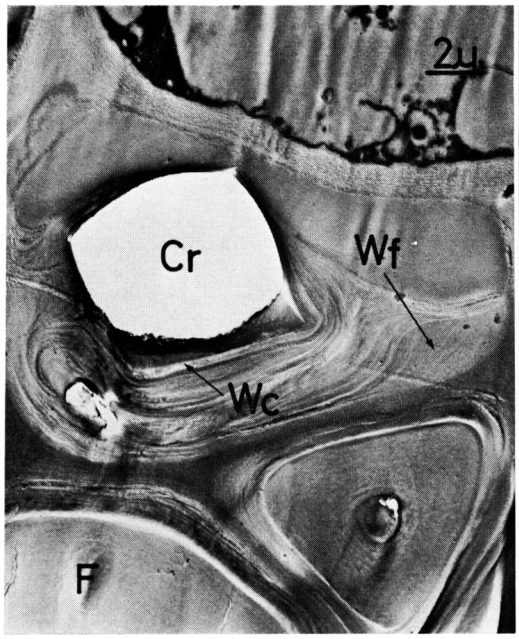

Photo 7. Cross section of a mature crystalliferous cell. Shadowed with Pt–Pd. Wf: protruding fringe, Wc: crystal-surface wall, Cr: space occupied by the crystal, F: fiber.

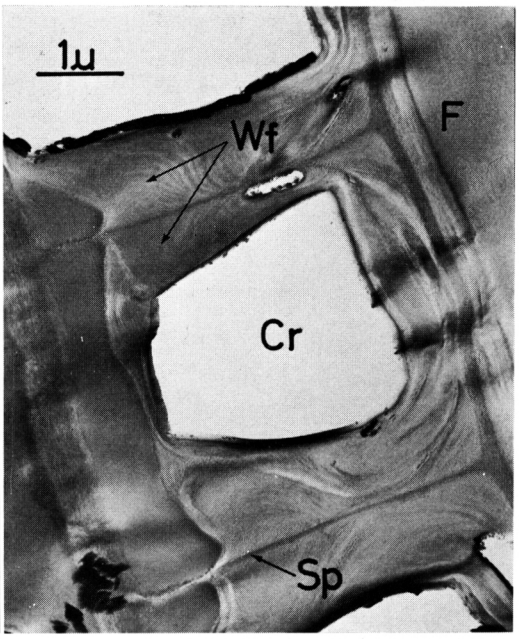

Photo 8. Radial section of mature crystalliferous cell. Shadowed with Pt–Pd. Wf: fringe, Sp: septum, F: fiber.

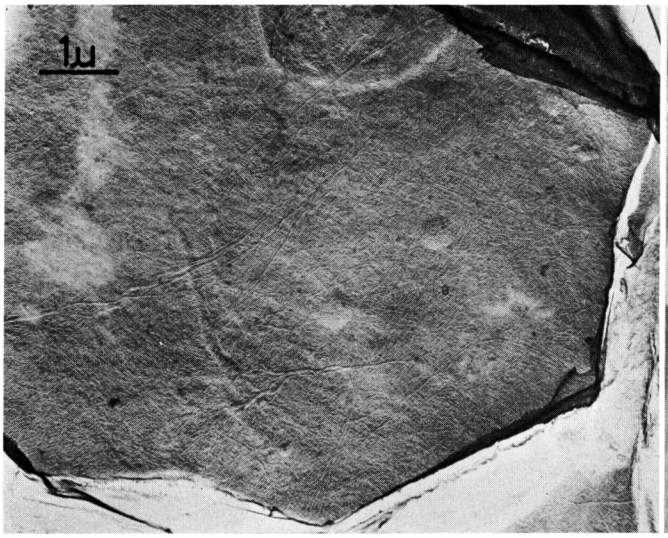

Photo 9. Direct carbon replica showing the crystal surface that away from the fiber. Delignified by chlorite method. Microfibrils are covering the crystal surface.

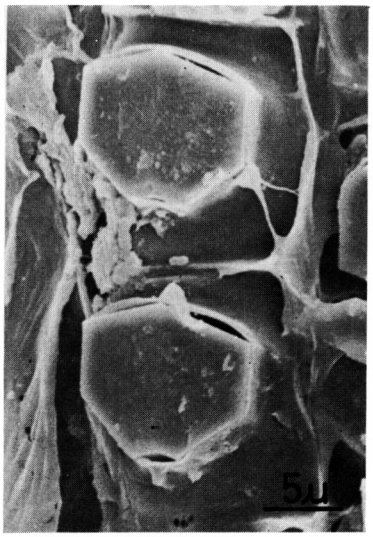

Photo 10. Scanning electron micrograph showing mature crystalliferous cells.

Photo 11. Radial section of mature crystalliferous cell. Shadowed after gas discharge etching. Thin layer (arrow) is shown inside of the parenchymatous wall. Wp: parenchyma wall, Sp: septum, F: fiber.

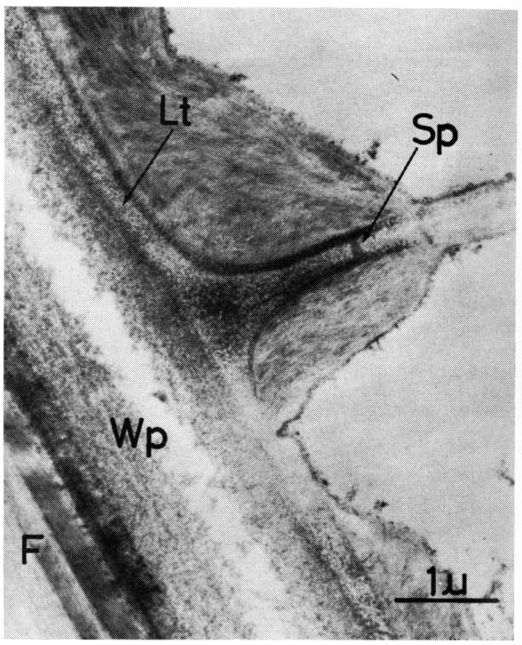

Photo 12. Septum of the developing chambered crystalliferous cell as seen in radial section. Stained with $KMnO_4$. Sp: septum, Wp: parenchyma wall, Lt: thin layer continuous with septum.

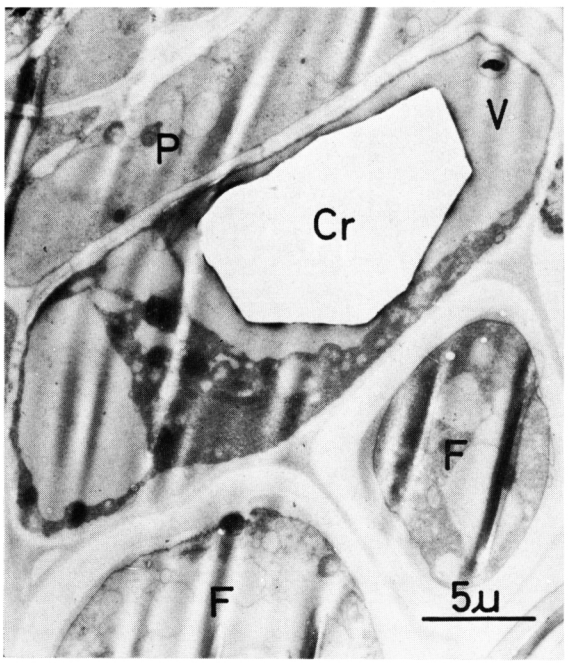

Photo 13. Cross section of a developing crystalliferous cell. Cr: space occupied by the crystal, V: vacuole, F: fiber, P: parenchyma cell.

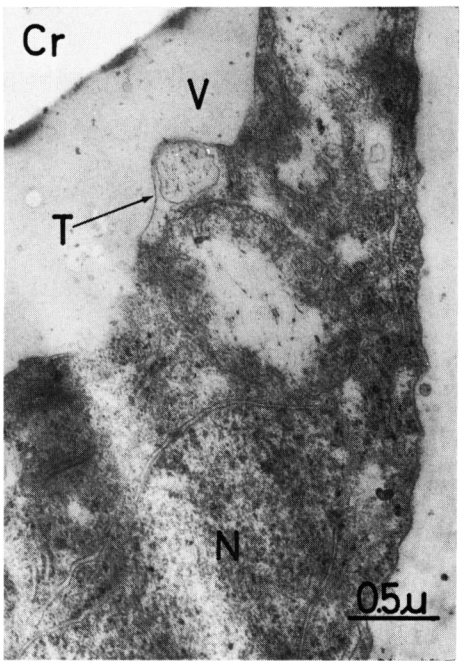

Photo 14. Enlarged view of the vacuole including a crystal. T: tonoplast, N: nucleus, V: vacuole.

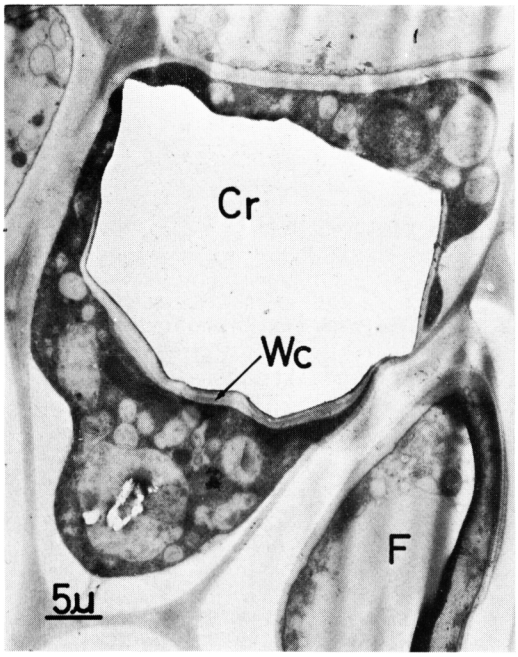

Photo 15. Crystal-surface wall deposition as seen in cross section. Wc: crystal-surface wall, F: fiber, Cr: space occupied by the crystal.

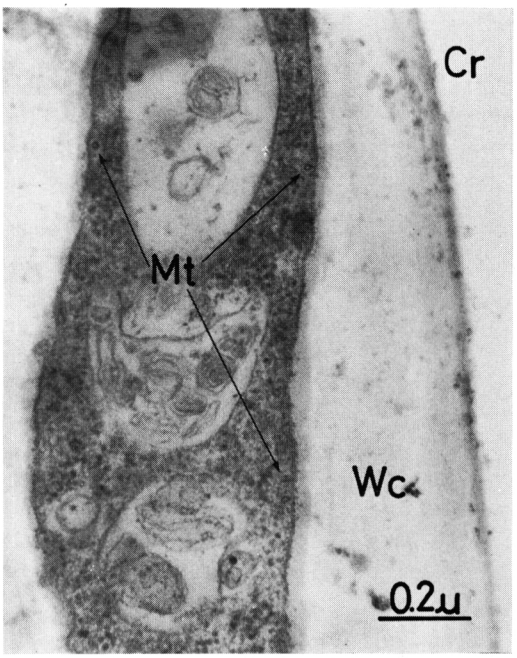

Photo 16. Enlarged view of the crystal-surface wall. Wc: crystal-surface wall, Mt: microtubule, Cr: space occupied by the crystal.

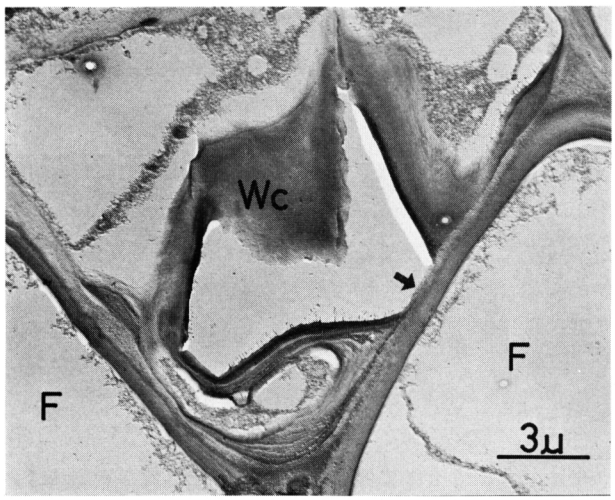

Photo 17. Similar to Photo 5. Secondary wall deposition cannot be found at the part shown with arrow. Wc: crystal-surface wall, F: fiber.