The secondary wall formation of compression wood tracheid. IV.

Cell wall structure of transitional tracheids between normal wood and compression wood.

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圧縮あて材仮道管の二次壁の形成 (第4報)

正常材とあて材との移行過程における仮道管の壁構造

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Résumé

The cell wall structure of transitional tracheids between normal and compression wood tracheids in *Pinus densiflora* and *Cryptomeria japonica* were examined, being related to the differentiation. The transition from normal wood to compression wood proceeds as follows. (I) Lack of S_3 and passing decrease of cell wall thickness were detected first of all. (II) Dense lignification of secondary wall and rapid thickening of S_2 proceeded in parallel. (III) Helical ridges and cavities developed. Cross shape of tracheids became round. (IV) Intercellular spaces occurred.

On the other hand, changes from compression wood to normal wood are as follows. (I) Degeneration of helical fidges and cavities led the changes. (II) Comeback of S_3 and decrease of S_2 thickness were followed. (III) Dense lignification of secondary wall dissolved. (IV) Intercellular spaces disappeared. Being lignification behavior as an exception, the sequences in the both cases are good consistent with the differentiation of normal or compression wood tracheids. Therefore the peculiarity of lignification was discussed.

要　　旨

アカマツとスギの正常材からあて材，あて材から正常材へのそれぞれの移行域にある仮道管の壁構造を調べた。樹幹を傾斜させて得た正常材からあて材への移行域では，(1) S_3 の欠落，(2)二次壁の木化度および壁厚さの増大，(3)らせん状のうねと裂目の発達，および仮道管断面の円形化，(4)細胞間隙の出現，の順にあて材仮道管の特徴が現われた。一方，傾斜した樹幹を直立させ

て得たあて材から正常材の移行域では，(1)らせん状のうねと裂目の衰退，(2)壁厚さの減少と S_3 の復活，(3)二次壁木化度の低下，(4)細胞間隙の消失，の順に正常材仮道管に復活した。これらの一連の変化を正常材とあて材仮道管の分化過程と関連づけて考察した。

INTRODUCTION

The peculiar cell wall structure of compression wood tracheid gives an impression that it is developed quite differently from normal wood tracheid. The structural difference between them, however, may not be so essential as one judges from its peculiar appearance, since the several types of transitional tracheids between them have been observed in the cases that the tree stem was tilted, or the inclined one was recovered vertically. Any important key which is covered in the differentiation of normal wood or compression wood may be found out in the transitional cells in relation to cell wall development, because these tracheids develop the cell wall under the peculiar conditions. However, such transitional tracheids was seldom examined, while the cell wall structures of typical compression wood tracheid have been studied numerously as much as those of normal one.

Kennedy and Farrer took the initiative in the observation.[1] They showed peculiar transitional tracheids in the infant seedlings of pine and tamarack which were grown under the tilting conditions repeated in growth chamber, and suggested that stimulus of tilting or recovery affected directly to the differentiating tracheids of several developing stages. However the seedlings used were so infant and they were tilted so often in a short period that the interpretations of the results are very complicated.

Fukazawa observed the lumen surface of the transitional tracheids of sagahalien fir, which was inlined artficially and then recovered naturally, using scanning electron microscopy, and showed some patterns of helical sculpture in the lumina.[2] He also observed the transition from center of compression wood arc to tip, namely the change from the lower side to lateral side of the inclined trunk, using UV microscopy.[3] However, the precise examination of the transitional tracheids relating to the differentiation of tracheid seems to remain still uncertain. We have been studying the sequence of secondary wall formation in the differentiating compression wood tracheids.[4-8] In this paper, we tried to develop the investigation started by Kennedy and Farrer using young trees of japanese red pine and japanese cryptomeria, applying the several microscopic techniques.

MATERIALS AND METHODS

The slender and vertical tree of japanese red pine (*Pinus densiflora* Sieb. et Zucc.) growing naturally on a slope at the Kamigamo Experiment Forest was used. It was

20 years old, 5 m height and 4 cm DBH. On May–16 of 1974, the trunk was tied with a rope at the upper part and then bended to take a bow shape in order to preserve horizontally the middle part of the trunk. It was recovered vertically on June–20 of the year, and cut down in the autumn. Wood blocks were collected from the middle part which had 11 annual rings. For the secondary observation, a young tree of japanese cryptomeria (*Cryptomeria japonica* D. Don) which was growing fast at the Experimental Nursery of the Kyoto University Forests was fixed with a splint and then inlined at 45° on May–9 of 1978 and recovered again vertically on June–20, and cut down in the autumn. It was 12 years old, about 2 m height and 1.5 cm DBH. Wood blocks were collected from the position at 1 m height where 5 annual rings were contained.

Wood blocks were embedded in epoxy resin by the ordinary method and then cross or radial thin sections of 2.5–3 μm thickness were sliced off from the blocks. These sections were mounted in Canada balsam directly or after safranin staining. This staining was attempted to examine the lignin distribution in addition to the Wiesner reaction. Thin sections of 3 μm were soaked in 1% safranin soln. for 30 min. and rinsed in water for 30 min. Then they were differentiated in 50% ethanol for 10 min. and more properly in 100% ethanol. The cell wall thickness, cell shape, intercellular space and lignin distribution were observed in the cross thin sections by the ordinary and phase contrast microscopes. The lack of S_3 was examined under polarizing microscope, using cross sections. The structure of inner-surface was observed by scanning electron microscope, using radial sections of 50 μm of non-embedded materials.

RESULTS AND DISCUSSION

In *Pinus densiflora,* typical compression wood tracheids induced by the artificial bending on May–16 were developed from about the 15th cell counted from the annual ring boundary in radial files, while about five normal early wood tracheids were formed before the bending. And about ten transitional tracheids were localized between them (Fig. 1A). On the other hand, the transitional zone from compression wood to normal wood was not so remarkable as the transition described above, because the late wood was already formed in that time. Therefore various structural changes from normal early wood to compression wood were examined of this material.

The cell wall thickness was measured first of all along several radial files, and diagrammed in Fig. 2A. It is interesting to note that the thickness is minimum in the 6th and 7th cells, and increased remarkably in the 9th–13th cells. S_3 detected clearly under cross nicols in the 1st–5th cells disappeared from the 6th or 7th cell (Fig. 1B). The passing decrease of cell wall thickness must be related to the lack of S_3 and the rapid increase from the 9th cell will be caused by the peculiar thickening of S_2 accompanied

by the larger fibrillar angle. These changes were ascertained by the scanning electron microscopy. The fibrillar orientation observed from the lumen side was about horizontal in the 1st–4th cells (Fig. 3A). It was very steep in the 7th cell (Fig. 3B), and became diagnoal from the 8th cell (Fig. 3C), but the helical ridges and cavities were developed gradually in the 9th–12th cells (Fig. 3D and E). The cross shape of tracheids began to change from about the 10th cell (Fig. 1A). The cell corner became round and the cells showed ellipse by degrees thereafter. The intercellular spaces appeared from about the 15th cell. The peculiarity of lignin distribution detected by Wiesner reaction was found in about the 8th cell and then lignin content increased rapidly, although the clear micrograph was not obtained due to the waving of thin sections caused by the staining procedure.

Thus the process of the transition from normal early wood to compression wood was examined in various aspects. But this material was not proper necessarily to consider the effect of inclination to the differentiating tracheids, because the differentiating tracheids were supposed to be not so many at that time of bending. On the contrary, the materials collected from *Cryptomeria japonica* seem to be more proper to examine the various reactions of differentiating tracheids to the change from vertical to incline and *vice versa*, because tracheid productions were very active during both treatments. About 25 normal early wood tracheids were already formed before the inclination on May–9, and also about 50 ones were formed after the recovery to vertical on June–20. The observations were proceeded mainly in the stained thin sections by safranin, because very sharp micrographs (Figs. 4A and 5A) were obtained and the safranin positive regions were in keeping with the result of Wiesner reaction. In this case, the transitional zones are so distant from the annual ring boundary that any other standard is necessary to count the cells in each radial file. When the cells were examined along radial files from normal wood to compression wood, the occurrence of a faint lignin-rich layer at the outer region of S_2 of about cell corner was watched (Fig. 4A). This cell was used as the standard cell in each radial file and then numbered (0) to the cell. Then the cells were numbered (1), (2) and so on to the cambium side, and (–1), (–2) and so on to the pith side. The new lignin-rich layer developed to radial and tangential walls, and increased rapidly both intensity and width from this standard cell, namely (0) cell, to the (7) cell. The lack of S_3 was detected from the (–5) cell. The cell wall thickness of the transitional cells was diagrammed in Fig. 2B. The passing decrease is found also in this case. The rapid increase proceeds in the (0)–(7) cells. The cross shape became round from about the (5) cell, and the intercellular spaces occurred from about the (10) cell (Fig. 4A). But the occurrence was rather variable.

The sequence of the transition from normal early wood to compression wood will be summarized with the following. (I) Lack of S_3 and passing decrease of cell wall

thickness were detected first of all. (II) Development of lignin-rich layer at the outer region of S_2, namely the dense lignification of secondary wall, and rapid thickening of S_2 proceeded in parallel. Fibrillar angle became larger. (III) Helical ridges and cavities developed. Cross shape of tracheids became round. (IV) Intercellular spaces occurred. It is supposed that the lignifying-stage of secondary wall, S_3-depositing-stage, S_2-thickneing-stage, S_1-depositing-stage, radially enlarging-stage and cambial stage are lined up orderly in the differentiating zone of normal wood from the mature xylem side to the phloem side. If these differentiating tracheids react quickly to the stimulus of inclination, and change the cell wall forming process from normal wood to compression wood, as pointed out by Kennedy and Farrer[1], a series of transitional tracheids will be lined up. The characteristics of compression wood tracheid will be appeared in the following order, (I) dense lignification of secondary wall, (II) lack of S_3, (III) thickening of S_2 and development of helical ridges and cavities, and (IV) round cross shape and occurrence of intercellular spaces. Although the dense lignification of secondary wall did not take the lead but proceeded in parallel with the S_2 thickening, the occurrence of other characteristics were good consisitent with the result of this observation. The so called "susceptible cell" to lignification named by Kennedy and Farrer[1] is supposed to be identical to the cell in which the new lignin-rich layer occurred, namely the just beginning cell of S_2 thickening. It is very strange that the dense lignification of secondary wall is delayed, because the secondary wall lignification is known to follow the S_2 thickening in normal wood[9,10] and also in compression wood.[5-8] It is also proved by our previous investigations that lignin precursors are already synthesized and stored in the cytoplasm in the cells of S_2-thickening-stage.[6),9] Therefore the just before cell of secondary wall lignification may not be able to react to the stimulus of further lignification, since it has finished the syntheses of lignin precursors in the cytoplasm.

It is very interesting whether the discussion described above is applied to the transition from compression wood to normal wood. In this observation, the last cell possessing the lignin-rich layer was used as the standard cell and numbered (0). And the cells were numbered (1), (2) and so on to the cambium side, and (–1), (–2) and so on to the pith side (Fig. 5A). (I) Degeneration of helical ridges and cavities began from the (–3) cell. (II) Decrease of cell wall thickness proceeded in the (–2)–(0) cells and comeback of S_3 was detected already from the (–1) cell which is still possessing cavities in the interior of S_2. (III) Dense lignification continued to the (0) cell. (IV) Intercellular spaces disappeared from about the (3) cell. Strange to say, the intercellular spaces were often plugged temporarily by lignin at about the (0) cell. It is interesting to compare this sequence to the developing stages of compression wood, namely, lignifying-stage of secondary wall, S_2-thickening-stage, S_1-depositing-stage, radially enlarging-

stage and cambial stage which are lined up from the mature xylem to the phloem. Although the lignification is an exception also in this case, other changes seem to be consistent with the differentiation of compression wood tracheid.[5-8] The peculiar behavior of lignin deposition, the continuance of dense lignification at the secondary wall and the temporary plugging of the intercellular spaces, also may be interpreted by the reason that much lignin precursors which are already stored in the cytoplasm in the cells of S_2-thickening-stage are supplied to wall. More the plugging will be caused by the surplus lignin precursors which could not move again to the rapid decrease of wall thickness. It is understandable that the "susceptible cell" is lignified densely even after the recovery from the short period inclination, if it is identical to the just beginning cell of S_2 thickening as described before and stores the much lignin precursors in the cytoplasm during the inclination.

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Explanation of Pigures

Fig. 1. A. Phase contrast micrograph of *Pinus densiflora*. The transitional zone from normal wood to compression wood is localized in about the 6th-15th cells counted from annual ring boundary. B. Polarizing micrograph of the transional zone. S_3 brightened at the 1st–5th cells disappears from the 6th cell.

Fig. 2. Thickness of double wall measured in the transition from normal wood to compression wood of *Pinus densiflora* (A), and of *Cryptomeria japonica* (B), and in the transition from compression wood to normal wood of *Cryptomeria japonica* (C). ● : radial double wall. ○ : tangential double wall.

Fig. 3. Scanning electron micrographs of the inner surface of the tra transitional tracheids from normal wood to compression wood in *Pinus densiflora*. A. The 1st cell from the annual ring boundary. The fibrillar orientation is almost horizontal in normal wood tracheid. B. The 7th cell. The orientation is steep. C. The 8th cell. The orientation is diagonal. D. The 9th cell. Helical ridges and cavities are occurred partially. E. The 11th cell. The helical ridges and cavities are well developed.

Fig. 4. Light micrograph of the transitional zone from normal wood to compression wood in *Cryptomeria japonica*. The number (0) cells are the first one possessing the new lignin-rich layer at S_2 (see text), and the layers are developed rapidly to the 7th cells (see arrows). B. Polarizing micrographs of the same part of Fig. A. S_3 brightened to the 5th cells disappears from the 4th cells.

Fig. 5. Light micrograph of the transitional zone from comprression wood to normal wood in *Cryptomeria japonica*. The number (0) cells are the last one possessing the lignin-rich layer at S_2 (see text). Note the plugging of intercellular spaces about the (0) cells (see arrows). B. Polarizing micrograph of the same part of Fig. A. S_3 is detected from the (−1) cells (see arrows).

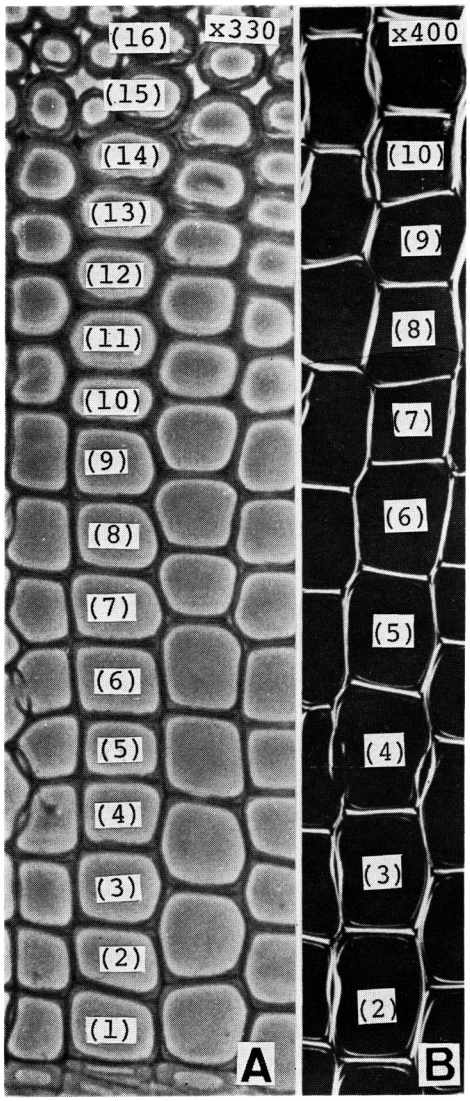
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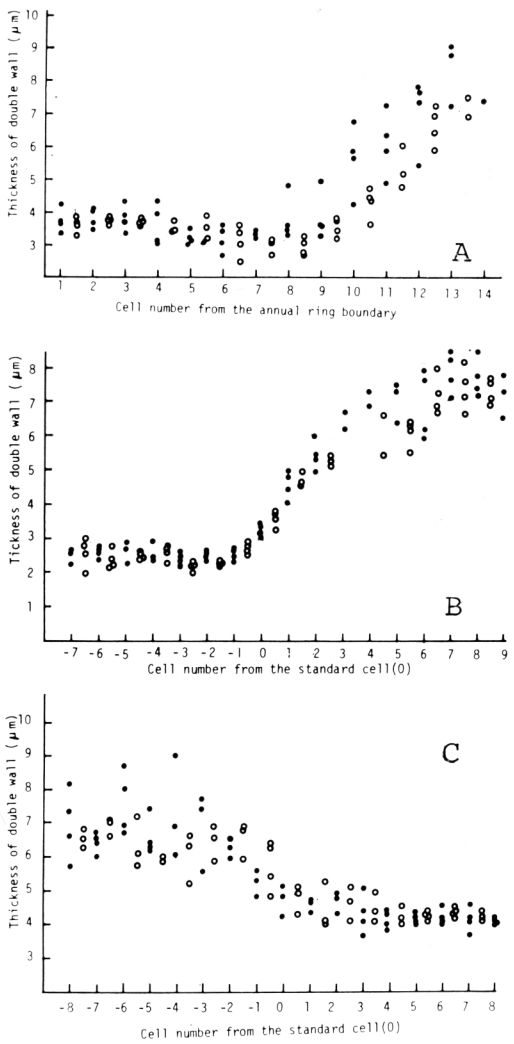
Fig. 1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Thickness of double wall (μm)
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Fig. 2

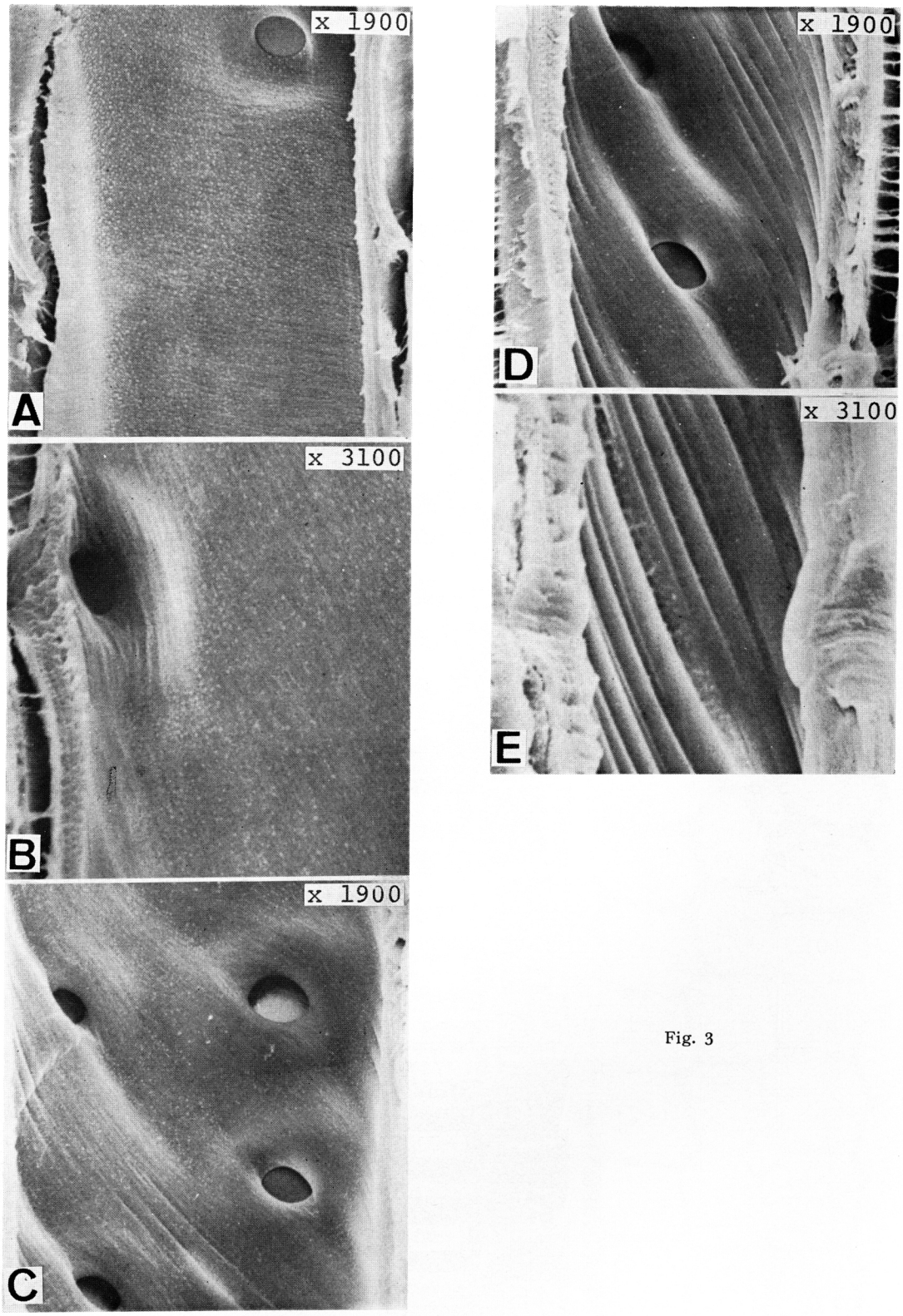
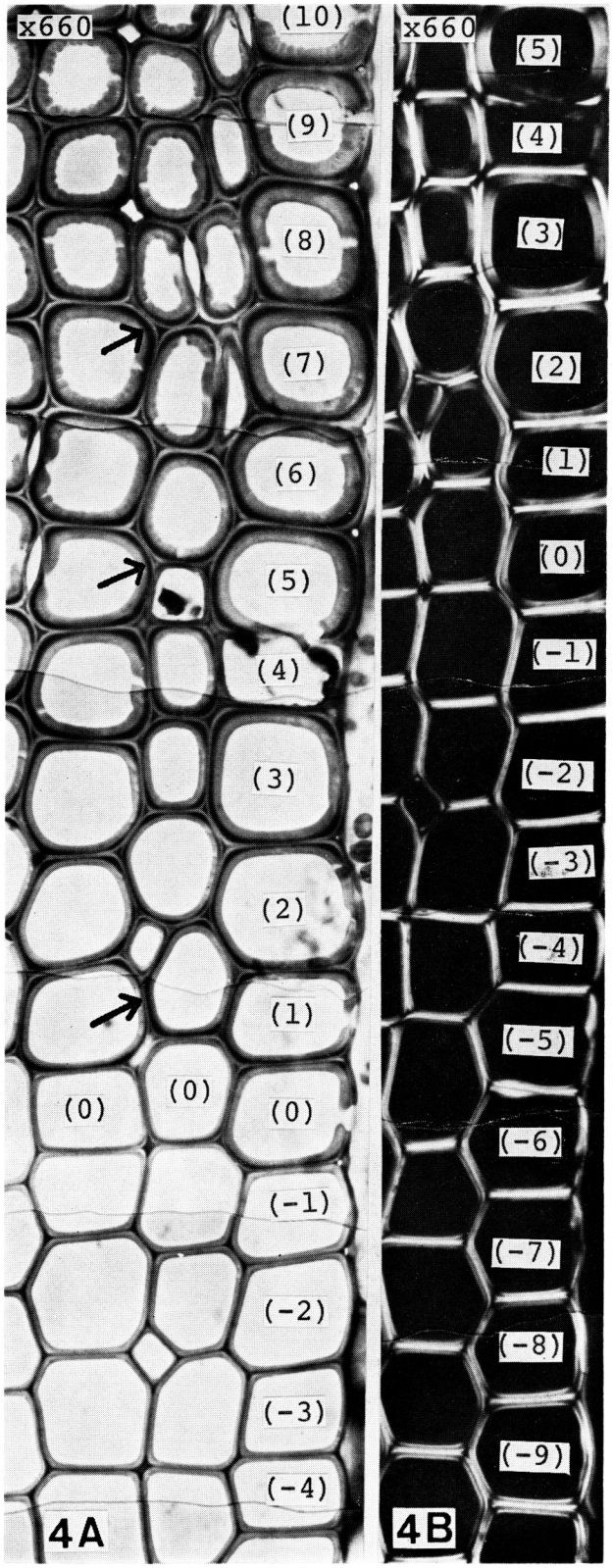


Fig. 3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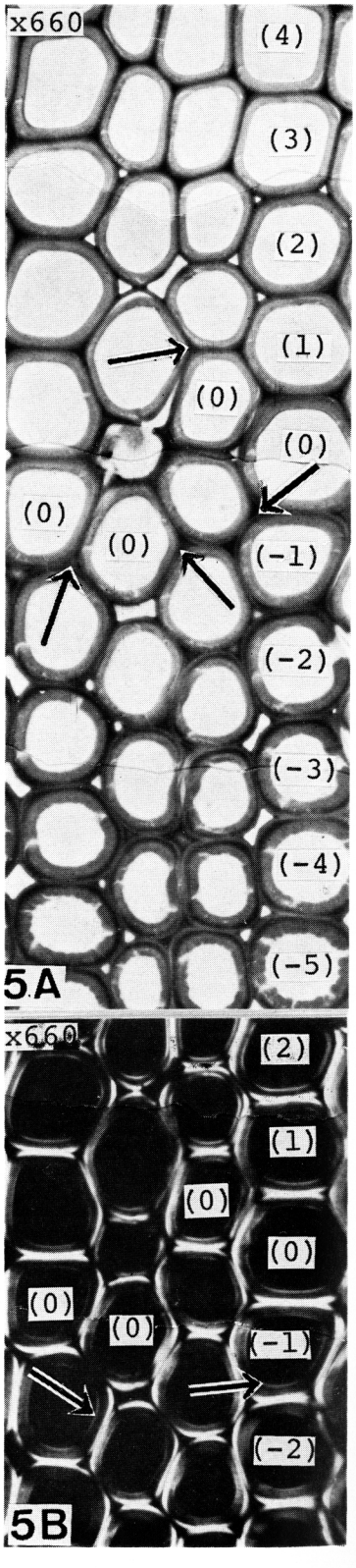
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