Lignification Process in Cryptomeria (*Cryptomeria japonica* D. Don) Tracheid: Electron Microscopic Observation of Lignin Skeleton of Differentiating Xylem.*

By

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スギ仮道管壁の木化過程：分化中木部のリグニンスケルトンの電顕観察*

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Abstract

Lignin skeleton of differentiating xylem obtained from the ultra-thin section was observed with TEM in order to investigate the lignification process of tracheid wall in cryptomeria (*Cryptomeria japonica* D. Don) at ultra-structure level.

Lignification was initiated at the outer surface of the primary wall in cell corner in the tracheid just before S_1 formation or beginning S_1 formation. Lignin was filled up step by step in the intercellular layer in cell corner with the tracheid maturation. Lignification of the intercellular layer and the outermost part of S_1 was initiated at cell corner in the S_1 formation stage, and gradually proceeded to the unlignified intercellular layer and outermost part of S_1, respectively. The lignification of primary wall began to spread in the tracheid beginning S_2 formation. When the tracheid came into contact with the ray parenchyma, outer surface of primary wall and intercellular layer in cell corner in ray parenchyma side were lignified earlier than those in the opposite side. Outermost part of

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S_1 in ray parenchyma side was also lignified earlier. Lignification of the secondary wall, which was initiated at the outermost part of S_1 in cell corner, proceeded to the unlignified outermost part of S_1, and spreaded out toward the lumen. In the tracheids after S_3 formation stage, lignin accumulation actively occurred all over the secondary wall. Consequently, lignin content of the secondary wall was fairly constant, though the warty layer was highly lignified.

1 Introduction

The investigation of the lignification of conifer tracheid was initiated by WARDROP (1957). Using an UV-microscope, he observed the differentiating xylem of radiata pine (*Pinus radiata*), and measured the silver density of UV-photonegatives due to the UV-absorption by lignin. In recent years, Imagawa and others (1976), and Fujita and others (1978) prepared for 0.5 μm thick sections and observed them closely under an UV-microscope. Although these observations were effectual in the study of lignification, this method had some problems. The data obtained by UV-microscopy are apt to be influenced by the artifacts of sectioning, such as cracks, wrinkles and unevenness of section. The resolving power of an UV-microscope is lower than that of TEM. In addition, it is still uncertain whether a trace amount of lignin can be detected under an UV-microscope.

A few workers (Hepler and others, 1970 ; Wardrop, 1971 ; Kutscha and Schwarzmann, 1975) also investigated the lignification of the cell wall with TEM using the specimen fixed with potassium permanganate. Kishi and others (1982), however, showed that the staining intensity with potassium permanganate was not always parallel to the amount of lignin in the cell wall. A cell wall where the cutting surface is parallel to the microfibril orientation is strongly stained with potassium permanganate, but the one where the cutting surface is perpendicular is weakly. Therefore, it is still ambiguous whether lignin is selectively stained with potassium permanganate. It is necessary to reexamine the lignification process of xylem element at the ultra-structure level by new techniques.

In recent years, Fujii and others (1981) developed the new method to obtain the lignin skeleton from the ultra-thin section. They cut ultra- thin sections and mounted them on carbon/formvar-coated molybdenum grids. After removing epoxy resin, they dipped the grids into 55% HF in order to hydrolyze polysaccharides without swelling. This method makes it possible to obtain the lignin skeleton from the fragile tissues such as the differentiating xylem. Lignin residue can be seen as the dense deposits within the cell wall under an electron microscope. In this study, the lignin skeleton of defferentiating xylem was observed with TEM in order to investigate the lignification process of tracheids at the ultra-structure level.

2 Materials and methods

2.1 Specimen preparation

Seven-or eight-year old cryptomeria(*Cryptomeria japonica* D. Don) grown at Kyoto Experimental Nursery, Kyoto University, Kyoto, was used. Small blocks were cut from the internode, fixed in 3% glutaraldehyde and 1% osumium tetroxide, and embedded in epoxy resin in the usual way.

2.2 TEM-observation of the lignin skeleton

Ultra-thin sections were cut on a LKB-ultramicrotome with a diamond knife from the

specimen block. They were mounted on 100 mesh Mo-grids, which were coated with a formvar support film and followed by evaporation of carbon. Epoxy resin was removed from the sections according to the method of Mayor and others (1961). The sections were hydrolyzed according to the method of Fujii and others (1981). They were observed with a JEM-7 electron microscope at 80 KV. Ultra-thin sections stained with uranyl acetate and alkaline lead citrate were also observed with TEM for checking the differentiating stage of tracheid.

3 Results

Figure 1 shows the tracheids from the final part of primary wall formation to the S_1 formation stage. Lignin residues were first observed at the outer surface of the primary wall in cell corner in the tracheid just before S_1 formation or the beginning part of S_1 formation. The outer surface of the primary wall was gradually accumulated with lignin residue, and the intercellular layer in cell corner was also filled up with the lignin residue with the tracheid maturation. When the tracheid was in contact with the ray parenchyma, lignin residue was observed earlier at the outer surface of the primary wall and intercellular layer in cell corner of the ray parenchyma side than those of the opposite side of the ray parenchyma. When the intercellular layer in cell corner was relatively wide, the bulk of lignin residue (this differs from the "bulk polymer" of lignin proposed by Sarkanen ; 1971) sometimes was observed (Figure 2).

Figure 3 shows the tracheids from the S_1 formation to the early part of S_2 formation stage. The lignin residue at the intercellular layer was spreaded from cell corners. In the early part of S_1 formation stage, the lignin residue was observed at the outermost part of S_1 in cell corner, and spreaded to the unlignified outermost part of S_1 with the tracheid maturation. The lignin residue at the outermost part of S_1 neighboring the ray parenchyma was also observed earlier than that not neighboring the ray parenchyma.

Figure 4 shows the tracheids from the S_2 formation to the S_3 formation stage. The lignin residues at the intercellular layer and the outermost part of S_1 had an appreciable density. The primary wall was gradually filled up with the lignin residue. The lignin residue at the secondary wall was spreaded toward the lumen of tracheid, lagging behind the cell wall thickening. At this time, the lignin residue at the outer portion of S_1 was much more dense than that at the inner portion of S_1 (Figure 5).

Figure 6 shows the tracheids at the S_3 formation stage. The lignin residue at the secondary wall was continuously spreaded toward the lumen, and reached to the lumen surface of the cell wall. At this time, the outer portion of the secondary wall showed a high density of the lignin residue. When the secondary wall lignification was completed, the secondary wall showed a uniform density of the lignin residue, though the warty layer had slightly more dense lignin residue than the secondary wall(Figures 7 and 8).

4 Discussion

The lignification of the tracheid wall was investigated by means of UV-microscopy (Takabe and others, 1981) and TEM adapting to the observation of lignin skeleton of differentiating tracheids. These methods have both advantage and disadvantage in order to investigate the lignin accumulation. UV-microscopy has an advantage for quantitative determination of lignin by measuring the silver density of UV-photo negatives, although it

has low resolving power. TEM adapting to the observation of lignin skeleton of the differentiating tracheid has a great advantage to observe closely the lignin accumulation because of the high resolving power. However, it is not possible to determine the amount of lignin from the silver density of TEM-negatives. Therefore, it is necessary to combine the advantages of these methods in order to elucidate the lignification process of cell wall more closely.

Lignin accumulation is initiated at the outer surface of the primary wall in cell corner in the tracheid just before S_1 formation or beginning S_1 formation. Wardrop (1957) and Imagawa and others (1976) reported that the lignin accumulation at cell corner occurs lagging behind the S_1 formation. The difference between their results and our result is probably derived from the low resolving power of UV-microscopy or the different fixatives. The detection of lignin at the outer surface of primary wall may be impossible by means of UV-microscopy. Lignification at the outer surface of the primary wall gradually proceeds with the cell wall maturation. Lignin is also filled up step by step in the intercelluar layer in cell corner with the tracheid maturation. When the intercellular layer in cell corner is relatively wide, a bulk of lignin is produced. Lignification of the intercellular layer and the outermost part of S_1 is initiated at cell corner in the S_1 formation stage and gradually proceeds to the unlignified intercellular layer and the outermost part of S_1, respectively. The lignin spreads at the whole of the intercellular layer and the outermost part of S_1. The lignification of primary wall begins to spread out in the tracheid beginning S_2 formation. When the tracheid comes into contact with the ray parenchyma, the outer surface of the primary wall and intercellular layer in cell corner, and the outermost part of S_1 are lignified earlier in ray parenchyma side than in the opposite side.

Lignification of the secondary wall is initiated at the outermost part of S_1 in cell corner, proceeds to the unlignified outermost part of S_1, and gradually spreads out toward the lumen. The amount of lignin accumulated to the secondary wall during the S_2 formation stage is relatively small. In the tracheids after the S_3 formation stage, lignin accumulation actively occurs all over the secondary wall. At this time, the lignin precursor is continuously supplied to all over the secondary wall, and the amount of lignin in the secondary wall is gradually increased by the continuous linking of the monolignol moieties. Consequently, lignin content of the secondary wall becomes fairly constant, though the warty layer is highly lignified.

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要　　約

スギ（*Cryptomeria japonica* D. DON）仮道管壁の木化過程を電顕レベルで調べるために，超薄切片から分化中木部のリグニンスケルトンを作製し，透過型電顕で観察した。

木化はS_1形成直前かS_1形成開始期の仮道管コーナー部の一次壁外表面で開始された。その後リグニンは仮道管の成熟にともないコーナー部細胞間隙に徐々に沈着された。細胞間層やS_1最外層の木化は，S_1形成期の仮道管においてコーナー部より開始され，それぞれ徐々に未木化の部位へ進行していった。一次壁の木化はS_2形成開始期の仮道管で進行し始めた。仮道管が放射組織に接している場合は，一次壁外表面やコーナー部細胞間隙，S_1最外層の木化は，放射組織に接している部位で早く開始されていた。二次壁の木化はコーナー部のS_1最外層で開始されたが，それは未木化のS_1最外層へと進み，その後内腔側へと進行した。S_3形成後の仮道管では，二次壁へのリグニンの沈着が活発に行われた。その結果，二次壁のリグニン濃度は，いぼ状層が高度に木化していた点をのぞけば，ほぼ均一となった。

Explanation of figures

Fig. 1. The tracheids from the final part of primary wall formation stage to the early part of S_1 formation stage. Lignin residue is first observed at the outer surface of the primary wall in cell corner (arrow head) in the tracheid just before S_1 formation. The density of lignin residue at the outer surface of the primary wall is gradually increased with the development of cell wall. The intercellular layer in cell corner is also filled up with lignin residue. When the tracheid comes into contact with the ray parenchyma, the lignin residues at the outer surface of the primary wall and the intercellular layer adjacent to the ray parenchyma (arrow) are detected earlier than those of the opposite side. Figure la represents the enlarged view of the cell corner shown in Figure 1. Small amount of lignin residue is observed at the outer surface of primary wall.

Fig. 2. When the intercellular layer in cell corner is relatively wide, a bulk of lignin residue (arrows) is sometimes observed.

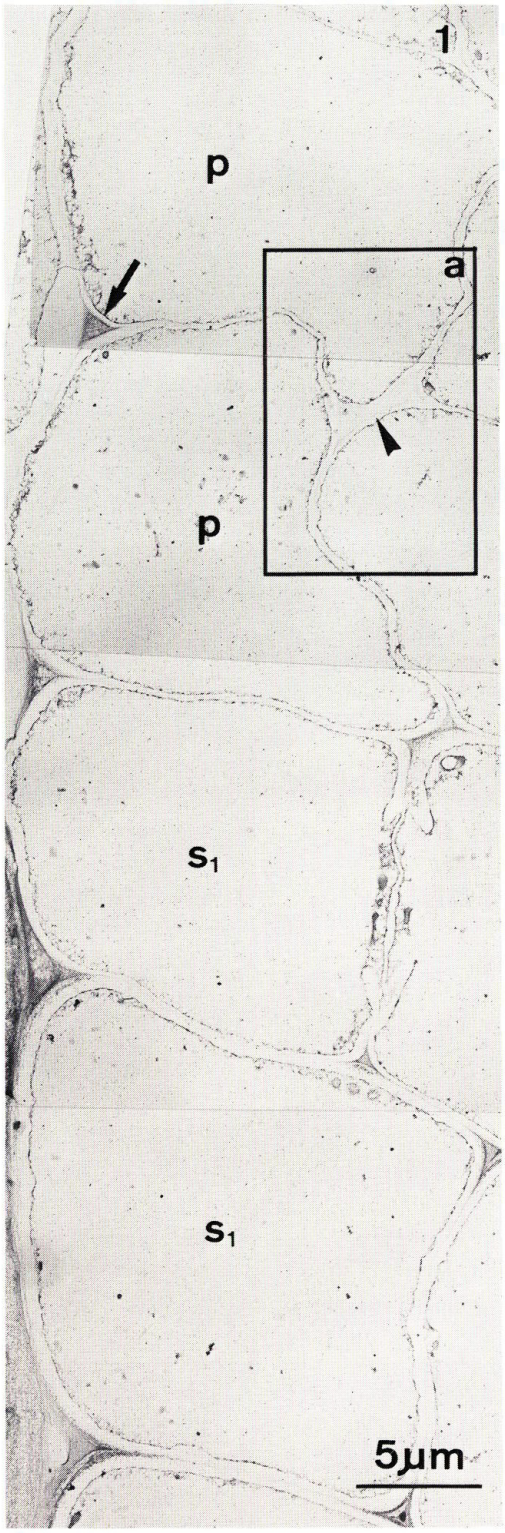
Fig. 3. The tracheids from the S_1 formation to the early part of S_2 formation stage. The lignin residue at the intercellular layer is spreaded from cell corners. The lignin residue is observed at the outermost part of S_1 in cell corner (arrow heads) in the early part of S_1 formation stage and is spreaded to the unlignified outermost part of S_1. The lignin residue at the outermost part of S_1 is also observed earlier in the ray parencyma side (arrow) than in the opposite side. Figures 3a and 3b are higher magnifications of cell corners in Figure 3. These figures clearly represent that secondary wall lignification is initiatied at the outermost part of S_1 in cell cormer. A pair of Figures 3c and 3d is also higher magnifications of cell wall shown in Figure 3. The lignin residue at the outermost part of S_1 can be observed in the ray parencnyma side, though not in the opposite side.

Fig. 4. The tracheids from the S_2 formation stage to the S_3 stage. The lignin residue of the secondary wall gradually proceeds toward the lumen, lagging behind the cell wall thickening.

Fig. 5. The tracheids in the S_2 and S_3 formation stages. Lignin residues of the compound middle lamella and the outer portion of S_1 gradually increase in their density with the maturation of tracheid. During these formation stages, the outer portion of S_1 has much more dense lignin residue than the inner portion of S_1. Lignin residue of secondary wall slowly spreads out toward the lumen, lagging behind the cell wall thickening.

Fig. 6. The tracheids in the S_3 formation stage. The lignin residue of the secondary wall proceeds toward the lumen and reaches to the lumen surface of the cell wall. At this time, the density of lignin residue at the outer portion of the secondary wall is still higher than that at the inner portion.

Figs. 7 and 8.
Mature tracheids. Secondary wall has approximately a uniform density of lignin residue, though the warty layer (arrow) has slightly higher density.
Compound middle lamella and ray parenchyma cell wall show a high density of lignin residue.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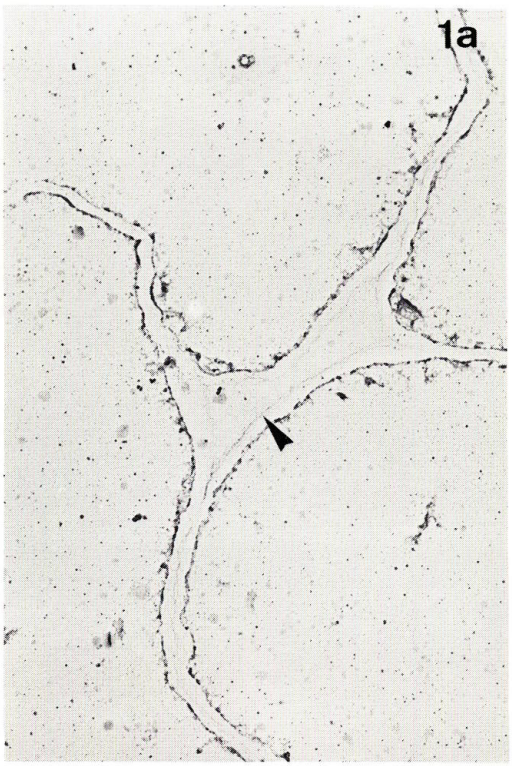
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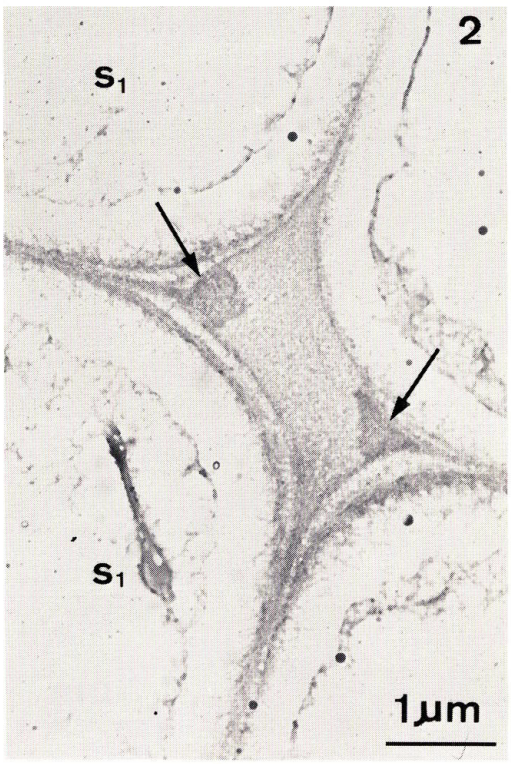
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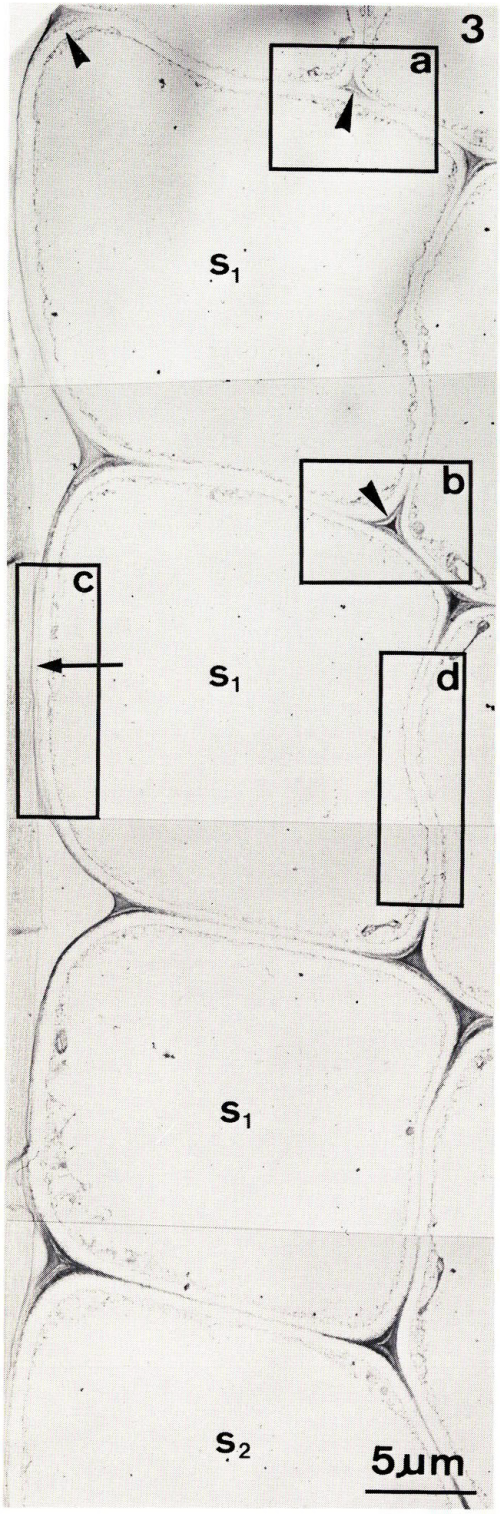
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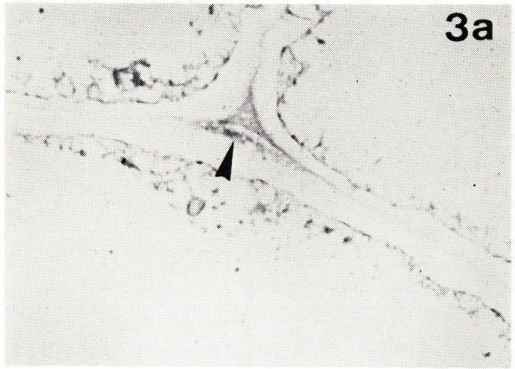
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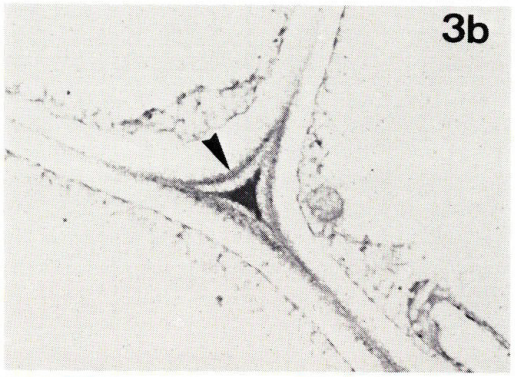
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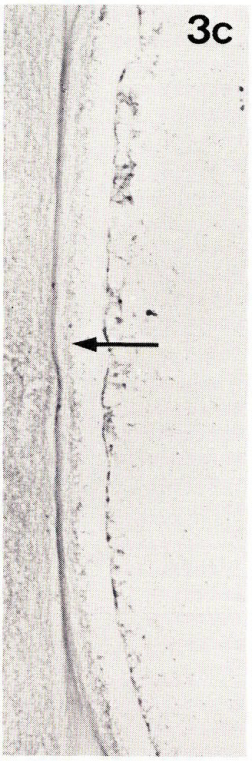
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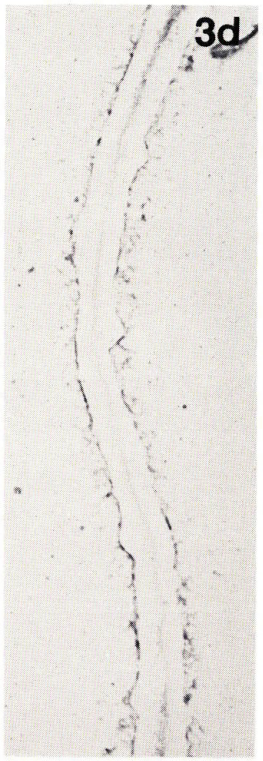
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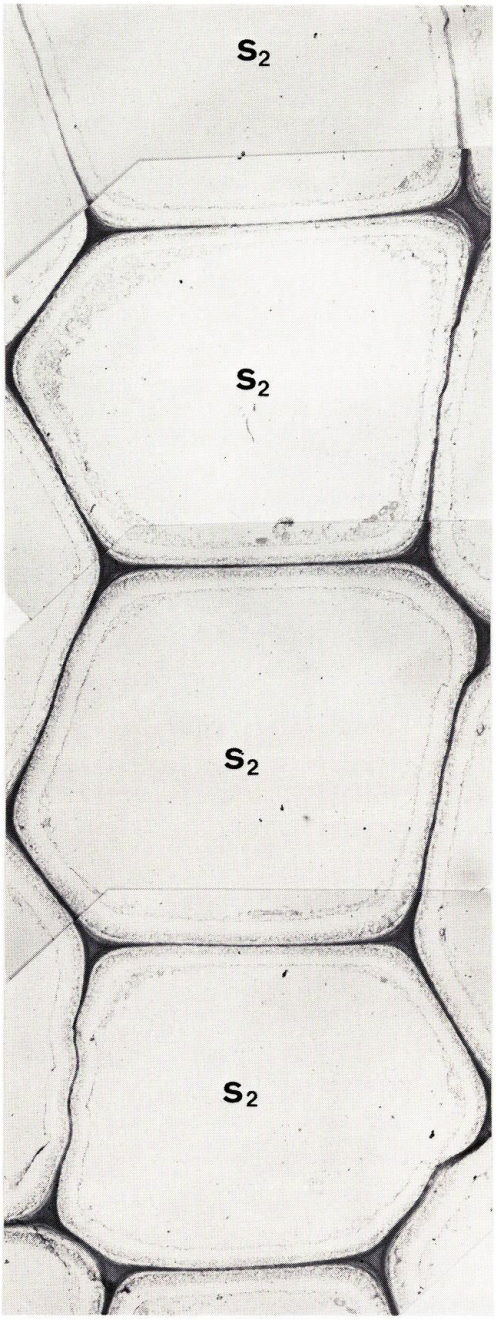
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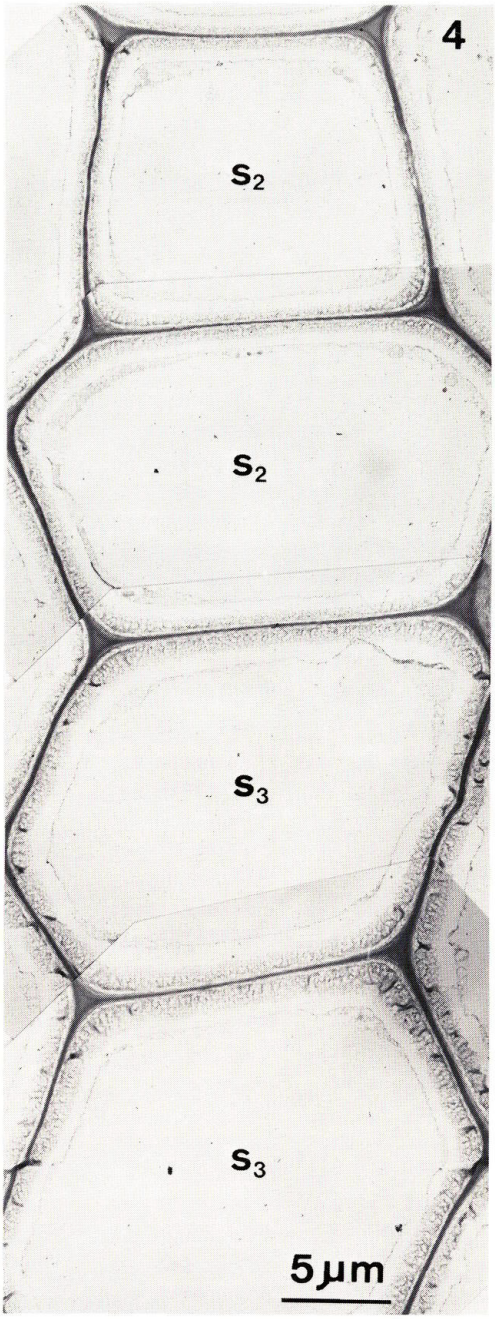
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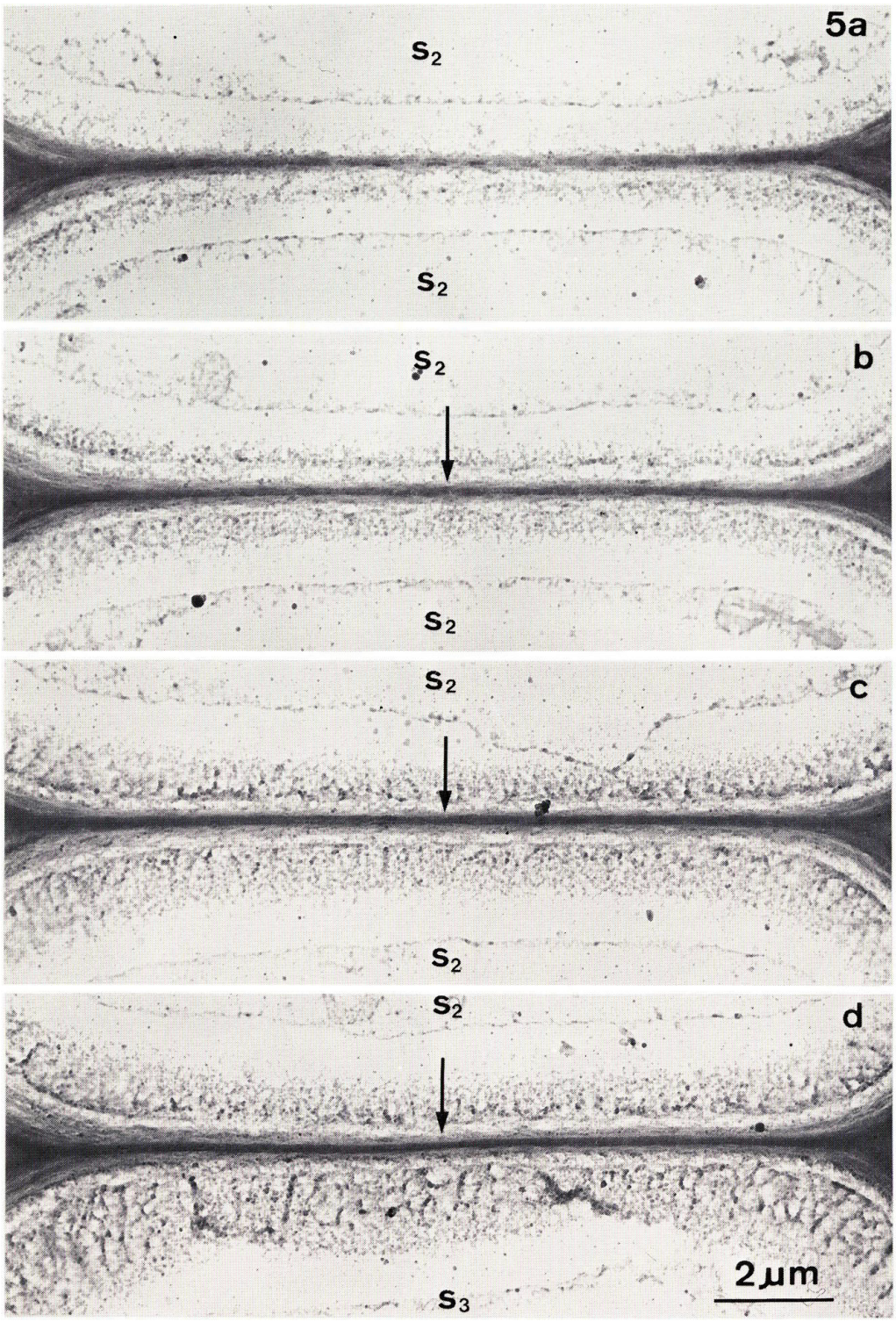
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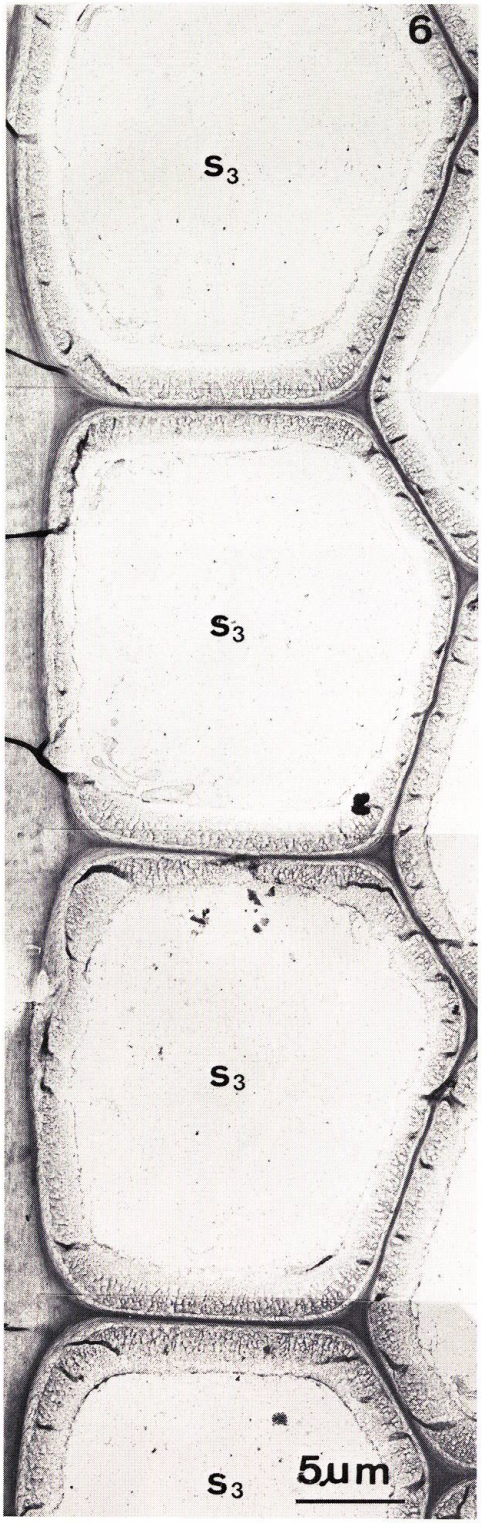
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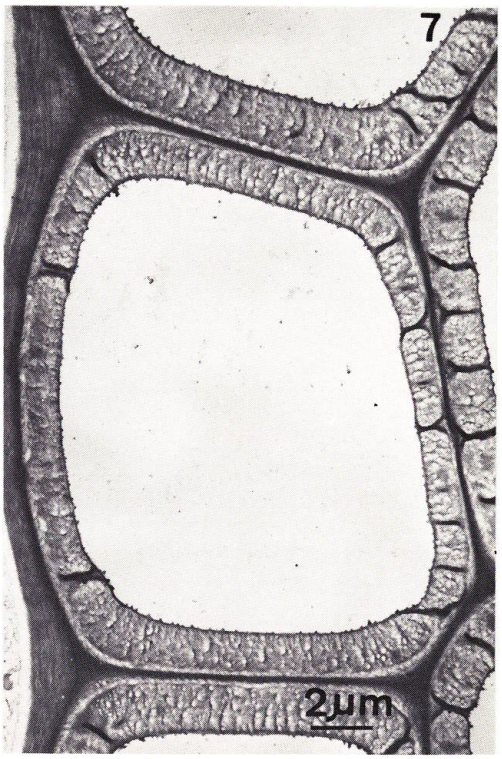
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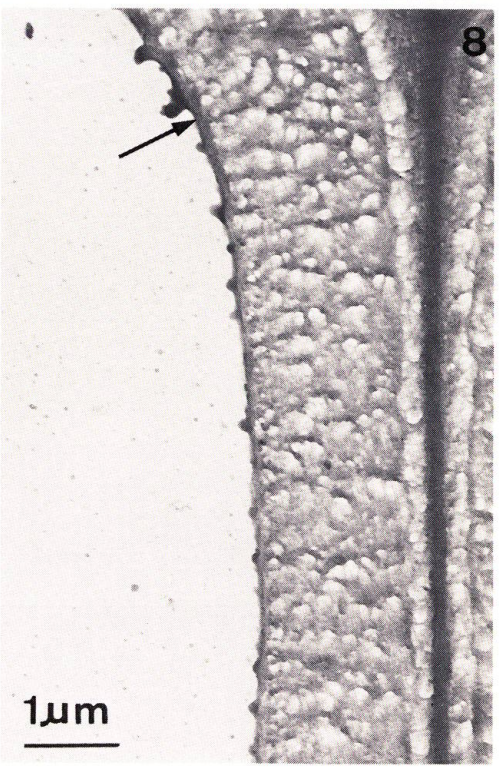
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