An Observation of the Cambial Cells in *Larix leptolepis* by Semi-Ultrathin Sections

By

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薄切片によるカラマツ, *Larix leptolepis* の形成層細胞の観察

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CONTENTS

Introduction

The radial growth of forest tree is accomplished by cell divisions in cambium. Many investigators have been concerned about this lateral meristem and numerous histological or cytological studies have been done. Thus, not a few models about the cellular structure of cambium have been proposed (BANNAN 1962, WILSON, WODZICKI and ZAHNFR 1966, Brown 1970, PHILIPSON, WARD and BUTTERFIELD 1971, STEEVES and SUSSEX 1972, BUTTERFIELD 1975, PHILLIPS 1976 and SCHMID 1976).

According to the fact that cambium produces xylem and phloem elements toward the both sides of it, it seems to be reasonable to assume so-called initial as a boundary between xylem and phloem. And practically, mainly in conifers, initial has been pointed out (SANIO 1873, NEWMAN 1956, BANNAN 1955, 1967, MAHMOOD 1968, MURMANIS 1970, 1971, 1977 and BARNETT 1973). Such initial is meaningly called as follows; permanent initial (PHILIPSON WARD and BUTTERFIELD 1971), distinctive initial (STEEVES and SUSSEX 1972), persistent cambial initial (KUTSCHA, HYLAND and SCHWARZMANN 1975), true or functional initial (PHILLIPS 1976) or initial proper (MURUMANIS 1977).

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On the other hand, some investigators have regarded the cells in cambium as homogeneous (WILISON 1964) or multiseriate (CATESSON 1974). And also the others have failed to distinguish initials from other cambial cells by ultrastructural observations (EVERT and DESHPANDE 1970 and TSUDA 1975).

As mentioned above, the cellular structure of cambium is not fully clarified. Therefore, more detailed studies muct be made. Since cambium is principally regulated by seasonal factor, the seasonal changes of cambium in Japanese larch stem are investigated in this study. On the basis of the results obtained, the cellular structure of cambium is discussed.

Materials and Methods

Specimens of wood and adherent bark were removed from the trunks of Japanese larch, *Larix leptolepis*, about 15 year-old. grown at the nursery in the campus of Hokkaido University. The collections were made periodically at about two week intervals from Apr. to Oct. in 1974 and May to Nov. in 1976. Moreover, specimens were also obtained from May to June in 1977. The materials taken in 1974 were same as those examined in previous study (IMAGAWA, FUKAZAWA and ISHIDA 1976). At each collection, one specimen was removed from each of two trunks.

Immediately after the removal, specimens were fixed in FAA solution, and then they were cut in pieces and embedded in epoxy resin for electron microscopy. Embedded specimens were cut transversely by an ultramicrotome with glass knives. Semi-ultrathin sections (1～3 microns in thickness) were stained with basic fuchsin as described by HUBER, PARKER and ODLAND (1968) and mounted. Such semi-ultrathin sections were examined by an ordinary light microscope with green filter.

Results

If semi-ultrathin sections used as a complement for electron microscopy are applied to light microscopy instead of usual light microscopical sections (BOUTELJE and ISHIDA 1963, ISEBRADS and LARSON 1973), it is expected that further detailed observations are possible and further precise knowledges can be obtained in comparision with usual light microscopy. In this study, it was confirmed that semi-ultrathin sections were very effective for the observation of the very thin walls of cambial cells. Moreover, in contrast with electron microscopical observations restricted to extremely smaller regions in an ultrathin section, entire regions in a semi-ultrathin section could be observed.

In light microscopy, it has been suggested that it is questionable to distinguish the very thin walls of cambial cells, especially newly formed partitions (MURMANIS 1970, BARNETT 1973). In this study, however, it was not troublesome to identify the partitions in the semi-ultrathin sections. Moreover, since specimens were fixed in FAA solution which tends to cause plasmolysis, even cells soon after cell divisions could be distinguished with more certainty comparing with complemental observations about such plasmolysis.

The term cambium used in this paper is not clearly defined as proposed by WILSON, WODZICKI and ZAHNER (1966), BUTTERFIELD (1975) and SCHMID (1976). Consequentely, for convenience sake, cambium implies cells between xylem and phloem cells which are radially enlarged. Individual cells in cambium are termed cambial cells. And ray initials are termed cambial ray cells.

Photo 1 shows the cambium in dormancy. Mature latewood tracheids produced in the previous growth period are found below, and mature phloem elements above. At the middle, tangentially flattened cells are regularly arranged in radial file. Such flattened cells are overwintering cambial cells. Walls (double walls) of these cells are considerably thick and in particular radial walls are very remarkable (BANNAN 1955, MURMANIS 1971 and PHILIPSON, WARD and BUTTERFIELD 1971). Among the cells adjacent to mature tracheids, there are cells of which the radial diameters are relatively larger than the others. They seem to be cells which have already lost the capacity for cell division and to initiate differentiation immediately in spring.

From the upper to the bottom cambial ray passes through the cambium. Cambial ray cell is found to be almost isodiametric in shape and has thick wall.

Photo 2 shows the cambium in transitional stage from dormancy to active period. Cambial cells are somewhat enlarged radially in comparision with those in dormancy (Photo 1). It has been well known that the swelling of cambial cells is the first morphologically visible event in spring (BANNAN 1955, 1962, PHILIPSON, WARD and BUTTERFIELD 1971 and PHILLIPS 1976). Protoplasms are changed to be lighter than those in dormancy (Photo 1). However, cell divisions have not occured at all.

Photo 3 shows the cambium shortly after the initiation of cell divisions. Three redial files of cambial cells and one cambial ray are observed. Many thin tangential walls and a few thick ones (arrows) are found in each file. The thick walls seem to be all the same to those of the cambial cells in dormancy (Photo 1) and transitional stage (Photo 2). However, the thin walls have not been found until this time. That is, the thick walls belong to the overwintered cambial cells while the thin ones belong to the newly formed cells which have resulted from the periclinal divisions of overwintered cambial cells. Most overwintered cells have been already partitioned into four new cells. The cell with six new cells is also found. The thickness of new walls may be more or less variable. However, the measurement of the thickness is difficult because of low resolving power of light microscope. Radial walls become thinner than those in non-active period (Photos 1 and 2), since the radial expansion of overwintered cambial cells causes the streching of radial walls.

It is apparent that cell divisions have already occurred in cambial ray cell. Five isodiametric cells are seen, but the end walls are not same in thickness. It is presumed that the overwintered ray cell with thick walls has been partitioned into four isodiametric cells.

Photo 4 shows the cambium later from the initiation of cell division. Four radial files (A, B, C and D) and one cambial ray are observed. In each radial file, some overwintered cells with thick tangential walls (arrows) and many new

cells with thin tangential walls are found. In the file A and C, the overwintered cells at almost middle portion have been partitioned into eight, while such ones in the file B and D into seven. Perhaps, eighth partition in the file B and D can be observed in the another section far from this section, since partition is formed from the center toward both tips of cell (FREY-WYSSLING and MÜHLETHALER 1965, MÜLETHALER 1965, LEDBETTER and PORTER 1970, ROBARDS 1970, STEEVES and SUSSEX 1972, GUNNING and STEERE 1975, DYER 1976, and PHILLIPS 1976).

Another overwintered cells at the xylem side have been also partitioned into two or five. Since some of them are considerably enlarged radially, they seem to advance into differentiating process. In the file A and B, two cells adjacent to mature tracheids appear to be matured without further enlargement (large arrows). In the other overwintered cells at the phloem side some cell divisions have occurred. However, thick tangential walls of the overwintered cell are not found as a pair, because some of new cells have proceeded into differentiating process. New phloem parenchyma cell in which cell contents are depositing is also observed (P).

Photo 5 shows the cambium at the same time as Photo 4. From the latewood tracheids, three overwintered cells are easily distinguishable. The fourth is not identified, because some of the new cells have already initiated the differentiation. In the two cells pseudotransvere divisions have occurred. Such doubling of radial file was observed at first after the initiation of cell divisions. It is noticeable that pseudotransverse divisions or doubling is found at such position, that is, at the phloem side of the overwintered cell which located at the middle of the cambium.

Photo 6 shows the cambium and the differentiating cells in most active period. The tangential walls of cambial cells are considerably variable in thickness as shown in the earlier period. Such difference is recognizable even in the differentiating cells.

In the file A, the tangential walls 1, 2, 3 and 4 are thicker than the others. Between the wall 1 and 2, five thin walls which belong to six new cells are observed, and these walls also more or less variable in thickness. Between the wall 2 and 3, and the wall 3 and 4, four cells are found respectively, which have already initiated the differentiation to some extent.

In the file B, the wall 1, 2, 3 and 4 are apparently thick. Between the wall 1 and 3, there are eight cells. However, the four cells between the wall 1 and 2 are not same state as those between the wall 2 and 3. The radial diameter of the latter seems to be somewhat larger than the former. Perhaps, the latter cells may advance into the differentiating process. And it is considered that in the former cells further periclinal divisions occur to become eight cells. In the file A, the six cells between the wall 1 and 2 seem to undergo cell divisions to become eight cells. Thus, in the file A, the cell which have been partitioned into those six cells is xylem mother cell, and also in the file B the cell with these four cells is same one. Such mother cells at this stage are not overwintered cells which have been found in the early period (Photos 1, 2, 3 and 4). Since a grear number of cells have been produced and many of them have been differentiated, it is not considered that the overwintered cells have been kept still in the cambium until

this time.

The doubling of radial file is observed at the phloem side of the wall 1 in the file B. Such doubling has occurred at the nearly same position as in the earlier period (Photo 5). It appears that the four cells near the wall 1 are undifferentiated and the two (arrows) adjacent to this four are differentiated to some extent because of the difference of radial diameter. In the file A, the two cells which are located near the wall 1 are undifferentiated and have relatively thick tangential wall, and the larger cell at the phloem side of the two seems to be differentiated. The phloem parenchyma cells with dense content are also seen (P).

Photo 7 shows the cambium at the almost same time in Photo 6. Radial files irregularly oriented are found. These cells show the appearance as if they are gathered to form groups of cells. Particularly, the eight cells between the thick tangential wall 1 and 2 seem to form a group according to the observation of overall configuration. This group corresponds to xylem mother cell. Some other groups are also recognized, but cells of which these groups consist are variable in number. Such groups were frequently observed in the cambium and ones with more than ten cells were found occasionally. In addition to the thick tangential wall, thus overall configuration of cambial cells is also effective to distinguish group of cells, that is, xylem mother cell which have been partitioned.

Photo 8 shows extremely thick tangential walls which are infrequently found at the position near or far from cambium (arrows). Such walls appear to belong to the overwintered cells, because they are considerably thicker than the usual thick walls of xylem mother cells in growth period. It is considered that they correspond to "extra thick walls" described by NEWMAN (1956) and MAHMOOD (1968).

Photo 9 shows the cambium and the maturing cells near the dormancy. Four or five cambial cells become flattened, and they resemble dormant cambial cells in appearance (Photo 1). Very thin tangential walls as in the growth period are not found. The walls of such cambial cells seem to become further thicker and the protoplasms denser in dormancy.

Discussion and Conclusion

In the growth period, the tangential walls of the cambial cells in the larchs examined were variable in thickness. Usually, a few thick walls and many thin ones were observed. The thick walls were located in pair, and several new cells with thin ones were found between them (Photos 3-6). These thick walls belong to original cells, i. e. mother cells, and the thin ones to new cells which are derived from the partitions of the mother cells. And also mother cells could be identified on the basis of the overall configuration of cambial cells, since in almost cases eight new cells were gathered to form a group. Such group was composed of the cells which resulted from the partition of a mother cell. The tangential walls of the mother cells were also markedly thick (Photo 7).

In regard to the difference of the tangential wall thickness in cambium, SANIO

(1873) mentioned that at each cell division, not only partition but also cell membrane which surround cell content is deposited. On the basis of this suggestion, several investigators indicated initial as a boundary in cambium (NEWMAN 1956, MAHMOOD 1968, MURMANIS 1970, 1971, 1977 and BARNETT 1973). However, it is considered that this suggestion is not fully proved or recognized (EVERT and DESHPANDE 1970, LEDBETTER and PORTFR 1970, ROBARDS 1970, PHILIPSON, WARD and BUTTEREIELD 1971, STEEVES and SUSSEX 1972, CATESSON 1974, GUNNING and STEERS 1975, DYER 1976, GREULACH and ADAMS 1976, and PHILLIPS 1976), although only a few investigators described the experimental evidence which might possibly support this (FREY-WYSSLING and MÜHLETHALER 1965, MURMANIS 1971, BARNETT 1973).

On the other hand, it has well known that the walls of cambial cells become thick near the end of growth period and extremely thick in dormancy (BANNAN 1955, MURMANIS 1971, and PHILIPSON, WARD and BUTTERFIELD 1971). In the larchs examined, such trend was also conspicuous (Photos 1 and 9). Near the dormancy, the frequency of cell divisions is reduced, and consequently cambial cells keep still in cambium for a longer time than in active period. While, since the activity of cambial cells may be possibly retained yet to some extent, thus walls may be thickened. Therefore it is assumed that the wall thickness of cambial cells is somewhat proportinal to the length of their stay in cambium. Applied this assumption to active cambium, it may be reasonble that the walls of mother cells become thick because they are existed in cambium for a relatively long time. And moreover, it may be possible to indicate the producing process of each new cell on the basis of the tangential wall thickness of them.

Assumed that the difference of wall thickness depends on the reason mentioned above, xylem mother cells can be identified in cambium and it seems to be easy to clarify the patterns of cell divisions in Japanese larchs. That is, at first mother cell is partitioned into eight cells. And then the four cells at the xylem side begin to differentiate, and the remainders are again partitioned into eight cells adding four. Thus, the repeat of such process seems to be the basic pattern of cell division in Japanese larchs. However it should be considered that some factors such as season, extent of cambial activity and so on may disturb this basic pattern.

According to this basic pattern, the existence of these four cells which retain the capacity for cell division may lead one to regard cambium as homogeneous (WILSON 1964) or multiseriate (CATESSON 1974). Moreover, these four cells may be indistinguishable each other at ultrastructure level (EVERT and DESHPANDE 1970 and TSUDA 1975).

It is very questionable that only one mother cell continues cell divisions infinitively. At a given stage, perhaps mother cell seems to lose the capacity for cell division and initiate the differentiation of themselves. In such case, another new mother cell must be inserted into the process of xylem production. The cells which may support this viewpoint were occasionally found out in the cambium. Such cells were located directly at the phloem side of the existing mother cells. They seem to be a part of the cells which produce phloem elements till this time. The tangential walls of these cells were relatively thick (file B in Photo 6). And

also it is noticeable that the doubling of radial file, i. e. pseudotransverse division was frequently observed at similar position (Photos 5 and 6). Although it is obscure whether such cells correspond to so-called initials or not, they are very interesting cells in future investigations.

In this study, the basic pattern of cell division in Japanese larchs were considerably clarified, but it was not enough to elucidate the cellular structure of the cambium. It may be necessary and significant to advance furthermore electron microscopical or cytochemical studies about cambium hereafter.

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要　　約

1. 生長期のカラマツの形成層帯を薄切片 (1～3 μ) を用いて光学顕微鏡観察したところ，細胞の切線壁における厚さの著しい相異が確認された。

2. この厚さの相異は，それぞれの細胞がそこに存在した期間の長さの相違によるものと考えることができる。

3. この考えにもとづくと，形成層帯細胞の分裂パターンは，細胞壁厚についての観察結果から次のように推定される。すなわち1つの紡錘形母細胞から8つの新細胞が生じる。その中の木部に近い4細胞は仮道管に分化し，残りの4細胞はさらに分裂して再び合計8細胞となる。

4. 基本的にはこのような分裂パターンから形成層帯細胞構成が定まり，その木部側細胞から分化帯に移行してゆくものと考えられるが，この modification，放射組織柔細胞分裂パターン等の問題をも含めてさらに新たな手法による研究が必要である。

Explanation of Photographs

Photo 1. The cambium in the dormancy. ; collected 17th Nov. 1976.

Photo 2. The combium prior to the initiation of cell divisions. ; collected 25th Apr. 1974.

Photo 3. The cambium soon after the initiation of periclinal divisions. ; collected 11th May 1976.

Photo 4. The cambium somewhat later from the initiation of cell divisions. ; collected 21th May 1976.

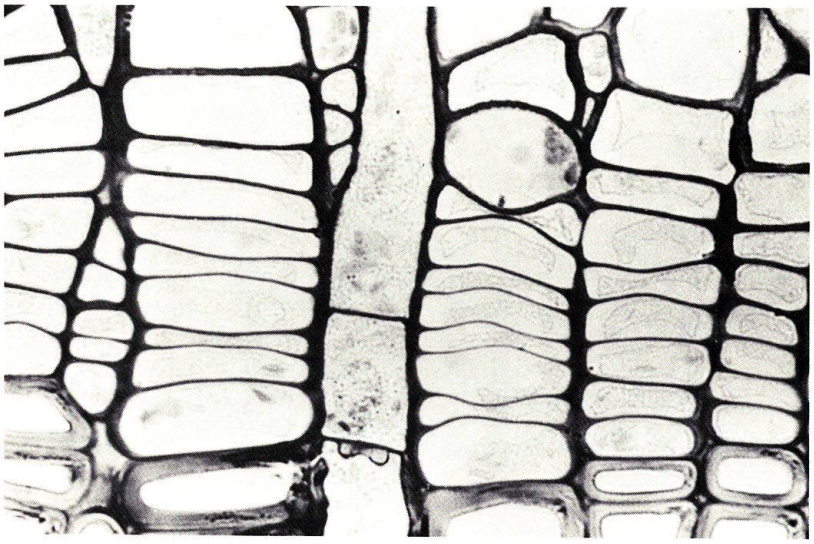
Photo 5. The doubling of the radial file. ; 21th May 1976.

Photo 6. The cambium in the growth period. ; collected 3rd June 1977.

Photo 7. The cambium in the growth period. ; collected 10th June 1974.

Photo 8. The cells with the extremely thick tangential walls. ; collected 29th May 1974.

Photo 9. The cambium at near the dormancy. ; collected 26th Sept. 1974.



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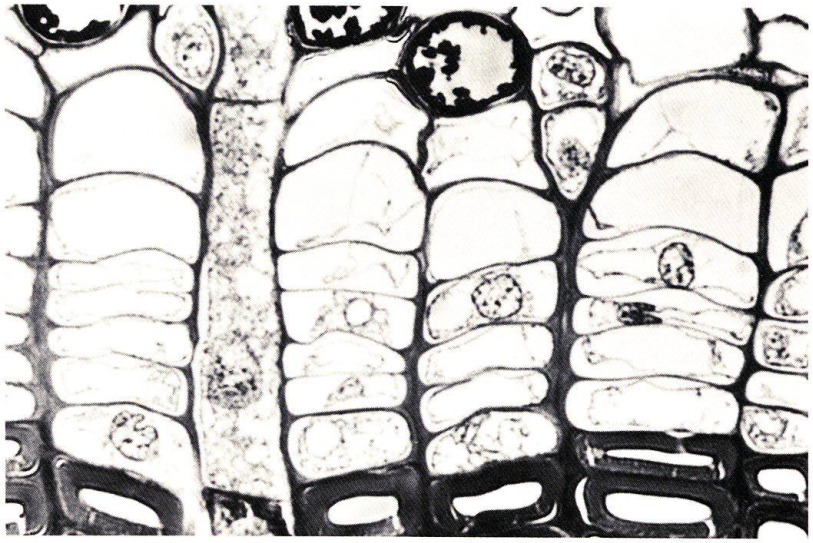

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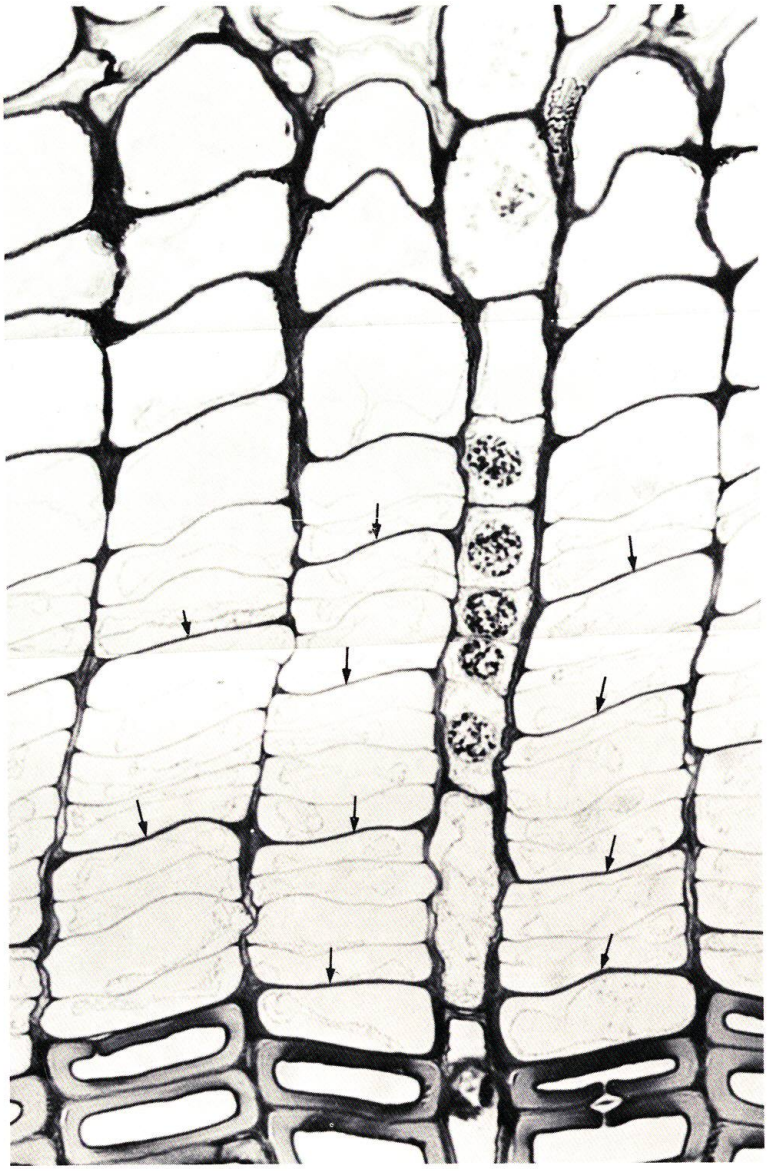

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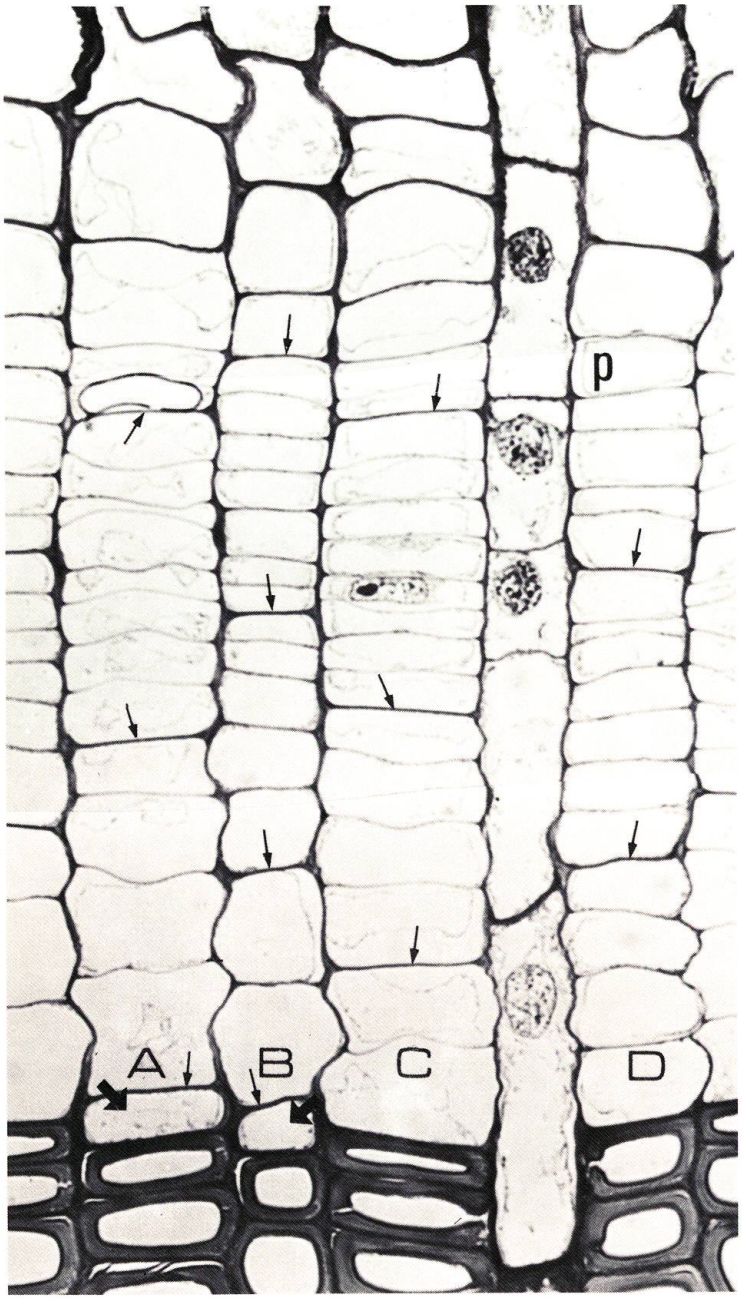

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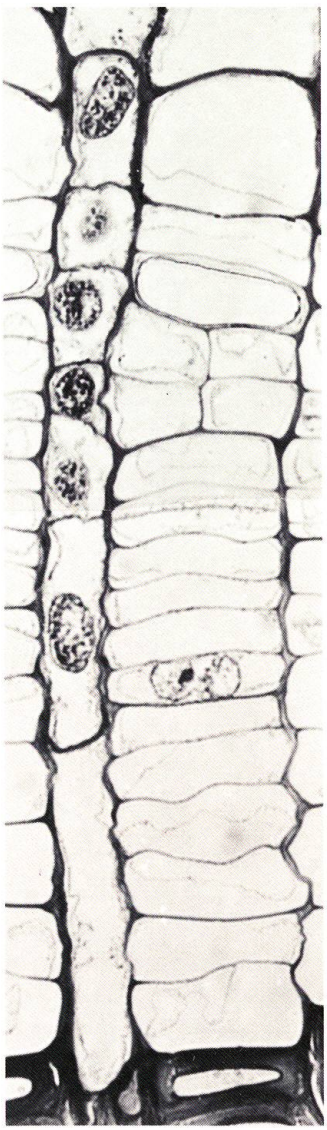

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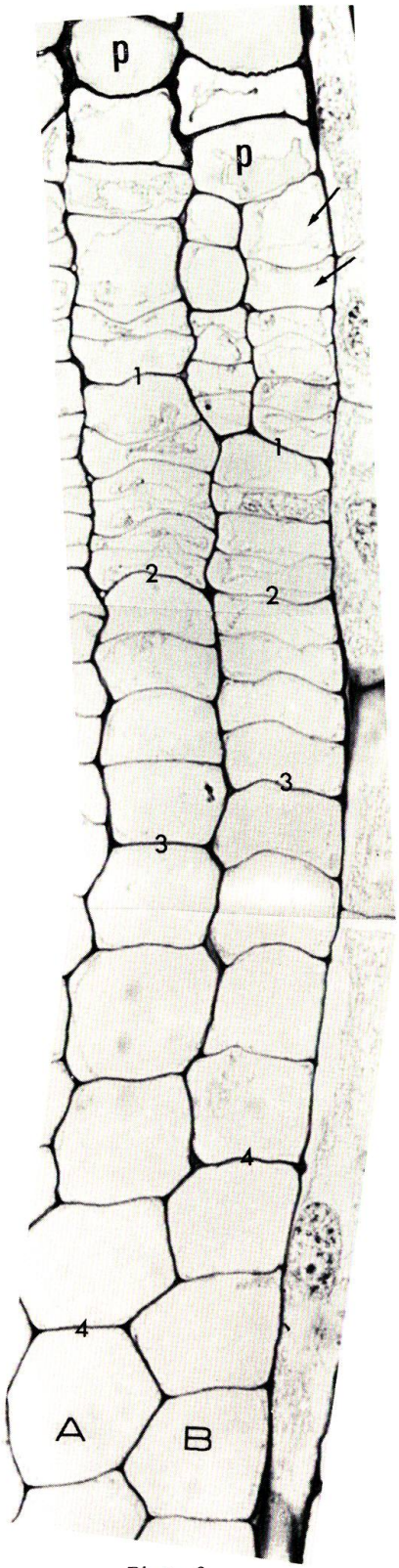

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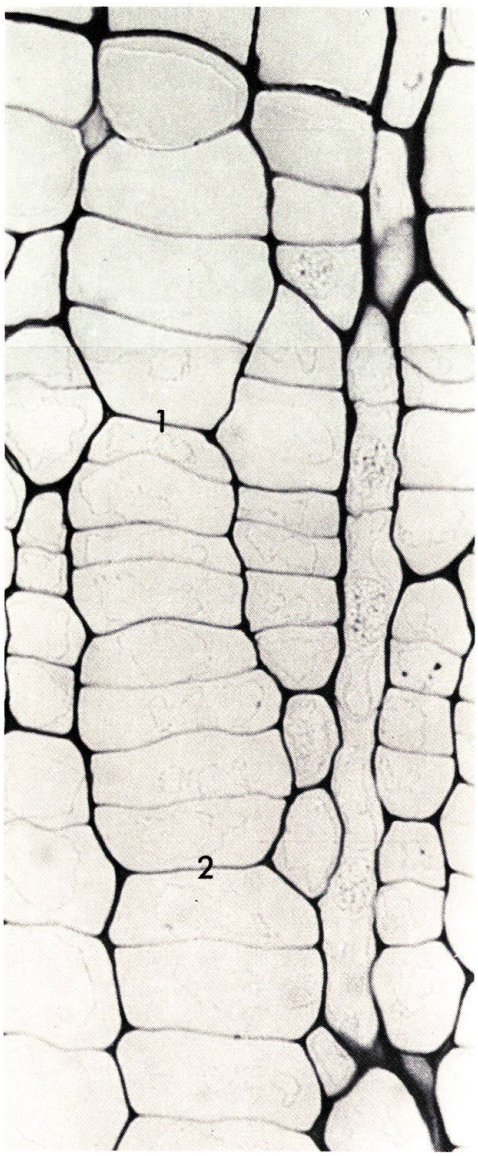

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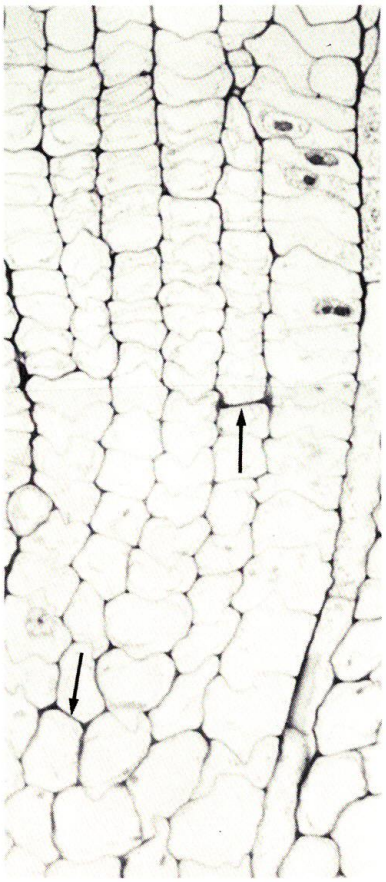

Photo 8

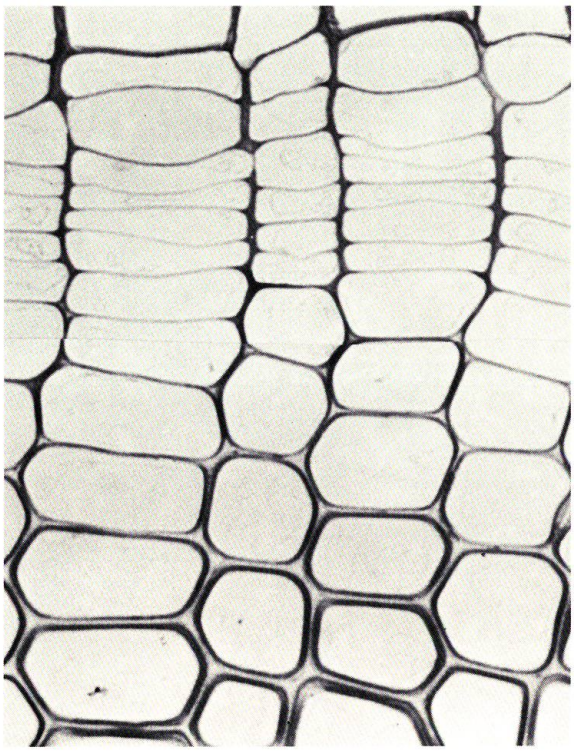

Photo 9