ELECTRON MICROSCOPY OF DEVELOPING PLANT CELL WALLS

by

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INTRODUCTION

The young cell wall of a plant consists of three layers; the middle lamella, which is laid down in the nuclear spindle during cell division and the two primary membranes which are deposited onto this lamella after the division into the two daughter cells. This threefold wall greatly increases in area as the cell grows; the important problem of how this occurs has been one that could not heretofore be followed either with the help of the optical microscope, the polarizing microscope or by means of X-ray diffraction. It can be studied, however, through electron microscopy.

The mechanism of this growth is made especially interesting by the fact that no decrease in the thickness of the cell wall has been seen as the cell enlarges. Consequently we are concerned with a process that involves the addition of new substances and not a mere stretching of the existing wall. Once a young cell has reached its ultimate size increase in wall thickness begins. Upon the completed primary wall the secondary wall is then quickly laid down as a series of lamellae one upon another. They can become so thick in fiber and stone cells that the wall lumen nearly vanishes and is recognizable only as a capillary space.

In a preceding paper[1] it was shown that primary and secondary wall fibers can clearly be distinguished from one another under the electron microscope. Cellulose in both walls is always in the form of very long, 250–400 Å, thick fibrils. In the primary wall these strands are intertwined while in secondary walls they lie more or less parallel to one another. The non-cellulosic materials, lignin, hemicelluloses, etc., lie between these elementary fibrils and amongst themselves together constitute another coherent system.

The present paper is devoted to a demonstration through a series of electron micrographs of how a cell wall is built up. Coleoptiles of corn (maize) and oats, whose tissues consist solely of young growing cells, have provided the necessary series of cell walls of different ages.

EXPERIMENTAL

In these experiments maize (golden bantam) and oat seeds were swollen for two

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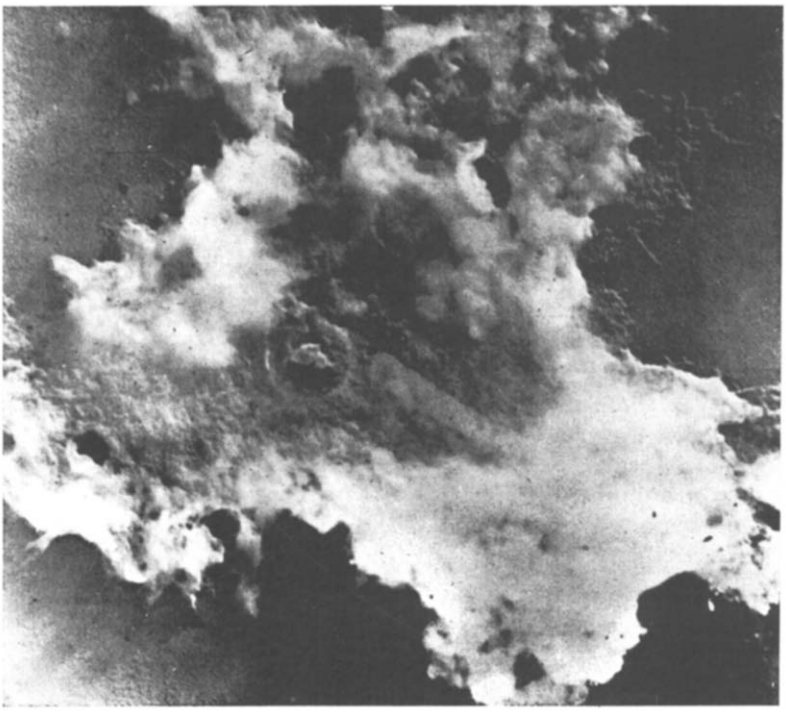

Fig. 1. Electron micrograph of a cell wall (maize coleoptile) covered with protoplasm. Magnification = 12000 ×.

hours, after which they were disinfected with "Semesan". In this way any spores of fungi present on the seed coat were killed without harm to the germinating seeds. The seeds were then germinated in the usual manner on moisted filter paper in a dark room, at a temperature of 25° C. Various stages of development of cell walls were obtained by using coleoptiles 5, 10, 15 and 20 mm long.

The very simple method of THIMANN AND BONNER[2] and BONNER[3] was employed to extract all non-cellulosic substances. By this process the tissues are treated first with hot, diluted sulphuric acid and then with hot, diluted sodium hydroxide to leave behind the purified structures composed of cellulose. In order to obtain the maximum interaction of these chemicals with the cell wall, the coleoptiles and roots were first cut in a Waring blendor. They were then exposed for about 15 minutes to 10% H_2O_2 and washed before being given the acid and alkali treatment outlined above. Tests show that this extraction frees the wall from all non-cellulosic substances but does not appear to attack the cellulose itself. Following extraction, the tissues were washed several times, cut again in the Waring blendor and suspended in water in the usual manner. Preparations were made by drying drops of this suspension on collodion covered grids and shadowing with chromium or palladium.

RESULTS

It is difficult to examine natural cell walls without first removing the thick layer of protoplasm which covers them. As shown in Fig. 1. this protoplasm is intimately

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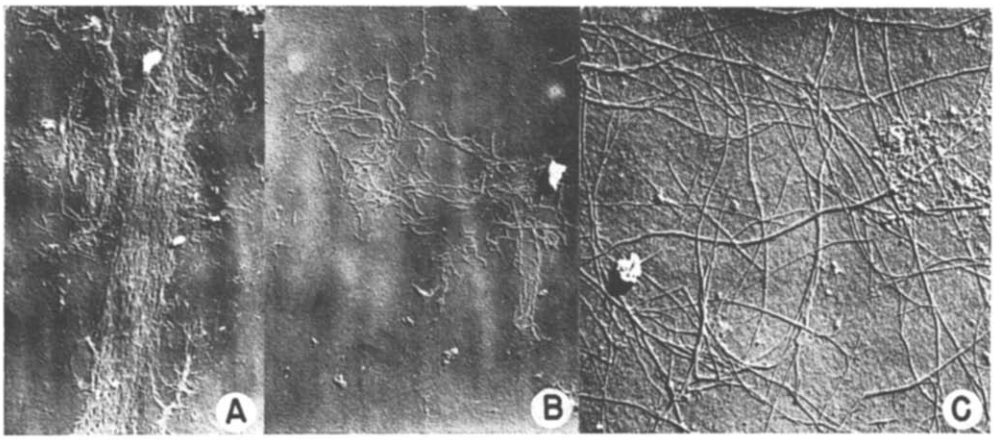

Fig. 2. Various developmental stages of cellulose fibrils (maize coleoptiles). a) first stage; b) in the state of development; c) completely developed. Magnification = 15000 ×

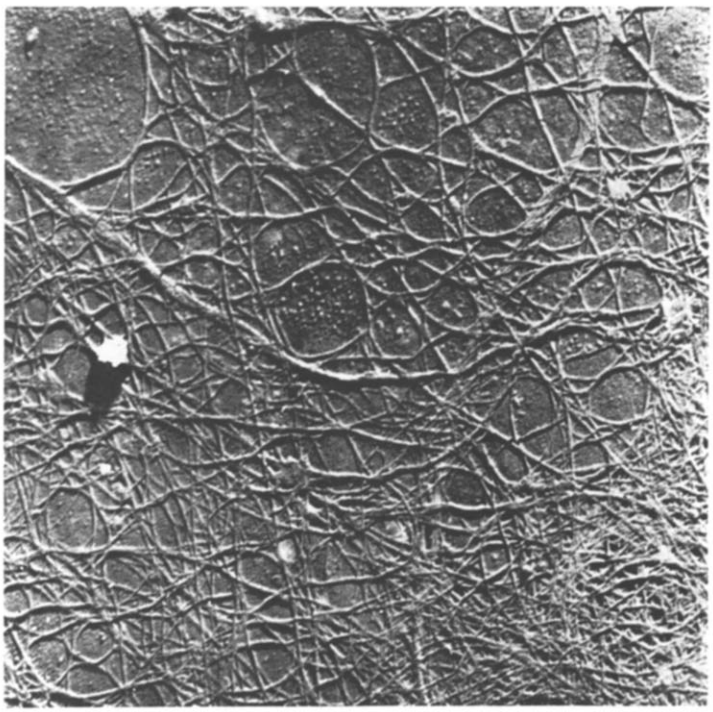

Fig. 3. Newly deposited primary wall in an oat coleoptile. Magnification = 20000 ×

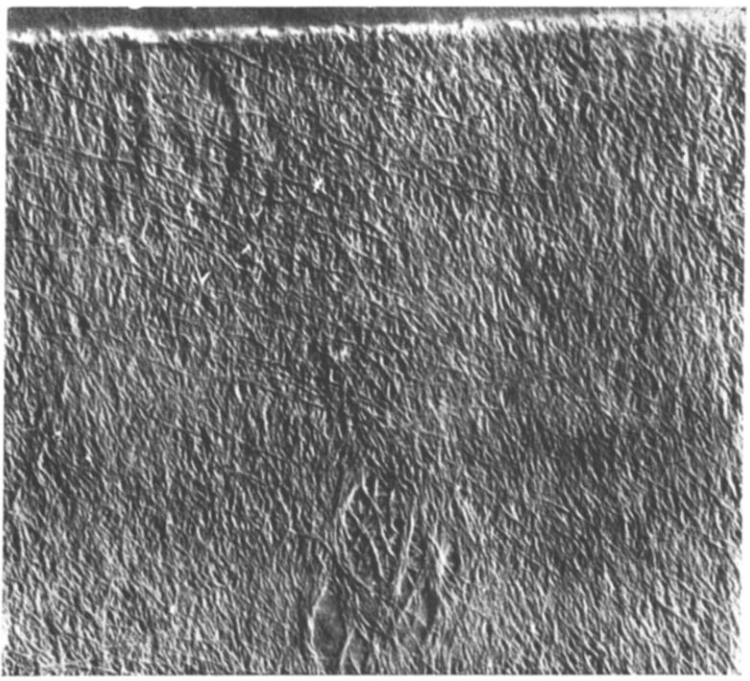

Fig. 4. Primary wall of a parenchymatic cell in oat coleoptile. Magnification = 20000 ×

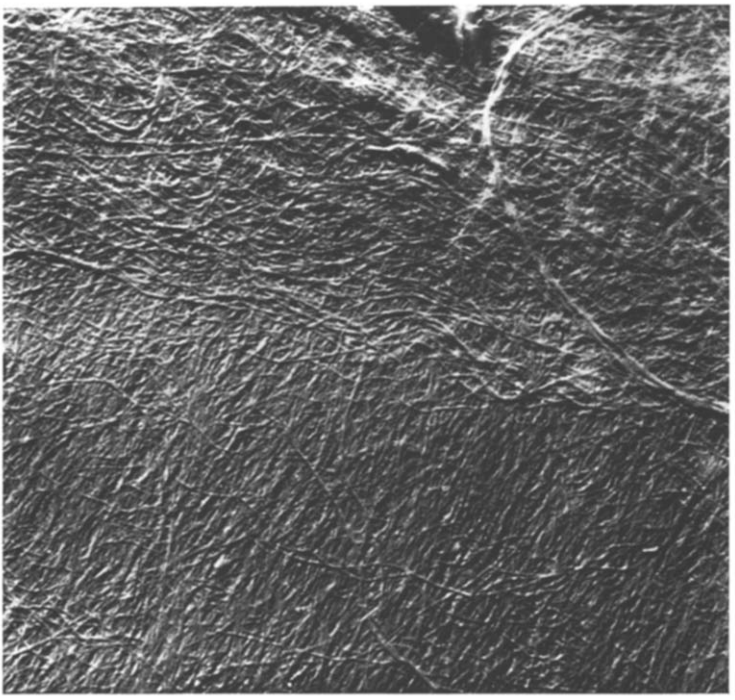

Fig. 5. Electron micrograph of a secondary wall in the state of development. The orientation of fibrils in subsequent layers has been displaced by 90°. Magnification = 20000 ×

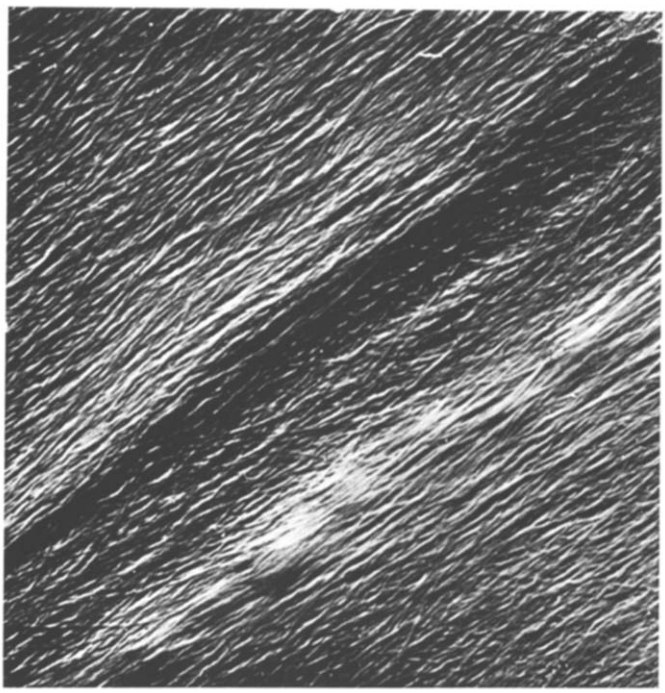

Fig. 6. Secondary wall of a cell of oat coleoptiles. Magnification = 20000 ×

connected with the developing wall, but it is no longer to be found in the completed layers. It does not enclose developed cellulose fibrils and this suggests that they may not develop within it but at its boundary with the already deposited wall.

Early steps in the formation of cellulose fibrils themselves can be observed by studying extracted samples of freshly germinated maize coleoptiles (5 mm long). Three such developmental stages are to be seen in Fig. 2. The first picture (Fig. 2a) shows a nearly homogenous substance having only a slight tendency to form fibrils. It is as if the cellulose were incompletely polymerized at this stage and had its short molecular chains randomly dispersed. In the next stage (Fig. 2b) the amorphous substances have disappeared and the cellulose is present as short fibrils. These apparently then elongate to yield the fully developed fibrils of Fig. 2c.

In a newly deposited cell wall (Fig. 3) such fibrils are interlaced as a very loose reticular network. During growth this reticulum is steadily strengthened by the addition of more cellulose fibrils, until after a short time part of the primary wall, as seen in the same figure, is completed. The occurrence of these loose reticular zones adjacent to dense finished areas suggests that primary walls grow very nonuniformly over its surface. When a primary wall is finished, however, it shows a structure like that of Fig. 4. New fibrils have become interwoven into the original loose framework till a thick membrane has resulted.

According to the usual concept the cell wall starts thickening when the cell itself stops elongating. The electron microscope, however, shows that the secondary wall may start to form before this. The two processes therefore are not strictly consecutive; instead one begins before the other has finished. Older evidence for this is to be found

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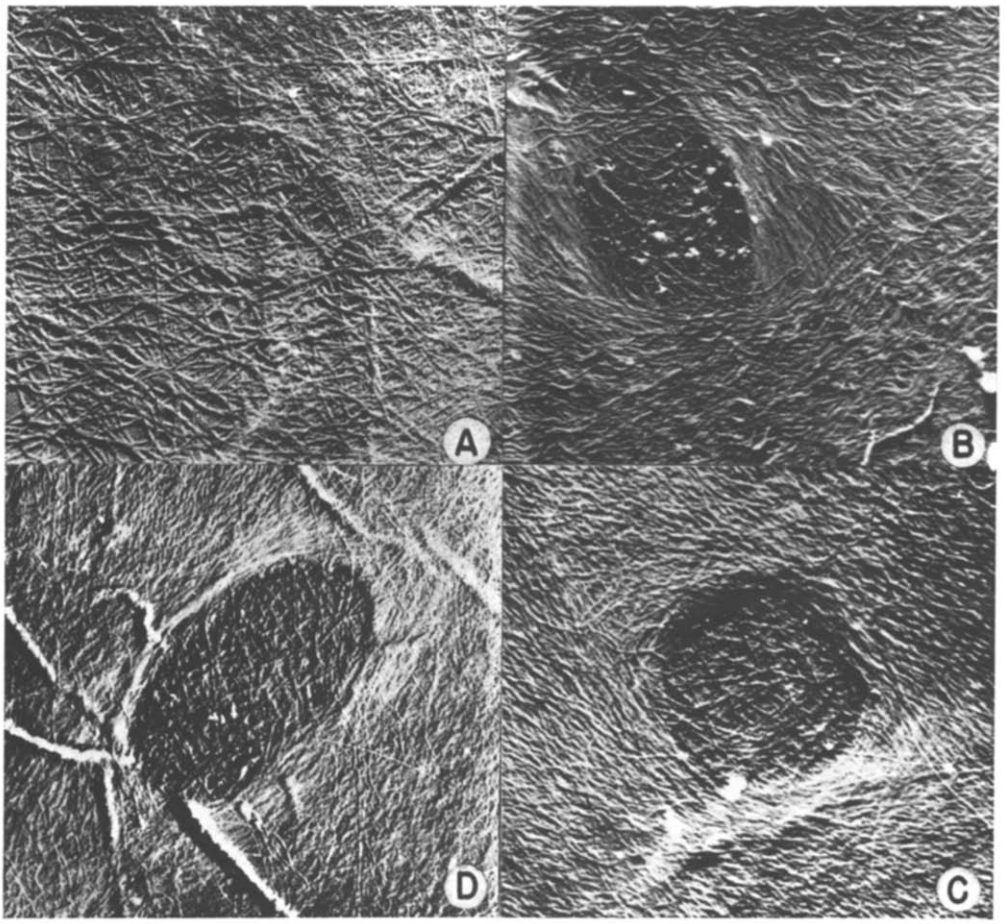

Fig. 7. Developmental stages of pits visible in the primary walls (explanation in text). Magnification × 15000.

in the local apical growth of fiber ends, where the ends of cells have continued to extend even though their middle parts were already enclosed in heavily thickened walls. It also shows itself in the slowness with which the fibrils of secondary walls achieve a parallel orientation. Thus the first layers of secondary wall deposited on primary wall commonly have their fibrils in considerable disarray (Fig. 5). This figure brings out the additional interesting fact that the two successive layers of secondary wall it shows are turned 90° with respect to one another. As additional lamellae are laid down they have fibrils in better orientation till, as Fig. 6 indicates, the latest layers are closely packed and nearly parallel to one another.

Electron micrographs of the walls of meristematic cells show many pits too small to have been visible before in the optical microscope. Such a finished pit appears in Fig. 7d. Photographs of developing walls show how these pits are formed. At first (Fig. 7a) they are areas containing few fibrils about which increasing numbers of fibrils are deposited in curving masses (Fig. 7b). Protoplasm is found in these areas which thus appear as channels, like the plasmodesmata, through which it can flow from one

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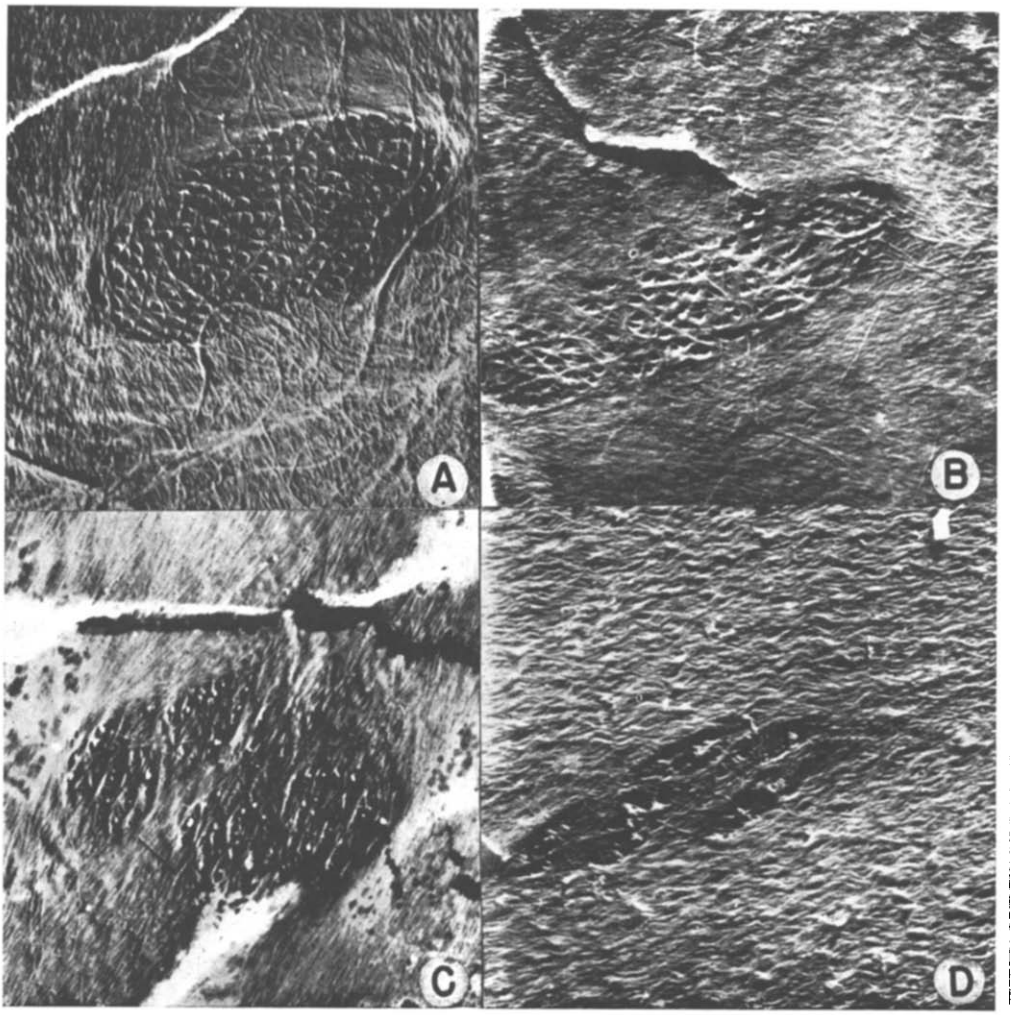

Fig. 8. Various stages of pits in primary and secondary walls. The pits are elongated ellipsoidically (a and b) and subsequently covered. Magnification = 15000 ×

cell to another. This flow during the period of growth of the primary wall keeps the channels relatively free of cellulose fibrils and their pitlike character becomes more and more evident (Fig. 7c). At first they are round but they gradually assume an elliptical form (Fig. 8a, b) probably as the cells elongate. During deposition of the secondary wall fibrils appear in increasing numbers within the elliptical pits which thus are broken up into a number of small holes that offer less and less area for the flow of the protoplasm they may contain (Fig. 8c, d). With the blocking of these holes by the deposited fibers, protoplasmic exchange between cells and growth presumably come to an end (Fig. 8d).

DISCUSSION

As already indicated, the first evidence of a developing cell wall is to be found in the spindle of the dividing cell. Working with the staminal hairs of *Tradescantia*,

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BECKER[4] concluded that the so-called cell plate of the phragmoplast first becomes visible as small droplets. These differentiating from the protoplasm become an isotropic membrane visible in the polarizing microscope. This initial membranous layer, the middle lamella, stains with ruthenium red and therefore presumably consists mainly of pectin. Cellulose dyes do not stain it. As soon as the cell plate extends through the spindle and reaches the cell wall of the mother cell, it appears faintly birefringent in the polarizing microscope. Since pectin has never shown birefringence we must conclude that the primary wall has begun to form at this moment, though the presence of cellulose cannot yet be demonstrated by microchemical tests. This failure of the micro tests[5] has been explained by assuming that cellulose is present but is masked by a protopectin and other substances to such a degree that direct contact with cellulose dyes cannot take place. It is to be concluded from such photographs as Fig. 2 that the failure of this microchemical test is due to there being a masking of relatively few fibrils at this stage, as well as to a masking of those that are present. There is insufficient cellulose to give rise to a marked colour reaction until the network has later been closely interwoven with additional fibrils.

Coleoptiles elongate so rapidly that they must build fibrils very fast. Thus it has been shown that their cell walls can grow at the rate of 7 micra per minute. Even this is slow compared with rye stamens[6] which can extend more than 2 millimeters per minute. Since there is no noticeable thinning of the wall when this takes place, new cellulose fibrils must be concurrently formed and incorporated into the existing primary wall.

It might be imagined that some of the extension was accomplished by existing fibrils becoming better and better oriented parallel to the long axis of the cell as a consequence of tension prevailing during growth; but the polarizing microscope gives no indication of such an effect. Thus during elongation the cell wall continues to show a faint negative birefringence with reference to the fiber axis. If the tissues were elongated by stretching, the birefringence of the cell wall would be changed and become positive with respect to the cell axis. In oat coleoptiles[3] a stretching of only 8% would be required to change the sign of the birefringence; nevertheless, no such reversal is manifested by rye stamens whose wall elongation amounts to more than 400%.

FREY-WYSSLING[5] has concluded that these optical properties require that the existing fibrils separate by parallel displacement and that new fibrils be continuously deposited in the gaps thus created. The present observations substantiate this insertion of new fibrils. They do not, however, establish if the open places are produced in finished cell walls by the fibrils slipping pass one another or if they develop during the extension of the cell. In the latter case primary wall would continue to be built from these localized regions of growth. The start of secondary wall before the primary wall is completed seems to favour the existence of such local areas.

The extent to which the protoplasm plays an active part in building up cell wall is still a matter of controversy. It is the generally accepted view today that cellulose fibrils are synthesized by the protoplasm and then deposited in the wall. There is also uncertainty concerning the factors that determine orientation of the fibrils. According to VAN ITERSON[7] it follows the direction of flow of the protoplasm. He has explained the crosswise layers in the cell wall of *Valonia* as the result of changes, through about 90°, in this flow after the deposition of each fibrous layer. Such a shift in orientation of the fibrils in successive layers is also evident in Fig. 5.

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SUMMARY

The development of the walls of meristematic cells (maize and oat coleoptiles) has been studied with the electron microscope. It has been found that the growing primary wall appears first as a loose framework which changes quickly into a dense network by the deposition of fresh cellulose fibrils. By the time the cell has reached its final size, deposition of a secondary wall has begun. The fibrils in its several layers are not interwoven but are deposited more or less parallel to one another. A discussion is given of the bearing of these results on previous knowledge of the mechanism of growth of cell walls

RÉSUMÉ

Le développement des parois de cellules méristématiques (coléoptiles de maïs et d'avoine) a été étudié au moyen du microscope électronique. Il a été trouvé que la paroi primaire en état de croissance apparaît tout d'abord sous forme d'un squellette lâche, se tranformant rapidement en un réseau serré par suite de dépôt de fibrilles fraîches de cellulose. Au moment où la cellule a atteint ses dimensions finales, le dépôt d'une paroi secondaire a déjà commencé. Les fibrilles qui forment ses couches successives ne sont pas enchevêtrées mais déposées plus ou moins parallèlement les unes aux autres. La portée de ces résultats sur nos connaissances antérieures concernant le mécanisme de la croissance de parois cellulaires a été discutée.

ZUSAMMENFASSUNG

Die Entwicklung der Zellwände von meristematischen Zellen (Coleoptile aus Mais und Hafer) wurde mit dem Elektronenmikroskop untersucht. Es wurde gefunden dass die wachsende Primärwand erst als loses Rahmenwerk erscheint welches sich rasch in ein dichtes Netzwerk umwandelt, durch Ablagerung von frischen Cellulose-Fibrillen. Zu der Zeit wo die Zelle ihre endliche Grösse erreicht hat, hat die Ablagerung einer Sekundärwand begonnen. Die Fibrillen in deren verschiedenen Schichten sind nicht ineinandergeflochten, sondern mehr oder weniger paralle zu einander abgelagert. Die Rückwirkung dieser Ergebnisse auf unsere bisherige Kenntnis des Mechanismus des Wachstums von Zellwänden wird besprochen.

REFERENCES

1 K. MÜHLETHALER, *Biochem. Biophys. Acta*, 3 (1949) 15.
2 THIMANN AND BONNER, *Proc. Roy. Soc. London*, 113 (1933) 126.
3 I. BONNER, *Jahrb. wiss. Botan.*, 82 (1935) 377.
4 W. A. BECKER, *Acta Soc. Botan. Polon.*, 11 (1934) 190.
5 FREY-WYSSLING, *Protoplasma*, 25 (1936) 261.
6 FREY-WYSSLING AND SCHOCH-BODMER, *Planta*, 28 (1938) 257.
7 G. VAN ITERSON, *Chem. Weekblad*, 24 (1927) 166.

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