Cellulose microfibril patterns in elongating cells of the Zea mays root tip

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BUSS, Warren Rumsey, 1937-
CELLULOSE MICROFIBRIL PATTERNS IN
ELONGATING CELLS OF THE ZEA MAYS ROOT TIP.

Iowa State University, Ph.D., 1968
Botany

University Microfilms, Inc., Ann Arbor, Michigan
CELLULOSE MICROFIBRIL PATTERNS IN
ELONGATING CELLS OF THE ZEA MAYS ROOT TIP

by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Plant Physiology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

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1968
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INTRODUCTION

Cell growth can be divided into two main processes, cell division and cell elongation or enlargement. Although a large amount of research has been carried out on both of these processes, many problems remain unresolved. One such problem is concerned with the mechanism by which the cell wall increases in area as elongation proceeds.

The problem is complicated by the nature of the cell wall which is a heterogenous mixture of cellulose, hemicellulose, pectins, proteins, and lipids. These components are found to occur in two rather distinct phases, a discontinuous phase consisting of cellulose microfibrils, and a continuous phase which forms an amorphous matrix composed of the other wall constituents. The cell wall can therefore be compared to reinforced concrete in which the microfibrils represent the "reinforcing rods" and the other components, the "cement." How then, is it possible for such a system to increase in area?

The approach of this study was to use the electron microscope to observe the arrangements of the microfibrils in the elongating primary cell wall of Zea mays root cells at various stages of enlargement. It was believed that changes in the microfibrillar patterns would indicate locations and mechanisms of wall growth. Both interior and exterior surfaces of the primary wall were observed in sections of cells and in whole-cell preparations.
REVIEW OF LITERATURE

Introduction

Historical background

The first microscopic observation of plant cell walls was recorded in 1665 when an Englishman, Robert Hooke, published a book titled, "Micrographia, or Some Physiological Descriptions of Minute Bodies Made by Magnifying Glasses." (Hooke, 1665). Hooke described his experiment as follows:

I took a good clear piece of Cork, and with a Pen-knife sharpen'd as keen as a Razor . . . cut off . . . an exceeding thin piece of it, and placing it on a black object Plate, because it was itself a white body, and casting the light on it . . . I could exceeding plainly perceive it to be all perforated and porous, much like a Honeycomb, but that the pores of it were not regular; yet it was not unlike a Honeycomb in these particulars.

First, in that it had a very little solid substance, in comparison of the empty cavity that was contain'd between . . . for the . . . walls (as I may so call them) or partitions of those pores were neer as thin in proportion to their pores, as those thin films of Wax in a Honeycomb . . . are to theirs.

To these small boxlike structures that he observed he gave the name "cells."

For almost two more centuries the wall remained the only part of the cell discovered. Then, beginning in 1831 with the discovery of the nucleus by Brown and the description of protoplasm by Dufardin in 1835, most research was directed to the study of cell contents rather than of the wall. However, some study of the wall did continue, for in 1874 Sachs formulated a theory concerning the mechanism of cell elongation. Basically it stated that turgor caused an elastic extension of the cell
which allowed intussusception of new wall material into the resulting spaces in the wall.

During the early part of the twentieth century many theories and counter theories were proposed, but it was not until the development of X-ray and later of electron microscope techniques that any real progress could be made in resolving the many problems concerning growth of the cell wall. (For a review of some of the more important early theories the reader could consult Heyn, 1940.)

Root Tip Development

General

As an apical meristematic region, the root tip is considerably less complicated structurally than its stem apex counterpart in that the root lacks developing appendages, nodes and internodes. Three growth regions or zones are generally acknowledged in roots: the meristematic zone, zone of elongation, and zone of maturation. The meristematic zone is characterized by mitotic activity in the apical meristem and the primary meristems (protoderm, cortical ground meristem, and procambium). The zone of elongation is the region of rapid and extensive increase in cell length. The third zone, the zone of maturation or differentiation, is distinguished by the development of root hairs, secondary wall thickenings, etc. However, the boundaries between these regions are not always clear. Esau (1965) states, "At the same level of the root, the processes of cell division, cell enlargement, and cell maturation overlap not only in the different tissue regions but also in the different cells of the same tissue region, and even in individual cells. The meristematic cortex
vacuolates and develops intercellular spaces close to the apex, where the central-cylinder meristem still appears dense. In the central cylinder the precursors of the innermost xylem vessels cease dividing, enlarge, and vacuolate considerably in advance of the other vascular precursors, and the first sieve tubes mature in the part of the root where cell division is still in progress. In individual cells, division, elongation, and vacuolation are combined."

Since division and elongation but not maturation are pertinent to this study, a detailed discussion of the first two growth regions only now follows.

**Meristematic zone**

The meristematic region is generally considered as being composed of the apical meristem, protoderm, ground-meristem, and procambium. The apical meristem is primarily an area of tissue initiation which determines the tissue patterns behind it. Esau (1965) characterizes the *Zea mays* root tip as having three tiers of initials in the apical meristem. The most distal tier constitutes the rootcap meristem, the calyptrogen. This meristem remains active during further growth of the root. Derivatives of the middle tier form the ground meristem or, by periclinal divisions of the outermost layer, the protoderm. The proximal tier of initials produces derivatives which form the procambium. These last two tiers of initials are concerned with the original organization of the root and may become quiescent during later growth.

In an extensive study of the onion root tip Scott et al. (1956) indicated that the apical initials were polyhedral in shape and that all
the cell faces appeared sievelike due to the presence of large numbers of very small, circular, primary pit fields. Slightly older cells in the meristematic zone were short cylindrical or "drum-shaped." The upper and lower walls of these cells were finely pitted with primary pit fields distributed radially over the cell surface. The vertical walls also contained primary pit fields which were distributed randomly at early stages, but soon became arranged in about 6 vertical bands. These bands were located where a cell was in contact with a neighboring cell. Within each band the primary pit fields were arranged in many ranks. The oldest cells in the meristematic zone of onion roots were characterized by the reorientation of primary pit fields into a single file within each band. Also, the distances between primary pit fields increased.

In working with *Zea mays* root tips, Baldovinos (1950) indicated that cells in the distal millimeter of the root were isodiametric and averaged 11μ in length. The cells were characterized by a high protein content (61% of dry weight), and a high dry matter content (20% of fresh weight). He reports that in the second millimeter of the root there was a rapid change from cell division to cell elongation. However, it was in the first half of this millimeter that the highest rates of cell division occurred—1 division every 6 to 10 hours at 25°C. In the last half of this region the division rate declined to zero. Also, he stated that even though the cells had not quite doubled in length in the second millimeter, the average protein nitrogen per cell was nearly twice that found in cells of the first millimeter, indicating rapid protoplasm synthesis. In addition, the per cent dry matter declined slightly as the cells began to take up more water.
Using photographic techniques and a different variety of maize, Erickson and Goddard (1951) and Erickson and Sax (1956b) found that most cell divisions occurred in the region 0.5 to 2.5mm from the root apex with the maximum rate of division occurring at 1.25mm (1 division/6.25 hours). The cells in the meristematic zone averaged about 18μ in length; had high dry weights and protein contents on a percentage basis; and showed rates of fresh weight, dry weight, total nitrogen, and protein nitrogen accumulation which kept pace with cell formation.

**Elongation zone**

Eames and MacDaniels (1947) state that the meristematic and elongation zones are markedly different in extent. Whereas cell division is confined to about a millimeter of the root, cell elongation may take place over approximately one centimeter, versus 8 - 10 centimeters in stem tips.

The exact location of the zone of maximum elongation is variable from species to species or even variety to variety. For instance, in *Zea mays* roots, Brown and Sutcliffe (1950) reported that the zone of most rapid elongation was 1.5 - 3.0 mm behind the root tip. Baldovinos (1950) stated that for the variety he used, the most rapid rate of elongation occurred in the last quarter of the second millimeter. Woodstock and Skoog (1962), using two different varieties of maize showed that maximum elongation occurred 3.3 mm from the apex in one variety and 4.8 mm from the apex in the other. It is interesting to note that in this same experiment it was reported that the growth region extended for 9.0 mm in the first variety and 11.0 mm in the second. Erickson and Sax (1956a) using a mathematical approach based on photographs of growing maize roots of a different variety, calculated that maximum elongation
occurred about 4.0 mm back from the tip. The rate at this point (for an infinitely small section) was 40% increase in length/hour. Elongation did not take place past the tenth millimeter.

As far as individual cell size is concerned enlarged cells are typically 10 - 20 times larger than meristematic cells but may be more than 100 times larger in some tissues. Siegel (1962) states that in the transition stage, "the open-work wall of the radial phase becomes more continuous, the gaps are filled in, and the primary pit fields are reduced in size." Scott et al. (1956) reported that in onion root cortex parenchyma the end walls remained sievelike and also that there was little change in the vertical wall patterns of primary pit fields.

Wanner (1950) found by measuring lengths of cells that epidermal, cortical, and central cylinder tissue began elongation at different times in root tissues. Brumfield (1942) studied the growth of epidermal cells of *Phleum pratense*, using photographic techniques, and could observe no sliding growth, i.e. one cell elongating more than its neighbor. This indicated that elongation in the epidermis at least was synchronous. He also observed that elongation showed no daily rhythm but instead occurred at a constant rate.

The composition of the individual cells also changes during elongation. Jensen (1955) reported that during transition to the elongation stage protein synthesis slowed down, cellulose synthesis stopped temporarily, and oxygen uptake began increasing. During the active elongation stage this same worker found that protein synthesis actually stopped and the total protein content of the cells declined. He also reported that oxygen uptake continued to increase, the non-glucose carbohydrate fraction
of the cell increased while the cellulose content remained constant. Frey-Wyssling and Blank (1940) estimated that in the *Zea mays* coleoptile there was a 1,670% increase in protoplasm during elongation. They also state that protein nitrogen increased up to 9.5 times during this same period. With maize roots Baldovinos (1950) found that total nitrogen per cell doubled in the third millimeter over that in the second millimeter. However, during this same period the average cell length tripled, indicating that protoplasm synthesis was beginning to fall behind. Soluble nitrogen and sugars were accumulated. As the rate of elongation slowed down protein, ash, dry matter and most cell constituents except cellulose and sugars decreased on a unit-cell basis as rapid vacuolation took place.

For their variety of *Zea mays*, Erickson and Goddard (1951) reported that cell length increased 5 or more times in the region of most rapid elongation; fresh weight increased 6 times; dry weight increased 3.6 times; but total and protein nitrogen accumulation declined considerably, with some cells losing protein nitrogen. At the conclusion of elongation the average cell length was greater than 230μ. The total nitrogen per cell was about twice that of cells undergoing division but the protein nitrogen was essentially the same as in dividing cells.

It should be obvious from the foregoing that there is some disagreement in the literature over these points. In this regard, Torrey (1956) states, "Although there is conflicting evidence as to the specific changes in cellular constituents, there appears to be general agreement that rapidly elongating cells show high metabolic activity and that such metabolic activity in some way determines the behavior of the cell wall during its extension."
Structure of the Cell Wall

Chemistry

General  The primary cell wall is segregated into two distinct phases. One is a discontinuous phase consisting of the fibrous materials, cellulose and noncellulosic polysaccharides. The other phase is a continuous one forming the matrix of the wall and composed of hemicelluloses, pectic substances, proteins, and lipids. The fresh primary wall is highly hydrated; water comprises 80 - 90% of the fresh cell wall weight according to Wardrop (1962). Frey-Wyssling and Mühlethaler (1965) indicate that the continuous phase or matrix is more strongly hydrated than the cellulose fraction.

In their review of cell wall structure and physiology, Setterfield and Bayley (1961) list a summary of quantitative chemical analyses of primary walls done by a number of workers. Average values of all the different tissues they list are as follows: Cellulose, 33.0% of total dry wall material; hemicelluloses, 26.6%; pectic substances, 16.2%; protein, 12.3%; and lipid, 10.7%. In this review they distinguished no noncellulosic polysaccharide fraction. Frey-Wyssling and Mühlethaler (1965) indicate that in the primary walls of most of the higher plants the fibrous materials, cellulose and noncellulosic polysaccharides (glucomannan and xylan chains), comprise about 30% of the dry wall weight.

Sixty per cent of the dry wall is composed of hemicelluloses (which on hydrolysis yield glucose, mannose, galactose, xylose, arabinose, galacturonic acid, and glucuronic acid) and pectic substances. The final 10% of the wall is composed of proteins and lipids.

The composition of the cell wall may vary between cells in different
tissues. For example, Jensen (1960) found that in the meristematic region of onion roots, the apical initials were low in all wall substances. Cortical cell walls were relatively high in noncellulosic polysaccharides and cellulose but relatively low in pectic substances and hemicelluloses. Protoderm cells during very early stages and later during elongation had the same relative balance of wall materials as cortical cells but during radial enlargement had just the reverse.

During cell development the ratio of one component to another may change. In his monograph on cell walls, Siegel (1962) states, "... in the cell wall the relatively constant cellulose is associated with a relatively constant interstitial aggregate whose composition varies, perhaps in a functional and dynamic fashion." In support of this statement he discussed the changes in composition of walls in growing Allium root cells. He pointed out that the apical initials contained small amounts of pectin, protopectin, and cellulose. Those cells enlarging radially had less protopectin than the apical initials but the ratio of cellulose to pectic substances was the same. Cells in the transition stage from radial enlargement to elongation showed an increase in all carbohydrates, with pectin, soluble noncellulosic polysaccharides, and cellulose all increasing faster than the surface area of the wall. Cells in the rapid elongation phase showed a filling in of the discontinuities of the wall with pectin, hemicellulose, noncellulosic polysaccharides, and cellulose. These components then became proportional to wall area during this phase.

Cellulose One of the earliest studies of the fibrous components of the cell wall was performed by Sponsler (1928) using X-ray analysis
techniques. He studied ramie fibers and concluded that the walls were composed of regularly-spaced structural units. These units were thought to be of indefinite length and were oriented longitudinally or transversely at different angles. He suggested that the structural units were polymers of glucose. Since that time it has been conclusively shown that the main fibrous component of plant cell walls is cellulose which is a polymer of β-D-glucose molecules joined in a 1-4 linkage.

A cellulose molecule is an unbranched polysaccharide which, according to Siegel (1962), has a degree of polymerization of 1,400 - 10,000, or a molecular length of 7,000 to 50,000Å. He also reports that in the wall the molecules are grouped together in definite arrangements. Approximately 100 cellulose molecules, each $33\AA^2$ in cross section, group together to form an elementary fibril, $3,000\AA^2$ in cross section. Twenty elementary fibrils form a microfibril which is $250\AA$ on a side or $62,500\AA^2$ in cross section. Wardrop (1962) declared that perfect three dimensional order exists along certain portions of the cellulose molecules forming the crystalline regions or micelles. These regions are separated by the amorphous regions where the molecules are not in an orderly array. Siegel (1962) states that the crystalline regions commonly measure 50 - 70Å wide and at least 600Å in length, thus "... individual extended molecular chains are so organized that they participate in many crystallites, fraying out in the intercrystalline regions."

It is evident from work cited by Preston (1961) that a microfibril actually consists of a central core of cellulose surrounded by chains of noncellulosic polysaccharides which yield xylose, mannose, rhamnose, and possibly other sugars on hydrolysis.
Probably the first direct observation of the presence of microfibrils in cell walls was made by Frey-Wyssling et al. (1948). In their study of corn root meristem, flax fibers, corn coleoptiles, and cotton hairs they indicated that when a plant tissue is treated by an appropriate fractionation method and then shadowed with a heavy metal, "a wonderful fibrillar structure is seen in the electron microscope." They declared that both primary and secondary walls are composed of microfibrils with nearly constant diameters of 250 - 300Å. That same year the work of Preston et al. (1948) with Valonia ventricosa showed that such structures could be found in the walls of algae. Since that time many workers have reported similar findings in other plants.

There have been some minor disagreements on the cross-sectional shape and size of the microfibrils. On the first point Preston and Kuyper (1951) affirmed that the microfibrils in Valonia ventricosa are "flattish ribbons rather than circular cylinders." Frey-Wyssling and Mühlethaler (1965) said that in ramie fibers microfibrils often appear flat with a width of 100Å and a thickness of 30Å. On the other hand Roelofsen (1954) states that the flat appearance is an artifact of preparation and that the microfibrils are really round in cross section.

The controversy over cross-sectional size of microfibrils is more easily solved. Manley (1964) stated that the microfibrils which he observed from cotton, ramie, wood, and bacteria were only 35Å in diameter. However, in the course of his preparation of samples he used ultrasonic vibrations to break up the cells. Wardrop (1962) points out that when microfibrils are treated with ultrasonic waves they break up into the constituent elementary fibrils which are 35Å in diameter.
Wardrop hastens to add that despite the fact that they can be broken up, microfibrils are still considered to be the basic structural unit of the wall on a morphological basis.

In regards to the structure of the elementary fibril, Manley (1964) disagrees with Siegel's (1962) model. Manley presents evidence that instead of the elementary fibril being composed of 100 straight cellulose molecules bundled together, it is composed of a single cellulose molecule twisted to form a helix of 35Å diameter. On this point Mühlethaler (1967) comments, "Today it is generally accepted that cellulose and its derivatives, which are recovered from solutions, do have a folded conformation." He further states this may not hold for cellulose in the wall. In fact evidence based on the formation of bacterial cellulose would tend to support the theory that there is a linear aggregation of microfibrils in cell walls. He also mentions the fact that mercerization of the naturally occurring cellulose tends to reduce the length of the fibrils, indicating that the molecules become folded just as the molecules precipitated from solutions are.

Wall ultratextures

In their pioneering work on microfibrillar orientation Frey-Wyssling et al. (1948) describe the primary wall as having microfibrils which are "mutually interwoven" but showing a "deviation about a primary course," this course being transverse to the longitudinal axis of the cells. Subsequent observations by other workers showed that there was a greater complexity of microfibrillar patterns in different cell types than was originally thought. To describe these various types of microfibrillar orientations or ultratextures, Frey-Wyssling (1962) proposed three terms:
fibroid texture in which the microfibrils are dispersed about a longitudinal axis, tubular texture in which the microfibrils are transverse, and foliate texture in which the microfibrils show a completely random orientation. He indicated that cylindrical cells generally have a tubular texture.

Meristematic cell walls generally have a tubular texture. Siegel (1962) indicates that when this type of wall is stretched mechanically, the normally optically negative wall becomes positive. Upon release of the tension the wall partially regains its negative birefringence. However, he states that an elongating cell does not show this change from negative to positive. Instead, it remains negative which is evidence that some microfibrils in the depth of the wall remain transverse even though the wall becomes more open and loose. Scott et al. (1956) suggest that microfibrils in apical initials form a loosely woven mesh. When these cells enlarge slightly, some of the microfibrils become longitudinally aligned at cell corners with primary pit fields developing in between. This confirmed the earlier work of Mericle and Whaley (1953) who in working with corn roots had found that on the inner surfaces of meristematic cells there were transverse bands or strands of microfibrils separated from each other by more open areas. They pointed out that these open areas later formed pit fields. Later as the cells elongated the pit fields became smaller and more widely separated.

Wardrop (1962) reports that cell types showing a tube texture are cambial cells, *Avena* coleoptile parenchyma, parenchyma of hypocotyls, stellate pith parenchyma of *Juncus*, staminal filaments, staminal hairs of *Tradescantia*, cotton hairs, and sclerenchyma fibers. Many, if not all
of these cell types have transverse microfibrils on the inner surface of the wall but random to longitudinal microfibrils on the outer surface. The "average" orientation is, however, essentially transverse.

Mühlethaler (1950b) observed that epidermal cells of coleoptiles showed a tubular texture also. He noted that the epidermal cells had many longitudinal microfibrils along the outer wall only. In further studies on epidermal walls, Bayley et al. (1957) reported that the outer wall contained 10 - 15 layers of microfibrils. These layers fused to make a more normal primary wall at the junction between two cells. All inner wall surfaces had essentially transversely oriented microfibrils.

Elongating parenchyma cells were reported at a very early date by Baranetzki (1886) to have corner thickenings which were later shown by Mühlethaler (1950a), Wardrop and Cronshaw (1958) and others to be longitudinally oriented microfibrils. Primary pit fields were located between these thickenings.

Root hairs seem to show an entirely different ultratexture. Newcomb and Bonnett (1965) describe the primary wall of radish root hairs as containing two layers of microfibrils. The outer layer covers the entire hair and consists of randomly oriented microfibrils. The inner layer is first detectable at a position about 25μ proximal to the tip and extends backwards over the rest of the wall. This layer consists of parallel, axially oriented microfibrils only. O'Kelley and Carr (1954) found that the microfibrils near the tips of root hairs were somewhat shorter than in other regions.
Mechanisms of Wall Growth

**Microfibril synthesis and initial orientation**

*Cellulose synthesis* Even though the composition of cellulose has been known for some time the biochemical reactions involved in its synthesis remain relatively obscure. However, recent work by Colvin (1959) with *Acetobacter xylinum* cells, Barber et al. (1964) with mung bean extracts and others have begun to clear up some of the mystery. In Colvin's work, ethanol extracts of active *Acetobacter* cells contained a substance which was polymerized into cellulose when placed in water even though no bacterial cells were present. He related that formation of the microfibrils was hastened by a heat-labile enzyme. He also felt that the precursor was not uridine diphosphoglucose nor short β-glucosan chains.

Enzyme extracts from etiolated mung bean roots and hypocotyls prepared by Barber et al. (1964) yielded a particulate enzyme system which synthesized cellulose when guanosine diphosphate D-glucose was fed into the system. Adenosine diphosphate D-glucose, the precursor of starch, was found to be completely inactive in the system.

Most workers, at the present time, feel that cellulose molecules are synthesized by end synthesis. This means that the cellulose is lengthened by the addition of glucose molecules one at a time rather than by the coalescence of cellobiose or cellodextrins into larger units. Thus it appears that cellulose synthesis may be at least partially analogous to starch synthesis with one of the main differences being that a different precursor is involved in each pathway.

The importance of cellulose synthesis in the overall process of
elongation was demonstrated by Brown and Sutcliffe (1950) who while studying *Zea mays* root segment elongation in a nutrient medium showed that elongation was dependent on external supplies of sugar. They noted also that cellulose synthesis increased during elongation, which led them to the conclusion that cellulose synthesis is the rate-limiting factor in elongation.

**Site of wall deposition** On the basis of X-ray studies Sponsler (1929) predicted that new wall material was added to the inner surface of the cell wall. He explained that the surface of the pre-existing wall (cellulose molecules) played an important role in the synthesis of new wall material since this surface made crystallization more favorable.

Similar conclusions were drawn by Green (1958) who used tritiated water to compare the incorporation of radioactivity throughout the thickness of *Nitella* cell walls. He summarized his results by stating, "... the isotope was present only as a thin layer at the inner surface. The decrease in thickness of the isotope-free wall layer, in relation to the surface expansion of the wall, followed the prediction based on growth by apposition. It was concluded that tritium-containing cell wall constituents were added to the growing wall at, or very near, the inner surface of the wall." Using tritiated sucrose, high resolution autoradiography and different plant material, Setterfield and Bayley (1958) reached a different conclusion. They found tritium in both cellulose and non-cellulosic materials throughout the thickness of elongating outer walls of epidermal cells of *Avena* coleoptiles. They considered the presence of tritium to be evidence of the deposition of cellulosic and noncellulosic materials throughout the wall, but they did not eliminate the possibility
Wardrop (1962) suggested a solution to this apparent conflict. He pointed out that microfibrils synthesized by end synthesis would probably extend some distance into the cell wall. This would explain how Setterfield and Bayley (1958) could observe incorporation of tritium throughout the wall when, in fact, synthesis of microfibrils may have been taking place only at the membrane surface.

Role of cytoplasmic organelles The ontogeny of the primary cell wall and the involvement of certain cytoplasmic organelles in this process have been recently reviewed by Frey-Wyssling and Mühlethaler (1965). They state that shortly following anaphase of mitosis, formation of a new wall begins to separate the two daughter nuclei. The plasma body involved in this process is called the phragmoplast, which is located in the equatorial plane of the mother cell. Along the edge of the phragmoplast golgi bodies accumulate and produce vesicles which become located along the surface of the phragmoplast. The vesicles then coalesce to form a semi-solid layer, the cell plate. The process of production, accumulation, and coalescence of the vesicles causes the cell plate to grow peripherally until it reaches the mother cell wall. By this time three distinct layers are evident: the middle lamella and two primary walls, one for each daughter cell. Birefringence of the primary walls indicates some incorporation of cellulose into the walls at this early stage.

Also, during the time of golgi vesicle deposition at the cell plate the rest of the wall becomes lined with new wall material arising from the golgi vesicles also. These walls do not show much of an increase in thickness due to simultaneous extension of the wall.
The role of golgi bodies in the addition of material to cell walls has been documented in many different cell types; e.g. outer root cap cells of maize (Mollenhauer et al., 1961), and of wheat (Northcote and Pickett-Heaps, 1966), root hairs (Sievers, 1963), pollen tubes (Rosen et al., 1964), developing xylem of sycamore (Wooding and Northcote, 1964), and epidermal cell walls of wheat (Pickett-Heaps and Northcote, 1966).

In the work of Northcote and Pickett-Heaps it was shown that in the presence of tritiated glucose, labeled material was synthesized in the golgi apparatus of wheat root cap cells. This material was then transferred in the golgi vesicles across the cytoplasm and through the plasmalemma where it was incorporated into the cell wall and slime layer. Analysis of the material showed it to be mainly polysaccharide, probably pectins and some hemicelluloses. Mühlethaler (1967) and Pickett-Heaps and Northcote (1966) also suggest that the golgi bodies are involved in pectin and hemicellulose synthesis rather than cellulose synthesis.

Another cytoplasmic component thought to be involved in wall formation is the endoplasmic reticulum (ER). Porter and Machado (1960) suggested that the position of the original plasmodesmata is determined by the presence of tubular elements of the ER which extend across the cell plate. These areas appear to develop into primary pit fields. Esau et al. (1962) discovered that the ER was consistently smooth near the wall in the region of sieve plate formation in phloem cells. Pickett-Heaps and Northcote (1966) state, "The endoplasmic reticulum is also to be found as a characteristic formation of tubules in close association with the pit fields of the parenchymatous tissue of the coleoptile and root."

They suggest that, "a possible role of the endoplasmic reticulum might
be to direct and channel material to the active sites of wall formation or to exclude it from other sites." Cronshaw and Bouck (1965) propose that both the endoplasmic reticulum and the golgi bodies are involved in the synthesis mechanism.

Microtubules were first discovered in plant tissue by Ledbetter and Porter (1963). They described the microtubules as being 230 - 270Å in diameter, of undetermined length, oriented circumferentially around the cell, and arranged in parallel arrays. Wooding and Northcote (1964) noted that the microtubular orientation was a mirror image of the microfibrillar orientation in xylem secondary wall thickenings. They suggest that these organelles may be involved in the synthesis or orientation of cellulose in the wall. According to them the microtubules could be: a) actually cellulose fibrils being spun into the wall; b) "extrusion moulds" through which the cellulose is "funneled, oriented, and polymerised"; or c) "purely a cytoskeletal system."

Cronshaw and Bouck (1965) and Esau et al. (1966) also noted a close relationship between secondary wall thickenings and microtubule orientation, with the two being parallel to each other. However, in the work of Newcomb and Bonnett (1965) with radish root hairs it was demonstrated that in the region of 3 - 25μ back from the tip the orientations of microfibrils and microtubules did not coincide. In this area the microtubules were arranged axially and parallel to each other while the microfibrils were randomly oriented. In other regions of the root hair, however, they did coincide. It is well to remember in attempting to analyze Newcomb and Bonnett's (1965) results that the radish root hair wall has a double layer, the inner of which is oriented axially and does not extend
completely to the tip.

It is interesting to note that in Pickett-Heaps and Northcote's (1966) discussion of the possible roles of microtubules they suggest that these structures may exert specific directional control over enzyme systems concerned with microfibril synthesis and orientation. Another possible role was the direction of materials to sites of wall synthesis by influencing protoplasmic streaming.

With the development of the freeze-etching technique it became possible to observe cells that had not been treated with various solvents and fixatives. Using this technique Moor and Mühlethaler (1963) discovered very labile proteinaceous particles about 150A in diameter, which were attached to the outer surface of the plasmalemma. Extending from the particles were fibrous structures closely resembling microfibrils. These authors also noted that the arrangement of the particles coincided with the microfibrillar arrangement. Thus they concluded that the particles are responsible for both the synthesis and orientation of the microfibrils. Esau et al. (1966) reported similar particles on the exterior surface of the plasmalemma in secondary walls of tracheal elements of *Beta vulgaris* and *Cucurbita maxima*. These particles, however, were located within invaginations of the plasmalemma.

**Theories of wall extension**

**General** According to Wardrop (1962) surface enlargement is accompanied by changes in thickness, mechanical properties, composition, metabolism, and organization of the cell wall. In many plant cells there is an initial thinning of the cell wall as elongation begins, but soon the wall regains its original thickness or becomes slightly thicker.
Because of these things it has been extremely difficult to formulate a theory of wall growth that adequately explains the observed phenomena.

In Heyn's (1940) review of early theories of cell elongation, he mentions that there are three theoretical possibilities of enlargement mechanisms. The first was that enlargement was caused by active wall synthesis which was independent of exterior forces. The second was that enlargement was caused by elastic stretching of the wall due to turgor pressure. New material was then deposited in the wall solidifying it. The third possibility was that enlargement was caused by plastic stretching due to turgor pressure. The wall particles slip past each other and then become re-attached in their new position. Heyn (1940) based his personal theory on the last of these possibilities. He stated that the primary factor in cell elongation is the plasticity of the cell wall and that the first step in elongation is an increase in wall plasticity. Then a plastic extension takes place during which the wall particles slide past each other. He considered that turgor pressure furnishes the energy for this surface extension.

Frey-Wyssling and Mühlethaler (1965) point out that it is the plastic nature of meristematic cells that makes them susceptible to surface tension forces which mold them into polyhedral shapes. The shapes and microfibrillar patterns in other cells are not as easy to explain. At the present time there are four main theories explaining growth in wall surface area (Wardrop, 1962). They are polar or bipolar tip growth, mosaic growth, "islands of synthesis" growth, and multi-net growth. Of these four, multi-net growth seems to have the more universal application. Even so, each theory warrants some discussion.
Polar or bipolar tip growth

This theory was proposed by Mühlethaler (1950b) who noted that early stages of coleoptile parenchyma cells showed corner thickenings of longitudinal fibrils which, he concluded, would inhibit extension of that portion of the wall. He also noted that the walls had a more open texture near the ends. He suggested, therefore, that the protoplast extended through the weak end wall and synthesized new microfibrils slightly behind the advancing front.

Immediately, the theory came under heavy criticism and Frey-Wyssling and Mühlethaler (1965) pointed out that the tracer experiments of several workers (O'Kelley, 1953; Wardrop, 1956a; and Setterfield and Bayley, 1957) indicated that in cotton fibers, coleoptiles, and root cortex cells that secretion of wall material occurs along the entire surface of the protoplast.

It does appear that in pollen tubes (Wardrop, 1962) and root hairs (Houwink and Roelofsen, 1954; Dawes and Bowler, 1959; and Belford and Preston, 1961) that tip growth does occur. For example, in their work Belford and Preston (1961) used $^{14}$C glucose in the medium in which Sinapsis alba seedlings were grown. They found through autoradiography that rapid wall synthesis took place only for a distance of about 120µ back from the hair tip. They concluded that the outer, randomly oriented layer was formed by intussusception at the very tip while the inner, axially oriented layer was formed by apposition a short distance back from the tip.

Mosaic growth

This theory was proposed by Frey-Wyssling and Stecher (1951) but was partially based on observations reported earlier (Frey-Wyssling et al. 1948). In this earlier report they concluded, on
the basis of their observations of microfibrillar patterns in a variety of cell types, that the primary wall was formed by intussusception from the living cytoplasm rather than by apposition at the surface of the cytoplasm. Growth in area takes place due to a loosening of the wall network which is then followed by the interweaving of additional new microfibrils which strengthen the wall. In the later paper, fortified with corn root parenchyma studies Frey-Wyssling and Stecher (1951) formalized the theory by stating, "The growth in area of plant cell walls consists in loosening local fields of the tubular texture of cellulose microfibrils. This loosening must be caused by intensive local plasm growth which pushes the existing microfibrils aside. This extension growth by local spots is termed mosaic growth."

Mühlethaler (1950a) also suggested that in corn and oat coleoptile cells the primary wall grows non-uniformly over its surface. He cites as evidence the occurrence of "loose reticular zones adjacent to dense finished areas . . . " and the fact that a finished primary wall has a more uniform arrangement of microfibrils. He also indicates that some of the loose areas developed into pits and that the plasmodesmata keep the pit areas free of cellulose microfibrils.

Wardrop (1954) disagreed with Frey-Wyssling and Stecher's idea that in mosaic growth thin areas develop temporarily where there is a localized displacement of the microfibrils by the protoplast but are then filled in with new microfibrils. Wardrop felt that the areas of wall synthesis, as suggested in the mosaic growth model, are identical with the primary pit fields. He states that, "the cell surface must be increased by an enlargement of the cytoplasm in the plasmodesmata. This may then be
followed by a peripheral encroachment of, or a subdivision of, the plasmodesmata by the synthesis of new microfibrils." He suggests that this process may be repeated in the same plasmodesma several times. Finally, he concludes, "Primary pit fields must be areas of cellulose synthesis."

In another series of experiments Wardrop (1955) studied the arrangements of primary pit fields in *Avena* parenchyma cells and concluded that growth took place over the entire length of the cell and not just at the tip. He states, "It has been observed that extension of the parenchyma involves a progressive separation of the primary pit fields accompanied by an increasing dispersion of the cellulose microfibrils about their preferred direction of orientation. On the basis of this . . . it is suggested that extension growth involves stretching of the cell with the intercalation of new microfibrils into the expanding cell wall framework from the regions of the primary pit fields and penetration of the wall by plasmodesmata."

In this same study Wardrop made counts of primary pit fields in elongating cells and showed that there was no significant change in the number per cell, but that the number per unit area decreased. In addition, the shape of the primary pit fields was observed to change during elongation. In young parenchyma cells the primary pit fields were elongated transversely to the cell axis, but in more mature cells were stretched in the longitudinal direction. In contrast to this observation, he noted that in enlarging, isodiametric parenchyma cells of apple cortex and potato tuber the primary pit fields remained nearly circular. He summarized these observations by saying, "Since growth takes place under conditions of turgor, stresses operative in the wall may govern the
orientation of the microfibrils. This is reflected in the shape of the primary pit fields."

Bayley et al. (1957) worked with epidermal cells of oat coleoptiles, and because of the persistence of distinct layers in the outer wall, came to the conclusion that new material must be laid down throughout the outer wall. This is similar to the mosaic pattern of growth model except that in this case, there appears to be no penetration of protoplasm into the outer layers. It is thus possible that this is a case of extension of pre-existing layers by end synthesis. This would require extra-cellular enzyme systems, however. Beer and Setterfield (1958) reported a similar situation in celery petiole collenchyma in which the wall thickenings were longitudinally oriented microfibrils.

**Islands of synthesis** This theory, advanced by Preston and Kuyper (1951), stated that microfibril synthesis and orientation were controlled by highly localized aggregates of cytoplasmic granules located on the inner surface of the wall. The theory was based on the observation of such particles on the inner lamella of *Valonia ventricosa* vesicle walls. The theory has been discounted because the wall becomes uniformly radioactive in tracer experiments (Wardrop, 1962).

**Multi-net growth** While studying cotton, *Ceiba*, and *Asclepias* plant hairs, Roelofsen and Houwink (1953) found that "... there is a gradual change from a compact transverse structure on the inside to a loose, more or less axial structure on the outside of the growing cell wall." They noted that the inside layer resembled a fishing net with the meshes elongated transversely while the outer layers showed meshes elongated longitudinally. They postulated that as the wall expanded the
net was stretched longitudinally, elongating the meshes in that direction. They termed this type of growth, "multi-net growth."

O'Kelley (1953) used autoradiographic techniques to determine the location of wall synthesis in cotton fibers and concluded that "cell elongation in the cotton fiber involves cellulose synthesis throughout the length of the fiber wall instead of at the tip of the fiber alone."

This result and others using the same method may be confounded by exchange reactions. Houwink and Roelofsen (1954) also studied cotton fibers (hairs) and described the outermost layer of microfibrils as being randomly oriented at the tip, but axially oriented in the tubular part of the cell. They asserted that this could be explained on the basis of multi-net growth since at the tip both radial and longitudinal enlargement are taking place simultaneously. Reorientation in the tube part of the cell indicates elongation is taking place uniformly over the wall. They pointed out that the microfibrils must be free to slip past each other since there is no decrease in cell diameter with microfibrillar reorientation. On this last point Frey-Wyssling and Mühlethaler (1965) suggest that it is possible for the fibrils to slip relative to each other because of the plastic nature of the matrix. deWolff and Houwink (1954) developed a mathematical model which would, within the framework of the multi-net theory, account for different fibril patterns in the primary walls of growing cells such as cotton hairs.

In a paper published in 1956 Wardrop (1956a) discarded the mosaic growth theory that he had previously favored (Wardrop, 1954, 1955) and accepted the multi-net model. As reasons for the change he cited his observations that *Avena* coleoptile parenchyma cells cultured in a medium
containing $^{14}$C glucose became uniformly labeled, suggesting that the pit areas alone were not the only sites of synthesis. Also he observed that the arrangement of microfibrils on the inner surface was transverse while the arrangement on the outer wall was more longitudinal. From another paper citing similar results with onion root parenchyma (Wardrop, 1956b) he concludes, "From this evidence . . . growth in coleoptile parenchyma is not the 'bipolar' or 'mosaic' types previously suggested, but corresponds to the 'multi-net growth' of Roelofsen and Houwink."

Setterfield and Bayley (1957) examined the location of labeled cellulose in parenchyma and epidermal cells of *Avena* coleoptiles and onion roots grown with $^{14}$C-labeled sugars. As had other workers, they found no localized incorporation in the vicinity of primary pit fields. In thin section studies of these plant materials, they observed a gradual change in orientation of microfibrils from transverse in the inner region to essentially longitudinal in the outer region of a growing cell wall. The degree of longitudinal orientation in the outer region increased with increasing cell length. However, they did point out that the multi-net theory alone does not adequately explain elongation in parenchyma cells which show longitudinal ribbing.

Wardrop and Cronshaw (1958) offer an explanation for the occurrence of these longitudinal bands in parenchyma cells. They postulate that these bands are formed at the cell corners because there are no plasmodesmata there to restrict the reorientation of the microfibrils due to multi-net growth. Later, as the protoplasm withdraws from the cell wall, more and more of the fibrils around the primary pit fields become reoriented to lie in a more longitudinal direction, thus giving the
appearance of a "filling in" of the pits. Also, cellulose synthesis would take place earlier on the inner wall where the plasmodesmata were not located, thus contributing to a localized thickening of the bands.

Houwink and Roelofsen (1954) indicate that some cells, e.g. Trades-cantia staminal hairs and Phycomyces sporangiophores, may have an intermediate type of growth between multi-net and tip growth. In both these cell types, the inner microfibrils are transversely oriented while the outer microfibrils are randomly oriented. This may indicate growth in more than one direction.

Wardrop and Cronshaw (1958) obtained evidence for the multi-net growth theory by using a different approach. They found that in Avena coleoptile parenchyma grown under conditions (2 - 4°C) that inhibited wall synthesis, but allowed cell extension, there was, "no uniform transverse microfibril orientation on either the inner or outer surfaces, the whole wall giving the impression of having been stretched."

Frey-Wyssling and Mühlethaler (1965) indicate the role that the microfibrils play in elongation by stating, "... the secreted microfibrils in the primary wall ... behave completely passively. They are inertly displaced and reoriented ... according to the laws of flow of viscous fluids, and in this way completely miss any morphogenetic impetus."

In summary then, Wardrop (1962) states that, "the multi-net mechanism appears adequate to explain the observed changes in microfibril orientation during growth in seed hairs and in parenchyma undergoing extension such as that of coleoptiles, pith, and roots. It also appears consistent with observations on the differentiating xylem of conifers." He concedes that growth of thick-walled cells such as colenchyma and epidermal cells is
not completely explained by the multi-net theory.

**Effects of auxins**

General Aberg (1957) has said, "It is true that root elongation is a complex phenomenon, but it is none the less true that it is a well integrated process which may be regulated as a whole." Deeply involved in the regulation of this process are auxins. One auxin, indoleacetic acid (IAA) is of considerable interest because it is a naturally occurring regulator.

Haber (1962) proposed that indoleacetic acid can have a double role in regulating growth in plants. These roles are: that of exerting either a positive or negative influence on growth by expansion, apart from action on cell division; or that of exerting either a positive or negative influence on mitotic activity, apart from expansion. According to Torrey (1956), in root sections cell enlargement in a radial direction as well as in a longitudinal direction is a common response to applied auxins. Thimann (1963) considers that cell enlargement is the most typical auxin function.

**Mode of action** In Heyn's (1940) review of cell elongation, he indicated that there were three possible mechanisms of wall enlargement. One of these required active wall synthesis to provide the driving force. The other two required turgor pressure to cause either an elastic or plastic extension of the wall. If elastic extension occurred, wall synthesis would be required to strengthen it in the extended position. Assuming these to be the only possibilities there are, therefore, several possible sites upon which auxins can act to stimulate enlargement. The
first is that auxins play a role in regulating wall synthesis (matrix fraction, cellulose fraction, or both). Secondly, they may be involved in altering the structure of the wall (make it more or less plastic). Thirdly, they may have an effect on the turgor pressure within the cell either by altering the permeability of the membrane or by changing the osmotic pressure of the cell sap.

The last of these possibilities has been considered by several workers. Ordin and Bonner (1956) concluded from heavy water uptake studies that IAA in optimal concentrations had no effect on uptake of water by Avena coleoptile sections. They showed that metabolic inhibitors such as 2,4-dinitrophenol and KCN in nonlethal concentrations had only a slight effect on permeability. Even killing the cells caused only a slight increase in permeability. Therefore, they concluded that the cell wall was the primary barrier to water uptake. Burström and Fransson (1957) decided from studies of water-saturated Avena coleoptiles that permeability could not limit auxin induced elongation even if there were no restriction on water uptake. It appears from the work of Burström (1942b) that the osmotic pressure does not change radically during elongation, since he observed no appreciable change in the turgor pressure of wheat root epidermal cells upon cell extension.

Indirect evidence against a modification of turgor pressure by auxins was furnished by Chao and Loomis (1947) in their temperature studies of elongation in dandelion scapes, leaves of Ricinus communis, and hypocotyls of Phaseolus vulgaris. Based on their observed $Q_{10}$ values, they concluded that chemical rather than physical reactions in the protoplast and/or cell wall were the limiting factors in cell enlargement where moisture was not limiting.
The possible effects of auxins on the synthesis of wall components have also been studied extensively. In his Ph.D. thesis on maize root growth, Baldovinos (1950) stated, "Cell enlargement would seem to be limited by enzymatic reactions dependent upon the presence of auxins. We may postulate that these reactions involve the lengthening of the cellulose micelles of the cell wall in such a way as to allow expansion of the cell by hydrostatic pressure." While working with potato tissues, Buffel and Carlier (1956) found that auxins caused a change in cell wall composition, with pectins increasing relative to cellulose. They proposed that through the hydration of pectins the wall becomes more extensible. The main conclusion of Bentley's (1958) research summary of the effects of auxin on the cell wall was that optimum concentrations (for growth) increased the content of pectic substances in the wall relative to cellulose; inhibitory concentrations enhanced cellulose synthesis leading to a more rigid wall.

In his work on oat coleoptiles Ray (1962) showed that auxin promoted elongation by a larger factor than wall synthesis. Auxin treated detached coleoptiles showed a decline in wall synthesis relative to elongation whereas nonauxin treated detached coleoptiles showed increased synthesis over elongation. He also noted that wall synthesis was depressed in the absence of sugar but elongation continued. From this information he concluded that wall synthesis and elongation were not directly related.

The final possible mode of auxin action is on the plasticity of the cell wall. In this regard Galston and Purves (1960) cite evidence in their review of auxin action mechanisms that "... auxin can produce its effects on plasticization and on elongation at low temperatures
(2 to 4°C) at which no increase in the weight of the cell wall material occurs. This leads to the conclusion that auxin acts on some protoplasmic system, this action leading to an altered arrangement of cell wall components, this in turn leading to a greater extensibility."

In very early work, Ursprung and Blum (1924) were able to separate extension of *Vicia faba* roots into plastic and elastic components by measuring cell sizes before and after plasmolysis at different stages of elongation in water at 0°C. Heyn (1931) was able to accomplish the same thing by measuring the reversible and irreversible angles of bending when a weight was placed on the unsupported end of a horizontal coleoptile. His procedure allowed him to test the effects of applied auxin on each of these components and he found that auxin increased the plasticity of the cell wall.

Thut and Loomis (1944) studied the rates of expansion of castor bean leaves at different times of day and different temperatures and found that cell expansion was dependent upon temperature as well as water supply.

In a series of experiments by Cleland and Bonner (1956) *Avena* coleoptiles were pretreated with auxin while held under non-expanding conditions (isotonic mannitol), given a transition treatment consisting of an auxin inhibitor (argon) in isotonic mannitol that stopped auxin action and expansion, and then allowed to expand in the presence of an auxin inhibitor (argon). They learned that: a) auxin action and expansion could be separated in time; b) an antiauxin in the expansion medium could partially reverse the effects of the auxin pretreatment; and c) some type of metabolism was required to maintain the pretreatment effect.
(inhibited by 2,4-dinitrophenol and KCN). Cleland (1958) proposed that extension requires an increase in plasticity, intussusception of new cell wall material, osmoregulation, and water for expansion. Of these, however, "only the loosening of the cell wall is auxin dependent."
MATERIALS AND METHODS

Plant Material

Young radicles of *Zea mays* L. were used to study the changing microfibrillar patterns in elongating cell walls. To eliminate as much genetic variation as possible, all seeds used for the study were from a single batch of the single cross hybrid WF 9 x M 14 supplied by Clyde Black and Son, Ames.

For germination, 25-100 seeds were arranged on a moist paper towel placed on a slanted sheet of glass in a plastic crisper. Care was taken to arrange each seed with the embryo against the paper towel and with the radicle pointing down the slope of the glass. An additional paper towel was placed over the seeds and arranged as to have the lower edge dip into 100 ml of distilled water in the bottom of the crisper. A lid was placed on the crisper to maintain high humidity inside the container. The crisper was then transferred to a dark incubator adjusted to 30°C. After 48 hours, germination was essentially 100% complete.

Growth Regions

Since varietal differences in the location and extent of the various growth regions have been reported for *Zea mays* roots, a marking experiment using the procedure of Baldovinos (1953) was performed to determine these parameters for the variety studied here (WF 9 x M 14). In this experiment eight corn seedlings of uniform size, previously germinated as above, were selected and the roots carefully marked with India ink at 1.0 mm intervals beginning at the base of the root cap and extending
backwards along the root for a distance of 10 mm. In each of four hori­
izontal staining dishes, two marked seedlings were positioned on sloping,
filter paper covered microscope slides, and covered with a single layer
of filter paper. The filter paper was so positioned as to dip into dis­
tilled water in the bottom of the dish thus keeping the seedlings moist.
The seedlings were incubated at 25°C.

At three-hour intervals for a period of twelve hours the length of
each marked segment on each root was determined with a millimeter rule
and a binocular microscope. After measurement a fresh mark was applied
in the center of each of the old marks. The experiment was repeated at
a later date.

To provide information on cell sizes and stages of development at
various levels in the root, longitudinal sections were prepared for light
microscopy. Root tips, approximately 10 mm long were excised from seed­
lings germinated by the procedure described above, fixed in FAA, dehy­
drated in an ethyl alcohol series (50,60,70,80,95,100%), stained in
chlorazol black E, changed to xylene, infiltrated with paraffin, and
sectioned (10μ thick) on a rotary microtome. Sections were floated on a
drop of xylene on a microscope slide to remove the paraffin and then
mounted in picolyte. An entire longitudinal section was photographed by
parts on 35 mm film (Adox KB-14) under phase contrast, using a Leitz
Orthomat automatic camera. The magnification was determined by photo­
graphing a stage micrometer under the same conditions. A composite
photograph was prepared of the entire root section which was then divided
into portions representing 1.0 mm segments of the intact root. Average
cortical cell lengths were determined at points corresponding to 0.1 mm
intervals in each segment. Notes were also made of relative cell developmental stages.

Sampling Procedure

At the end of the germination period, five to ten, serial 1.0 mm segments were cut from the radicles of 10 – 25 seedlings. This was done with a device consisting of six single-edge razor blades, spaced with narrow strips of aluminum and bolted together so that the blades made cuts 1.0 mm apart. The roots were laid on the cutter with the distal edge of the meristematic zone directly over the first blade and then pushed down over the blades by a rubber stopper or block of paraffin. Segments taken from the same location on each of the roots were combined and placed in 13 x 100 mm test tubes.

Digestion

The procedure used to remove the protoplasm and the non-cellulosic components of the cell walls was a modification of the method described by Wise, et al. (1939) which involves heating the plant tissues in anhydrous ethanolamine at a temperature slightly below the boiling point of the solvent for an extended period of time. They report that this method removes essentially everything but cellulose.

In the present study, 3.0 ml of anhydrous ethanolamine (Matheson, Coleman, and Bell) was added to each of the test tubes containing root segments. The test tubes were filled with 25 mm funnels and covered with a watch glass of the same diameter. The funnel and watch glass served as reflux condenser. A rack containing five or more test tubes was then
lowered into an oil bath maintained at 168°C. A refluxing period of 5.0 hours was found to be adequate for removal of noncellulosic materials from the tissue segments. At the end of this time the funnels and watch glasses were removed, the tubes lifted from the oil bath, and the ethanolamine diluted with distilled water to cool the tubes and stop the reaction. The solvent was then poured off and the tissue rinsed with several changes of distilled water to remove the ethanolamine. Segments were immediately given a maceration treatment for whole cell studies or prepared for embedding in paraffin for sectioning.

Specimen Preparation

Macerated tissue

The procedure used to separate the tissue into individual cells was adapted from Letham (1962) and involved simply storing the tissue in a macerating solution consisting of 20 g sodium hexametaphosphate (sodium metaphosphate, Fisher S-333) per liter (dissolved without heating, pH adjusted to 4.1 with dilute HCl).

Four ml of this solution was added to each test tube containing digested and washed tissue. Then 1.0 ml each of chloroform and toluene were added, the tubes stoppered with rubber stoppers, and shaken vigorously, thus sterilizing the tube contents. Finally, the stoppered tubes were placed in an incubator at 25 or 30°C for 1 to 4 weeks.

At the end of this period the lower chloroform-toluene layer was withdrawn with a syringe. Next, the macerating solution was poured off and the tissue rinsed in several changes of distilled water. After the final rinse the tissue was transferred to 10 x 75 mm test tubes to which
approximately 1.0 ml of distilled water were added. The tissue segments were still intact at this point, so small stirring rods were used to break them up into individual cells and suspend them in the water.

A sample of the cell suspension from any given tube was withdrawn with a 1.0 ml syringe having an 18 gauge, blunt needle. A small drop of this suspension was applied to each of several formvar-coated, 150 mesh copper grids which served as specimen supports in the electron microscope. The grids were allowed to dry in a dust-free container at 60°C and were then ready for shadowing with a heavy metal.

For details pertaining to the preparation of formvar-coated grids, a good handbook of electron microscopy such as Kay (1965) or Pease (1960) should be consulted.

Sectioned tissue

Following digestion, 1.0 mm long segments to be used for observing the inner surface of the primary wall were dehydrated in an ethyl alcohol series, stained with chlorazol black E while in 100% ethyl alcohol, transferred to xylene, infiltrated with paraffin, and then embedded in paraffin using the techniques of Sass (1958). Longitudinal sections of 10μ thickness were cut on a rotary microtome.

Mounting the paraffin sections on the grids and removing the paraffin support posed several problems. First of all, the solvent for the paraffin, xylene, tended to weaken or tear the formvar film. Secondly, the sections were frequently washed off the grid while applying the solvent. Finally, traces of xylene left in the cell walls would cause the cells to shrink in the beam of the electron microscope. These problems were overcome by the following procedure. Short lengths (1 or 2 sections) of the paraffin
ribbon were floated on a small amount of xylene in a spot plate for about 1 minute to remove the paraffin. The sections were next transferred with a small wire loop to a 1:1 mixture of xylene and absolute ethyl alcohol for about 1 minute, then placed in absolute ethyl alcohol for the same amount of time, scooped up on a formvar-coated grid, and allowed to dry. At this point the grids were ready to be shadowed.

Adjacent portions of the paraffin ribbons were mounted on standard microscope slides by floating them on a drop or two of xylene on the slide, adding several drops of picolyte in xylene, and finally adding a cover slip. After a few minutes the slides were ready for observation under a phase contrast microscope.

Electron Microscopy

Shadowing

To render the microfibrillar patterns visible in the electron microscope, all grids were shadowed with platinum-carbon in a Mikros model VE 10 Vacuum Evaporator. The instrument was equipped with a Ladd Platinum-Carbon and Carbon Evaporation Unit which used Pt-C pellets as the metal source. This allowed essentially point source evaporation and produced an extremely fine-grained coating.

The grids were placed in shallow depressions on a small sheet of plastic in concentric semicircles about the point of evaporation so as to give shadowing angles of from 30 to 45 degrees. As soon as the evaporator was at operating vacuum (approx. $0.5 \times 10^{-4}$ mm Hg) a single 1/8 inch long Pt-C pellet was evaporated. After shadowing, the grids were placed on labeled blotters in petri dishes until they could be observed in the
Preparation of micrographs

For each treatment, shadowed grids containing cells or sections of cells taken from each region of the roots were observed in an RCA EMU 3F Electron Microscope at 50 kV. The magnifications used ranged from 1,930x to 34,200x with most work at the lower end of the magnification scale. Each grid was scanned carefully and micrographs were taken of representative cells or parts of cells on 2 x 10" Kodak Projector Slide Plates (Contrast) or 4 x 10" sheets of Cronar film (DuPont). Careful notes were taken on unusual features of the cells, position of cells in a section, longitudinal axis, etc. Both plates and film were developed in D-19 developer (Kodak) diluted 1:1 with water, fixed, washed, and dried.

Since micrographs taken of shadowed material are positives, it was necessary to make internegatives before photographic enlargements were made. The internegatives were made by contact on 4 x 10" sheets of Kodak Gravure Copy film. This film was used because it produced negatives of more normal contrast than the original and because it was insensitive to red light and therefore could be handled readily in the darkroom. The internegatives were developed in DK 50 Developer (Kodak), fixed, washed, and dried. Contact positives on photographic paper were made from the internegatives for examination and study. In general, 8 x 10" enlargements were made for clarification of details.
Light Microscopy

Phase contrast photomicrographs were made of the sectioned material using a Leitz Ortholux microscope fitted with an Orthomat automatic camera. Exposures were made on Adox KB 14 35 mm film which was then developed in Kodak D-11 developer to increase the contrast. Prints were made on photographic paper using standard techniques.
RESULTS

The first step in this investigation was to determine, for the maize variety used, the growth rates of different regions of the root tip. This was accomplished by a marking experiment and making cell measurements on longitudinal root sections. Once this information was obtained, electron micrographs were made of representative cells from regions beginning to elongate, regions elongating rapidly, and regions ceasing to elongate. These micrographs were then analyzed for changes in microfibril patterns during the course of elongation to clarify the mechanism of cell wall expansion during growth.

Regions of Growth

Germinated corn seedlings were marked with India ink at millimeter intervals beginning at the base of the root cap (most distal point of the apical meristem) and extending backwards for eight millimeters. At three hour intervals over a period of twelve hours the length of each original marked segment was determined. The data from this experiment, summarized in Table 1 and graphed in Figures 1a and 1b, indicate that root growth in the maize variety WF 9 x M 14 was primarily confined to the distal six millimeters of the tip. The rate of growth during the 0-3 hour period (Figure 1a) was low in the first millimeter segment, increased in the second and third segments, reached a peak in the fourth segment, and then declined, reaching an insignificant level in the seventh and eighth segments. An analysis of the growth rate of each original segment compared to its position relative to the root cap base at the end of succeeding time
Table 1. Mean segment lengths and absolute growth rates of consecutive 1.0 mm segments of maize root tips

<table>
<thead>
<tr>
<th>Segment</th>
<th>Mean segment length in millimeters after growth periods of 3 hrs</th>
<th>6 hrs</th>
<th>9 hrs</th>
<th>12 hrs</th>
<th>Absolute growth rates in millimeters per 3 hours during the growth periods, 0-3 hrs</th>
<th>3-6 hrs</th>
<th>6-9 hrs</th>
<th>9-12 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.03±0.047</td>
<td>1.31±0.047</td>
<td>1.61±0.063</td>
<td>1.84±0.059</td>
<td>0.08</td>
<td>0.23</td>
<td>0.30</td>
<td>0.23</td>
</tr>
<tr>
<td>2</td>
<td>1.40±0.025</td>
<td>2.05±0.090</td>
<td>3.76±0.246</td>
<td>8.03±0.492</td>
<td>0.40</td>
<td>0.65</td>
<td>1.71</td>
<td>4.27</td>
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Figure 1a. Absolute growth rates of consecutive 1.0 mm maize root tip segments during the 0-3 hour period
Figure 1b. Mean length of consecutive 1.0 mm maize root tip segments at various intervals during a 12 hour growth period.
intervals also indicated that the most rapid growth was located 3-5 millimeters back from the tip.

Over the twelve hour period (Figure 1b) the first segment showed a total growth of only 1.8 times, whereas the second segment increased eight times. The third, fourth, fifth, and sixth segments in turn showed decreasing total growth with time. The seventh and eighth segments remained essentially unchanged during this period.

Cell measurements made from photomicrographs of longitudinal sections (Figures 1c, 2a, 2b) indicated a similar trend, with gradually increasing cell lengths throughout the first segment, more rapidly increasing lengths in the second and third segments, and a leveling off in the fourth and fifth segments. Some error, no doubt, was inherent in this procedure due to shrinkage of cells during fixation and dehydration of the tissue, with the longer, more highly vacuolated cells being affected the most. This shrinkage can be observed in Figure 2b by comparing the root width at the fourth millimeter mark with that at the sixth.

Electron Microscopic Observations

Method

To observe the microfibril patterns on both the exterior and interior surfaces of elongating cells, root tip segments 1.0 mm in length were excised from corn seedlings, digested to remove noncellulosic components, and then either macerated for whole cell preparations or embedded in paraffin and sectioned to allow observation of the inner surface of the walls. All preparations were shadowed with platinum in carbon previous to examination in the electron microscope.
Figure 1c. Mean cell length and cell length range at various points along the maize root tip
Figure 2. Longitudinal section of a *Zea mays* root tip. a: Location of the study segments 1-4. b: Location of segments 5-8.

Marked intervals represent 1.0 mm. 36X.
First segment

A photomicrograph (Figure 3) of the entire first millimeter-long segment shows a remarkable consistency in cell size and shape with only a few cells in the central cylinder showing any pronounced enlargement. However, observation of the exterior surfaces of cells taken from this segment show two somewhat different microfibril patterns. The first pattern is shown by the cell in Figure 4 which, based on its size and shape, was judged to be a newly divided cell and also by the somewhat larger cells in Figures 5 and 6. This pattern consisted of two elements: a) microfibrils oriented longitudinally, forming bands or corner thickenings as described by Muhlethaler (1950a), Wardrop and Cronshaw (1958), and others; and b) microfibrils oriented in a curving, random fashion forming a loose, open network in the areas between the bands. In these interband areas there was, however, a thin surface layer of scattered, longitudinally oriented microfibrils.

In the second pattern (Figures 7, 8, 10) longitudinal bands were also present. However, they were considerably wider, thicker, and therefore more distinct (Figure 7). The interband areas were also more distinct (Figure 8) because of the development (or persistence) of thin areas in the wall which were surrounded by multiple layers of microfibrils. These thin areas probably represent primary pit fields at a very early stage. It should also be noted in Figures 7 and 10 that between bands and interband areas there is a continuity of microfibrils which possibly indicates that forces of different types or directions were operative upon a given microfibril in different regions of the cell wall.

A modification of this microfibril pattern which was observed in
Figure 3. Longitudinal section of the first segment of the maize root tip. 168X.

Figure 4. Electron micrograph of the outer surface of a newly divided cell taken from the first segment. Note the bands of longitudinally oriented microfibrils separated by regions of more randomly oriented microfibrils. The arrow indicates the longitudinal axis of the cell. 10,800X.
Figure 5. Outer wall surface of a first segment cell which was somewhat larger than the one shown in Figure 4. A similar pattern of microfibrils can be observed in both cells. 14,530X.

Figure 6. Outer surface of a cell taken from the first segment showing the microfibrillar pattern in the interband area of the wall. 14,530X.
Figure 7. Outer wall surface of a first segment cell showing the microfibrillar pattern in a longitudinal band. 14,530X.

Figure 8. Interband area of the outer surface of the same cell shown in Figure 7. 14,530X.
Figure 9. Outer surface of a first segment cell showing a distinctly different microfibrillar pattern than was illustrated in the previous figures. 14,530X.

Figure 10. Outer surface of a first segment cell. Note the cluster of what might be protoplasmic granules on the longitudinal band. 14,530X.
only a few cells of the first segment showed a drastic reduction in the proportion of the wall occupied by primary pit fields (Figure 9). Thus the wall texture appeared to be more uniform, having a predominantly longitudinal microfibril orientation.

Of the two exterior patterns described above, the first was consistently found in the smallest cells while the second was definitely the most common and is probably the one in cells that have begun some radial enlargement and elongation.

Figures 11-14 reveal that microfibrils on the interior wall surfaces of cells of the first segment were transversely oriented, even on areas of the wall directly inside the longitudinal bands. This last point is illustrated in Figure 11 where both inner and outer wall surfaces are shown because of the plane of sectioning. The pattern of the interband areas was very similar to that of the corresponding exterior surfaces, indicating very little reorientation of microfibrils around the plasmodesmata of the primary pit fields.

It may also be noted in Figures 13 and 14 that removal of the non-cellulosic materials from the inner surfaces of the wall was not complete in some cells. However, the microfibril orientation is still apparent.

**Third segment**

Cells of the cortical region of the third segment (Figure 15) varied in size from an average of about 45 μ near the distal end to about 75 μ near the proximal end. In the central cylinder considerable enlargement of a few cells was evident. The microfibril pattern on the exterior wall surface of the cell shown in Figure 16 is very similar to the second
Figure 11. Inner (I) and outer (O) wall surfaces of a first segment cortical cell which was sectioned in paraffin. 24,740X.

Figure 12. Inner surface of a wall interband area. This cortical cell was taken from the first segment of the root tip. 24,740X.
Figure 13. Inner wall surface of a first segment cell showing the inner surface of a longitudinal band. 14,530X.

Figure 14. Inner wall surface of a cell from the first segment. Note the distinct, transversely oriented microfibrils in the interband region. 14,530X.
Figure 15. Longitudinal section of the third segment. The epidermal cells can be distinguished along the lower edge of the micrograph. Note the range in cell sizes. The distal end of the segment is to the left. 168X.

Figure 16. Outer wall surface of a cell taken from the third segment. 18,750X.
pattern type found in the first segment except that there appeared to be a reduction in the number of primary pit fields per unit area. Also, there appeared to be a definite increase in wall thickness even within the thinner areas. Longitudinal bands were evident in cells of this segment (Figures 16 and 17) and some microfibrils in the interband areas were also longitudinally oriented (Figure 18). There were, however, transversely oriented microfibrils in the deeper layers.

Microfibrils on the interior surface were oriented transversely (Figures 19-22) except for a few scattered longitudinal microfibrils in some cells (Figures 19 and 20). Additional microfibril deposition around the edge of primary pit fields varied from almost none (Figure 21) to a very large number (Figure 22).

**Fifth segment**

Measurements taken from Figure 23 indicate that some cortical cells in the fifth segment were still elongating but a few had reached their maximum lengths. On the average, these cells were 20-25 percent longer than their counterparts in the third segment. The cells in the stele were more variable in length than those in the cortex, ranging from slightly shorter to much longer, with the majority being as long as or longer than those in the cortex.

The outer wall surface of most cells of this segment was characterized by a nearly uniform arrangement of microfibrils (Figures 24-28). Primary pit fields were more scattered than in the shorter cells of the previous segments and in many cases were partially covered with microfibrils of longitudinal to random orientation. However, there were a few cells in
Figure 17. Outer surface of a third segment cell. 32,340X.

Figure 18. Outer surface of a third segment cell. Note the thin areas (dark spots) that are partially covered with longitudinal microfibrils. 32,340X.
Figure 19. Sectioned cell from the third segment showing both inner (I) and outer (O) wall surfaces. 24,280X.

Figure 20. Inner wall surface of a sectioned cell from the third segment. 24,280X.
Figure 21. Inner (I) and outer (O) wall surfaces of a sectioned third segment cell. Note the change in orientation of microfibrils between the two surfaces. 24,280X.

Figure 22. Inner wall surface of a sectioned third segment cell. Note the heavy deposition of microfibrils around the few thin areas. 24,280X.
Figure 23. Longitudinal section of the fifth segment. The distal end of the segment is to the left. 168X.

Figure 24. Outer wall surface of a fifth segment cell. Note the greater uniformity of the microfibrillar pattern over the surface of the wall. 10,790X.
Figure 25. Outer wall surface of a cell taken from the fifth segment of the Zea mays root tip. 14,530X.

Figure 26. Outer wall surface of a fifth segment cell. 14,530X.
Figure 27. Outer wall surface of a fifth segment cell. The slightly darker areas represent thin areas of the wall which have been partially covered with microfibrils. 14,530X.

Figure 28. Uniform outer wall surface of a fifth segment cell. 14,530X.
which distinct primary pit fields were still maintained (Figure 29).

Figure 30 shows both inner and outer wall surfaces and illustrates the fact that microfibrils on the outer surface were generally longitudinally oriented while those on the inner surface had transverse orientation primarily, with only a very few surface microfibrils being longitudinally oriented. Figures 31-34 indicate that on the inner surface distinct primary pit fields remain through much of the thickness of the wall. Around these areas the microfibrils were transversely oriented.

**Eighth segment**

In the longitudinal root section pictured in Figure 35 it may be noted that while there was some variation in cell length between files of cells, along any given file there was little variation, indicating that elongation had virtually ceased. Secondary wall thickenings are evident in a few cells of the vascular cylinder. Long cells of the type seen in the central region in previous segments were undoubtedly present in this segment but were not in the plane of the section pictured.

Figure 36 shows that some of the primary pit fields were well developed on the exterior wall surface while others (Figures 37-40) were more or less obscured by microfibrils of various orientations, the majority being essentially longitudinal. In this regard these cells did not differ significantly from cells in the fifth segment.

Figure 41 shows a striking comparison between inner and outer surfaces, with the former consisting of transverse microfibrils and the latter of longitudinal ones. In this segment primary pit fields occupied a much smaller proportion of the interior surface of cortical cells (Figure 42), while most of those present were well developed (Figure 44).
Figure 29. Outer wall surface of a fifth segment cell. This type of pattern was very rare among cells in this segment. 23,440X.

Figure 30. Inner (I) and outer (O) wall surfaces of a cell taken from the fifth segment. 24,740X.
Figure 31. Inner wall surface of a sectioned cell taken from the fifth segment showing distinct primary pit fields. 24,740X.

Figure 32. Inner wall surface of a sectioned fifth segment cell illustrating well developed primary pit fields. 24,740X.
Figure 33. Inner wall surface of a cell from the fifth segment. 24,740X.

Figure 34. Inner wall surface of a fifth segment cell. Note the few scattered longitudinally oriented microfibrils lying on the surface. 24,740X.
Figure 35. Longitudinal section of the eighth segment. The distal end of the segment is to the left. Note the nearly uniform length of the cells along any given file. 168X.

Figure 36. Outer wall surface of an eighth segment cell. 29,750X.
Figure 37. Outer wall surface of a cell from the eighth segment. Note the localization of the primary pit fields. An enlargement of the central portion of the micrograph is shown in Figure 38. 3975X.

Figure 38. Outer wall surface of the same cell shown in Figure 37. Note the scattered microfibrils covering the primary pit fields. 13,600X.
Figure 39. Outer wall surface of an eighth segment cell. 18,750X.

Figure 40. Outer wall surface of an eighth segment cell. 18,750X.
Figure 41. Inner (I) and outer (O) wall surfaces of an eighth segment cell. Note the longitudinal orientation of microfibrils on the outer surface and the predominantly transverse orientation on the inner surface. 24,280X.

Figure 42. Inner wall surface of a sectioned cell from the eighth segment. 24,280X.
In the cell shown in Figure 43 a very unusual pattern can be observed. The microfibrils of the interior of this cell were oriented longitudinally rather than transversely.

The final photograph, Figure 45, a micrograph of a cell from the tenth segment, shows a much later stage of primary pit field development. At this stage the wall is more resistant to the action of the digesting solution so that the pores through which the protoplasmic strands protrude still remain visible.
Figure 43. Inner wall surface showing well developed primary pit fields in an eighth segment cell. 24,280X.

Figure 44. Inner wall surface of a sectioned eighth segment cell. 24,280X.
Figure 45. Tenth segment cell outer wall showing an advanced stage of primary pit field development. The arrow indicates the longitudinal axis of the cell. 57,250X.
DISCUSSION

Regions of Growth

Marking experiments in the present study showed that root growth in the *Zea mays* variety WF 9 x M 14 was primarily confined to the distal six millimeters of the tip, with the maximum rate of elongation occurring in the fourth millimeter segment. In addition, very low rates of elongation were found in the first, seventh, and eighth segments with intermediate rates occurring in the others. These results correspond closely with those of Boss (1955) who found maximum elongation in the same segment while using the same variety in a shorter term experiment. He also found the same relative rates in the other segments with the exception of the sixth which showed a somewhat lower relative rate than in the present study.

In comparing these results with those of other researchers it is evident that varietal differences do occur with regard to the extent of the region of growth and the location of most rapid cell elongation in *Zea mays* root tips. For instance, Baldovinos (1950) found for his variety that the growth region was confined to the terminal five millimeters and that the region of most rapid elongation occurred in the last quarter of the second millimeter segment (1.75-2.0 mm from the tip). Brown and Sutcliffe (1950) reported the most rapid rate of elongation for their variety in the region 1.5-3.0 mm behind the root tip. Woodstock and Skoog (1962) showed for two different varieties of maize that one had maximum elongation at a point 3.3 mm from the tip and the other at 4.8 mm. These same two varieties had growth regions of 9.0 and 11.0 mm respectively. For their variety, Erickson and Sax (1956a) calculated that the maximum
rate of elongation occurred 4.0 mm back from the tip.

It may be concluded, therefore, that the results of the present study fall within the range of values reported for other varieties of *Zea mays*.

**Electron Microscopy**

The methods used in this study to prepare root cell walls for electron microscopic observation made it possible to compare the inner and outer wall microfibrillar patterns of cells at different stages of elongation, of cells at the same stage of elongation, and in a few cases of the same cell. Also, by applying the results of the marking experiments to the electron microscope observations it is possible to compare the wall patterns of cells which were undergoing rapid elongation with those of cells elongating more slowly or not at all.

The most obvious feature in these elongating cell walls was the alternation of distinctly longitudinal bands of microfibrils with areas of more randomly oriented ones. These latter areas had the appearance of a delicate filigree at the beginning of elongation (first part of the first millimeter segment) but then as elongation proceeded became organized into distinct primary pit fields, which in turn may change in shape and arrangement during the later stages.

Such a rearrangement of primary pit fields was reported by Scott et al. (1956) in onion root cortical cells. They indicated that primary pit fields were randomly distributed at early stages then became aligned in vertical bands (i.e. between corner thickenings) and still later became oriented in a single file within each band. This final reorientation was observed in some cells in the corn roots presently studied. Also, in these
cells the primary pit fields were elongated in the direction of the longitudinal cell axis. In the majority of the cells, however, there was only a slight tendency for reorientation into a single file.

Although no specific counts were made it is readily apparent from the micrographs that the number of primary pit fields per unit area of the wall decreased during elongation. This observation is supported by Mericle and Whaley (1953) who reported similar results in the corn root tissue which they studied. It seems likely that part of this decrease is due to the lack of formation of new primary pit fields, coupled with elongation of the wall between pre-existing ones. It is also possible that the plasmodesmata retract from some primary pit fields which then become covered over with microfibrils.

Evidence from the micrographs indicates that cellulose microfibrils are laid down in a direction transverse to the long axis of the cell in those areas of the wall not occupied by the plasmodesmata. In the regions of corner thickenings these transverse microfibrils appear to become reoriented and to lie in the longitudinal direction. This would lend some support to the multinet theory of wall growth as proposed by Roelofsen and Houwink (1953), at least in these areas. It was noted, however, that microfibrils were reoriented in their entirety which is not what would be expected in the multinet model. This model suggests that the microfibrils are essentially straight and transversely oriented forming a series of transversely-elongated meshes. Then as the wall expands these meshes become elongated in the longitudinal direction. If cross-linkages between microfibrils of any type are present it would be expected that any given microfibril would become progressively more zigzagged in appearance.
However, this was definitely not true in the cells studied. It would appear more likely that the microfibrils are passively reoriented as individual units rather than as components of a network.

In the regions of the primary pit fields the changes in microfibrillar patterns are more difficult to assess because of the interference of the plasmodesmata. It seems logical to assume that wall synthesis is taking place at all cell wall-cytoplasm interfaces, which would include those extending through the primary pit fields connecting adjoining cells. Thus new microfibrils are possibly being added to the outer surface of the wall at about the same rate as to the inner surface. This would form an entirely different pattern of microfibrils which would be less affected by the longitudinal extension of the cell. Such a pattern is apparent in the micrographs studied.

As elongation progresses and some of the plasmodesmata retract from the wall it is possible that the primary pit fields would be filled in from the outer surface, with randomly oriented microfibrils through the thickness of the wall but then with transversely oriented ones on the inner surface. If the cell is then capable of further elongation these microfibrils would also become passively reoriented along the longitudinal axis. On the other hand if elongation has essentially ceased when the protoplasm retracts the walls would tend to have more randomly oriented microfibrils in these areas. This would account for the more uniform pattern of microfibrils as observed in most cells of the fifth and later segments.

In conclusion, it appears that the multinet theory does not completely explain the pattern of wall growth in Zea mays root tip cells. Instead,
the plasmodesmata seem to play a more dynamic role in modifying the pattern of cellulose microfibril deposition than this theory would suggest.
SUMMARY

Growth rates of successive 1.0 mm root tip segments of the *Zea mays* variety WI 9 x M 14 were determined at three-hour intervals for a total period of twelve hours. Cell counts and measurements were made from photographs of untreated root tips prepared for photomicrography using standard histological techniques. It was found that growth was limited to the terminal six millimeters of the root with most rapid elongation occurring in the fourth segment.

Root tips from newly germinated corn seeds were prepared for electron microscope observation by cutting them into 1.0 mm segments, removing the noncellulosic components by digesting in ethanolamine, macerating the tissue to form a cell suspension which was applied to copper grids, and finally by shadowing with platinum in carbon. This procedure made it possible to observe the cellulose microfibril patterns on the outer wall surfaces of cells from each segment. To observe the inner surfaces the digested segments were imbedded in paraffin and sectioned on a microtome. Single sections were treated to remove the paraffin, placed on grids and shadowed.

The results of these various experiments made it possible to compare rates of growth and cell size with inner and outer wall microfibril patterns. At early stages of elongation cells had very open, lacy patterns which, upon elongation, changed into a pattern of developing primary pit fields in certain areas and corner thickenings of longitudinal microfibrils in other areas. Rapidly elongating cells tended to have a transverse orientation of microfibrils on the inner surfaces and longitudinal orientation on the outer ones except in the location of the primary pit fields.
Cells completing elongation had a more uniform pattern composed of fewer primary pit fields and a random to longitudinal orientation of microfibrils. It is considered that the multinet model of cell wall elongation is not completely adequate to explain the observations made in this study. Instead it is suggested that cellulose microfibrils are synthesized at all cytoplasm-cell wall interfaces, including primary pit fields. In most areas of the wall the microfibrils are laid down transversely to the cell axis and then are passively reoriented as turgor pressure enlarges the cell. In the primary pit fields the microfibrils are deposited throughout the thickness of the wall and are therefore less susceptible to reorientation. In time as the primary pit fields are covered over the main orientation of microfibrils on the outer surface is longitudinal.


ACKNOWLEDGEMENTS

The author wishes to express appreciation to Dr. Walter E. Loomis for suggesting this problem, for guiding the early and final stages of research, for carefully editing the manuscript, and especially for offering his encouragement during the entire project. Special thanks are also due to Dr. C. C. Bowen for serving as Major Professor during the absence of Dr. Loomis and for allowing unlimited use of the electron microscope facilities of the Cell Biology group.

The author is also grateful to his colleagues in cell biology and plant physiology at Iowa State University for making valuable suggestions and for instruction in techniques relative to electron microscopy; to the National Science Foundation and the Department of Botany and Plant Pathology at Iowa State University for financial support through an NSF Cooperative Graduate Fellowship, Teaching Assistantship, and Instructorship; and finally to his wife, Beverly, and family for their encouragement and sacrifices to make this all possible.