Multinet Growth in the Cell Wall of *Nitella**

By PAUL B. GREEN, Ph.D.

(*From the Division of Biology, University of Pennsylvania, Philadelphia*)

PLATE 138

(Received for publication, October 18, 1959)

ABSTRACT

Plant cell walls typically consist of crystalline microfibrils embedded in a non-crystalline matrix. The growing cylindrical *Nitella* cell wall contains microfibrils predominantly oriented in the transverse direction. The present study has shown that the transversely oriented microfibrils are primarily located toward the inner surface of the wall and that, proceeding outward from the inner surface, the wall contains microfibrils of ever poorer transverse orientation, the fibrils being randomly or axially arranged in the outermost regions of the wall. Because cell expansion is primarily in the axial direction, the texture of the fibrillar elements of the wall can be explained by assuming that new microfibrils of transverse orientation are added only at the inner surface of the wall and that they become passively reoriented to the axial direction during cell elongation. The described structure corresponds to that proposed by Roelofsen and Houwink for cells showing "multi-net growth." The demonstration of a continuous gradient of microfibrillar arrangement and its partial quantitative description was accomplished by the analysis, with the polarized light and interference microscopes, of wedge-like torn edges of developing cell walls which were 1 micron or less in optical thickness.

INTRODUCTION

The growth of the plant cell may be viewed as the product of an interplay between an expanding cell vacuole and a yielding cell wall. There are many reports which show that the turgor pressure of the vacuole either remains constant or falls during growth (1), and thus the ability of the wall to yield to vacuole pressure becomes the more important factor in cell expansion. There is an extensive literature (2) concerning the question of whether the plastically extending cell wall is a dead secretion of microfibrils and polysaccharide matrix (growing by apposition), or whether it is, at least in part, a living structure permeated by protoplasm and growing in mass throughout its thickness (growing by intussusception) (Text-fig. 1 *a*). Recent evidence, to be described below, is in favor of the view that the wall is an inert accumulation of complex secretions from the protoplast (3).

The electron microscope has permitted the direct viewing of the orientation of microfibrils on the inner and outer surfaces of cylindrical cell walls and from such pictures Roelofsen and Houwink (4) have proposed the "multi-net growth theory." They have suggested that the protoplast continually secretes, during growth, a mesh of transversely oriented microfibrils onto the inner surface of the cylindrical wall. Because the cell extends in an axial direction, the deposited microfibrils will be in a structure that is expanding primarily in a direction at right angles to their original orientation. The microfibrils will thus undergo a passive reorientation from a transverse to an axial arrangement during growth (Text-fig. 1 *b*). Because new microfibrils are continuously added to the inner surface during expansion, a given group of microfibrils will undergo (*a*) passive reorientation to the axial direction, (*b*) apparent migration toward the outside of the wall, and (*c*) attenuation in relation to cell surface expansion. The above workers, and

* This work was supported in very large part by a grant (G-5944) from the National Science Foundation. The technical aid of Mr. James C. W. Chen is gratefully acknowledged.

others (5, 6), have found that a variety of walls show axial microfibrils on the outer wall surface and transverse microfibrils on the inner surface, in accord with multinet growth. The author has shown (7) that the incorporation of tritium from the medium into the cell wall of *Nitella* takes place only at or very near the inner surface of the cell wall, and thus support, based on a second method, has been added to the view that the wall is a passive structure.

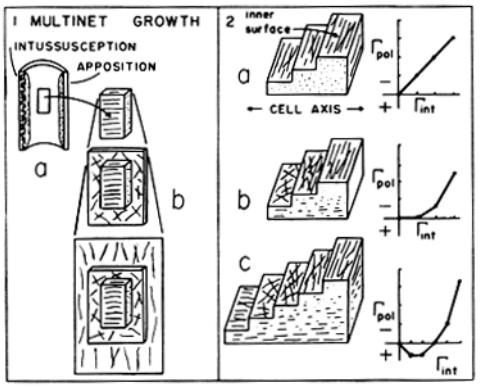
The multinet growth hypothesis, proposing the passive reorientation of originally transverse microfibrils, was based on the assumption that the transversely arranged microfibrils seen on the inner wall surface and the random or axial ones seen on the outer wall surface represented the two extremes of a *gradient* of microfibrillar arrangement. Testing the actuality of this gradient in the cylindrical *Nitella* internodal cell was the object of the present research.

This cell is advantageous because growth is evenly distributed along the cell axis and the structure of a given cell is similar along the axis (8, 9). It was known through electron micrographic studies that the *Nitella* internodal cell wall had a transverse arrangement of microfibrils on the inner surface (11) while the outer surface unfortunately showed no microfibrillar texture in replicas. That the majority of the wall's microfibrils ran transversely was indicated by studies with polarized light and with ultrathin cross-sections of the wall examined in the electron microscope (9). This latter method, which had been quite successful in demonstrating the orientation of microfibrils in lamellae in the alga *Valonia* (10), gave only a general impression of a transverse arrangement in *Nitella* probably because of the lack of lamellae and greater dispersion of microfibrils in the last named alga. The clear demonstration of the presence of the gradient of structure required a quantitative approach and it was found possible to describe the gradient with two optical parameters, one measured in the polarized light microscope, the other in the interference microscope. (The author several years ago unfortunately published the opinion that multinet growth appeared not to occur in *Nitella* (9)).

Experimental Methods

The mode of action of the two microscopes used in these studies will be briefly described. For further details see (11) and the references therein. Cell walls are comprised of many crystalline microfibrils dispersed in an amorphous matrix. The action of a given piece of wall upon a beam of polarized light is a function of both the number and orientation of the microfibrils. Each crystalline region, having two refractive indices, splits the incident beam into two components which travel at different speeds through the crystal. Upon leaving the crystal the two wave trains interfere giving elliptically polarized light which can pass through the analyzer. The retardation of one wave train with respect to the other (Γ_{pol}) is a distance and is measured with a compensator. When many microfibrils are superposed, parallel to each other and normal to the beam, the action on the beam is additive. When the microfibrils are superimposed but scattered at random, the effects on the beam cancel out and there is zero retardation. The compensator also gives the sign of retardation. If the direction of the high index of refraction coincides with the long axis of a body, the body is said to show positive retardation. If the higher index is at right angles to the axis, the body shows negative retardation. Retardation divided by thickness of the crystal gives birefringence which is correspondingly positive or negative. Individual microfibrils are positively birefringent. When they are transversely arranged in a cylindrical cell wall, the wall is termed negatively birefringent. When the microfibrils run in the axial direction the wall is positively birefringent. It will be realized that the retardation of a piece of wall divided by its thickness will give a measure of the degree of scatter of the constituent microfibrils, provided the percentage of crystalline material is the same in all samples. The higher the value of this quotient, the better the alignment of the crystalline fibrils. When the value is negative but of large magnitude, there is good transverse alignment. When the value is zero the crystallites are at random. When the value is positive the alignment is in the axial direction. All these conditions were found in pieces of wall especially prepared for this investigation. The quotient is not identical with birefringence because the thickness measurement includes the non-birefringent matrix between microfibrils. The term birefringence is usually applied to single crystals.

The measurement of thickness, necessary for determination of the quotient, was carried out in an interference microscope. Here again retardation is measured, but this time the retardation is between two halves of a split beam which has been split in the microscope itself and not in the specimen. The specimen encounters one wave train and retards it in comparison with a wave train which passes through the mounting medium. The retardation is a linear function of the thickness of the specimen provided the refractive index of the specimen and mounting medium are constant for all samples. The retardation is a measure of mass/area of the specimen. When retardation is divided by the difference between the refractive index of the specimen and mounting medium, a distance called optical thickness



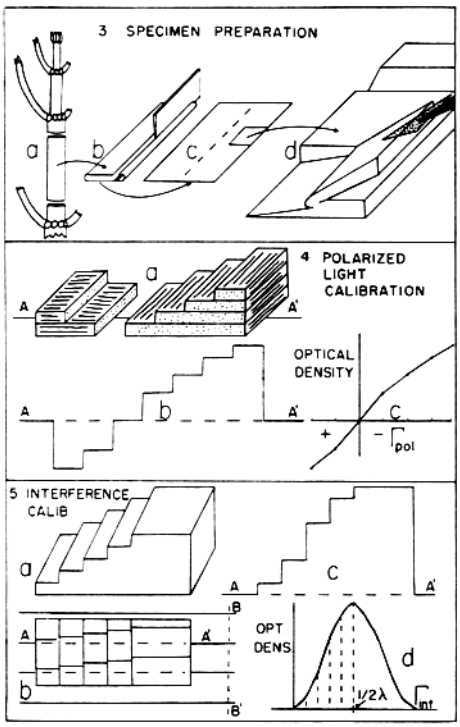
TEXT-FIGS. 1 and 2. Text-fig. 1 *a* shows opposing views of cell wall growth: intussusception where new mass is added in the interior of the wall by penetrating protoplasm, and apposition whereby the protoplast does not penetrate but deposits new mass only at the inner surface of the wall. Text-fig. 1 *b*. The sequence of cell wall development by multinet growth. The original group of transverse fibrils deposited at the inner surface undergoes: reorientation in the direction of cell elongation, apparent migration toward the wall exterior, attenuation in inverse proportion to cell area increase. Text-fig. 2. Staircase-like pieces of single wall thickness, inner wall surface up. In *a*, wall texture is uniformly transverse. The graph at right is a plot of negative (transverse) retardation in polarized light as the ordinate, optical thickness (retardation in the interference microscope) which is equivalent to mass/area as the abscissa. Note that loss of mass is accompanied by a proportionate loss in negative retardation. In *b* the model wall at left has a gradient from inner transverse structure to outer isotropic structure. The corresponding graph, with the same conventions as above, shows a curve with a sag toward the abscissa. In *c* the wall shows the complete reorientation of fibrils at the outer surface of the wall to the axial direction—the typical multinet growth structure. The graph at left, with conventions as above, now crosses the X axis.

is obtained. This is the distance through the "dry" part of the specimen, the part not permeated by the mounting medium.

The basic idea of this investigation is given in Text-fig. 2. It shows how the combined measurement of action on polarized light (Γ_{pol}) and mass/area (Γ_{int}) at various levels inside cell walls can quantitatively describe the internal structure. The method worked with cell walls less than one micron in optical thickness which were torn so as to present tapering edges giving areas at various depths in the wall. Assume the edge to be an ideal staircase as in Text-fig. 2 *a*. Here the wall is imagined to have a uniform transverse texture. Note that plotting one variable against the other for the various steps yields a straight line. This is because the removal of a given mass of wall reduces the action on polarized light in exact proportion. This is not the case when the model in Text-fig. 2 *b* is examined along with the corresponding graph. Here there is a gradient of structure from transverse on the inner surface to random on the outer surface. The graph shows a sag toward the X axis (the outer random "step" shows no Γ_{pol}). Finally, Text-fig. 2 *c* shows the typical multinet structure with the gradient of structure proceeding from transverse, to random, to axial as passage is made from the inside to the outside of the wall. That part of the torn edge with an axial arrangement will show positive birefringence and hence the graph for this model sags further and crosses the X axis. The values for each step give the sum of the optical properties for the material under each step. It is more interesting to know the orientation at any given step (or depth) within the wall. This can be deduced from the *slope* of the curve. If the slope is positive, the orientation is transverse; if zero, the orientation is isotropic; if negative, the orientation is axial. With the assumption of multinet growth and a passive reorientation of structure, the *slope should continually increase* with departure from the origin (provided the inner surface of the wall is up). This crucial expectation is met in the results (Text-fig. 6).

The practical details of obtaining the necessary measurements follow. An internodal cell wall (Fig. 1) was cut and opened as shown in Text-fig. 3 *a* to *c*. The edge of the dry wall was roughened with a needle and then the tip of a small triangle of Scotch tape was adhered to the edge. The tape was pulled with a fine forceps as shown in Text-fig. 3 *d*. The torn areas were never so smooth as drawn and varied in orientation from the two extremes shown in Text-fig. 3 *c*. Because of the irregularity, measurements of the two types had to be made along the same line down an individual tear using densitometric methods.

Polarized Light Microscopy.—A piece of wall with torn wedges in it was placed at the 45° position in a Zeiss research polarized light microscope and then a 1/30 wave length compensator was rotated to give about 90 Å retardation, darkening much of the wall and brightening the field. Under these conditions there was a gradient of intensity from the whole wall (dark) to the thinnest edge (light). Between the two marks in Fig. 3 four distinct intensities can be seen. The meaning of these intensities in terms of retardation was deduced from a known model preparation shown in Fig 2. The model is a folded staircase of *Nitella* leaf internode cell wall. In the area with four steps, all darker than the field, the high index of refraction (microfibrillar direction) is perpendicular to the long edge of the page; in the two steps lighter than the field the orientation is parallel to the long edge. The same model is diagrammed in Text-fig. 4 *a* where the fibrillar orientation is drawn in. The sign and magnitude of the retardation of the individual levels in the staircase



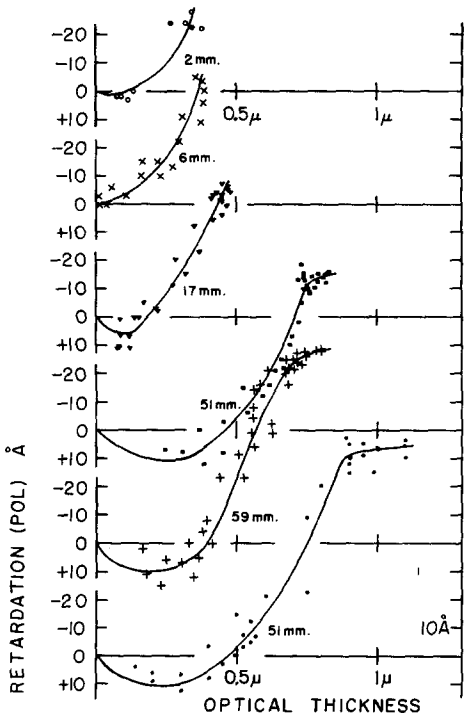
TEXT-FIGS. 3 to 5. Method. Text-fig. 3. A sketch of the method by which *Nitella* internodes were cut (*a* and *b*), opened (*c*), and finally torn (*d*). The edge of a single thickness of wall was gripped by a small triangle of Scotch tape (dotted) which was in turn pulled by a fine forceps (black). The angle of the tear was irregular, tapering either axially or transversely as drawn. Text-fig. 4. Calibration of optical density to retardation of polarized light. The model system at *a* was made by folding thin walls of leaf internodes, with mean microfibrillar orientations as shown. At the 45° position, with the compensator rotated, a range of intensities from lighter to darker than the background resulted (Fig. 2). A typical trace across the model is shown in *b*, the calibration curve in *c*. Text-fig. 5. Calibration of optical density to optical thickness in negatives taken in the interference microscope. An idealized torn edge of wall is at *a*, the displacement of field fringes for this object, vertical view, is shown at *b*. A densitometric trace of the negative from *A* to *A'* would be as in *c*. The calibration trace, *d*, is part of a trace *B* to *B'* across the field fringes. Γ_{int} stands for retardation in the interference microscope and is a measure of mass/area.

were measured directly with the compensator; a calibration curve relating the optical densities along the trace *A* to *A'* to the actual sign and magnitude of retardation of the steps was made as in Text-fig. 4 *b*, *c*. In this study negatives of Kodak panatomic X film were analyzed with a Walker-type automatic recording microdensitometer (Joyce, Loebl, and Co., Ltd., Newcastle-on-Tyne). The calibration curve was used to measure retardation at close intervals along a trace such as that between the two marks in Fig. 3.

Interference Microscopy.—A Dyson-type interference microscope was used in this study. In this instrument the beam is split by half-silvered mirrors and the birefringence of the specimen does not affect the measurement of optical thickness. The microscope was adjusted so that a series of parallel dark and light fringes crossed the field. The fringes result from a uniform gradient of optical path across the field. The specimen displaces the fringes in direct relation to the optical path difference between areas of the specimen and the surroundings (11). In Fig. 4 a typical torn edge is shown, with the thin edge toward the top of the page; fringes are displaced to the left. The densitometric method for measurements used is diagrammed in Text-fig. 5. Given a model object as in Text-fig. 5 *a*, the fringe displacements are as in 5 *b*. The fringes are actually high and low points along sine curves running at right angles to the fringes. Thus a trace from *A* to *A'* in Text-fig. 5 *b* yields intensity changes as drawn in Text-fig. 5 *c*. While lateral displacement is a direct function of the mass/area of the specimen, the intensity changes are a function of the sine curve and the exposure conditions for the negative. The calibration of optical density on the negative to retardation (optical path difference) of the specimen was made by recording a trace at right angles to the first, as *B* to *B'*. Provided the retardation did not exceed 1/2 wave length, this second trace could be used directly to calibrate the first as in Text-fig. 5 *b*. Note that sensitivity is related to the steepness of the slope. The trace *A* to *A'* was usually made in the center of a dark fringe (down the same line as the previous study in polarized light) but extra sensitivity was occasionally gained by using the midpoint between light and dark fringes. In the above manner, data for both retardation in polarized light (Γ_{pol}) and in the interference microscope (Γ_{int}) were obtained for individual points along a line running from areas of total cell wall thickness down to vanishingly thin torn edges. No data were taken from the very margins of the preparation, where the Becke line diffraction effect was noticeable. A developmental series of cells was studied, using successive internodes from a single shoot. Usually two or three of the best tears from each cell were analyzed and the data combined. In the case of cells 2 and 6 mm. long, the original growing shoot supplied insufficient material and cells of similar length from other shoots were also used.

RESULTS

The wall structure produced by multinet growth should show a gradient of microfibrillar arrangement from axial to random to transverse, the



TEXT-FIG. 6. Six curves of retardation in polarized light (ordinate) plotted against optical thickness (abscissa). The ordinate is a succession of regularly spaced origins and the ordinate is linear for all curves. The curves are a developmental series and the length of the cell from which the curve was derived is given beside the curve. The slopes of the curves reveal ever increasing transverse orientation from the outside of the wall (zero optical thickness) into the interior. The flat ends of the lower three curves represent an isotropic secondary cell wall deposited after elongation has ceased.

gradient going from the outside to the inside of the wall. The results, showing this, are given in Text-fig. 6. Γ_{int} has been converted to optical thickness.

If the left hand part of the four lower curves in Text-fig. 6 be examined, it is seen that the slope, from the origin outwards, is negative, zero, then positive. The origin represents the outside of the wall and as one proceeds from left to right along the X axis one approaches the inner surface. A negative slope indicates an axial arrangement, a slope of zero indicates random arrangement of microfibrils, a positive slope, a transverse arrangement. These changes in sign of the slope show that the gradient of orientation postulated by multinet growth exists in these cells which were 17, 51, 59, and 51 mm. in length. This figure is an assemblage of curves representing, from top to bottom, a developmental series from a single shoot. Cell length is given beside each curve. The ordinate is a succession of equally spaced origins and the scale is linear for all curves. If it be assumed that the percentage crystalline material is constant throughout the wall (and thus throughout cell elongation) then it is possible to interpret the *value*, as well as the *sign*, of the slopes in all six figures. In brief, an increase in slope from the origin out along the X axis always shows an increase in transverse orientation. If the flat right ends of the bottom three curves be ignored for the moment it is seen that in all six curves there is a continual increase in slope from the origin outwards, meaning a continual increase in transverse orientation from the outside of the wall inwards. This is exactly the expectation for the passive reorientation of microfibrils in multinet growth. In the cells 2 and 6 mm. long no axially oriented outer zone could be demonstrated beyond the limits of experimental error and the gradient of structure inward proceeds only from random to transverse. The cell 17 mm. long is in the midst of its elongation and shows the multinet structure clearly. The bottom three curves are of cells which have ceased elongating. Deposition of wall material continues after elongation as an isotropic layer of mutually compensating fields of microfibrils is laid down (11). This explains the horizontal right ends of these curves. It is noteworthy that these very long cells have longer portions of the curve with a negative slope, indicating the accumulation of axial microfibrils at the outside of the wall. These can be seen in Fig. 5 where the inner edges of the tears are brighter than the field and hence of axial orientation.

The above test of multinet growth based on an ever increasing slope of the experimental curve is actually only a test of the passive reorientation of microfibrils (poorer and poorer transverse orientation from the inside outward). The same curve could result if hypothetical lamellae of microfibrils simply rotated in the plane of the wall from transverse to axial orientation during elongation with no change in dispersion within the lamellae. In pictures with walls at the 45° position there is no way of distinguishing increased dispersion from a rotation, on the part of certain wall lamellae, to an oblique direction with reference to the cell axis. This possibility of the rotation of lamellae explaining the curves was removed by examining torn edges with the cell axis at the 0° position

where obliquely oriented lamellae would appear bright. No bright regions in the torn edges could be detected and the view of passive reorientation by the multinet method was sustained.

The similarity in slopes of the inner wall surface part of the curves (at right) indicates that the degree of transverse orientation of newly deposited material is roughly constant during elongation and changes rather abruptly for isotropic secondary wall formation. This latter change is seen at right in the three bottom curves. Further interpretation of the slopes is equivocal because scatter was considerable and the curves were fitted by eye. Tears from different parts of the same wall showed similar but quantitatively different structure. The walls did not tear well when the outer wall surface was up. A few such tears were analyzed, however, and gave points which, after calculation, yielded data for Text-fig. 6.

The texture of the wall is seen in Fig. 5 and this became the limiting factor in the technique. Averaging out the intensity to get smooth traces involved increasing the diameter of the densitometer probe, thus lowering resolution. The texture is most striking in the region of the two linear "striations" (8, 9). These areas are linear "stillwaters" between ascending and descending protoplasmic streams. These striations are seen in Figs. 6 and 7. They persist even in the outermost region of the wall seen above in both figures. The light lines must be near-parallel microfibrils running in direction of the striation (just a few degrees off the axial) and the adjacent dark lines must be transversely oriented fibrils, a remarkable structure to survive multinet growth.

DISCUSSION

The present report demonstrates the passive reorientation of birefringent microfibrils inside the elongating wall of *Nitella*. It thus provides evidence for multinet growth by a quantitative method in a new cell type.

The multinet hypothesis was proposed by Roelofsen and Houwink (3, 4) for the growing hairs of *Gossypium* (cotton), *Asclepias* (milkweed), *Ceiba*, *Tradescantia* stamens, *Zea* roots, the sporangiophores of *Phycomyces*, and the stellate pith cells of *Juncus*. They postulated the addition of new microfibrils by apposition and the passive reorientation of microfibrils during growth. They had observed that the above cell types generally showed a transverse orientation at the inner wall surface and either an isotropic or axial arrangement on the outside. (In the case of the root hair only the hemispherical tip was involved in growth and the originally randomly deposited fibrils retained a random arrangement.) Support, also based on the comparison of the orientation at the inner and outer surfaces of walls, has come from a variety of laboratories. Multinet growth images were found characteristic of *Avena* coleoptile parenchyma by Wardrop (6) and by Setterfield and Bayley (5).

Various authors have reported structures at variance with the multinet growth theory. The elongating *Avena* parenchyma cell contains longitudinal ribs of microfibrils both outside and within the typical transverse structure (5). Collenchyma cells can exhibit predominant longitudinal orientation in all stages of growth (12, 13). The outer epidermal walls of *Avena* coleoptiles also have a predominant longitudinal arrangement and, further, evidence has been presented to indicate that new layers originate inside the wall proper (14) although no extension of the protoplast into the wall was noted. Roelofsen (15) deals with these exceptional cases, presenting the view that tissue tensions may well create a predominating axial tension in certain tissues, which might explain the presence of excessive longitudinal orientation. In the case of *Nitella* the question of tissue tensions does not arise and it is noteworthy that the "classical" multinet structure is present. There is some spiral growth in *Nitella* (8) and the multinet effect would be expected to draw out microfibrils in the slightly off-axis vector of elongation rather than strictly axially.

Electron micrographs of ultrathin sections of growing plant tissue cells (16) generally show the wall as a simple structure, perforated by plasmodesmata, lacking the dense granules and membranous structures characteristic of the cytoplasm. Thus, from several lines of evidence, the structure of many cell walls may be viewed as an accumulation of microfibrillar secretions of original transverse orientation whose joint physical properties undoubtedly influence the direction and rate of cell extension.

BIBLIOGRAPHY

1. Hackett, D. P., *Plant Physiol.*, 1952, **27**, 279.
2. Küster, E., Die Pflanzenzelle, Jena, Gustav Fisher Verlag, 1956.

3. Houwink, A. L., and Roelofsen, P. A., *Acta bot. néerl.*, 1954, **3,** 385.
4. Roelofsen, P. A., and Houwink, A. L., *Acta bot. néerl.*, 1953, **2,** 218.
5. Setterfield, G., and Bayley, S. T., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 377.
6. Wardrop, A. B., *Australian J. Bot.*, 1956, **4,** 193.
7. Green, P. B., *Am. J. Bot.*, 1958, **45,** 111.
8 Green, P. B., *Am. J. Bot.*, 1954, **41,** 403.
9. Green, P. B., and Chapman, G. B., *Am. J. Bot.*, 1955, **42,** 685.
10. Steward, F. C., and Mühlethaler, K., *Ann. Bot.*, 1953, N. S. **17,** 295.
11. Green, P. B., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 505.
12. Majumdar, G. P., and Preston, R. D., *Proc. Roy. Soc. London, Series B*, 1941, **130,** 201.
13. Beer, M., and Setterfield, G., *Am. J. Bot.*, 1958, **45,** 571.
14. Bayley, S. T., Colvin, J. R., Cooper, F. P., and Martin-Smith, C. A., *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 171.
15. Roelofsen, P. A., *Acta bot. néerl.*, 1958, **7,** 77.
16. Whaley, W. G., Mollenhauer, H., and Kephart, J., *J. Biophysic. and Biochem. Cytol.*, 1959, **5,** 501.

EXPLANATION OF PLATE 138

FIG. 1. A living *Nitella* shoot with internodes about 2 and 12 mm. long. The faint *X's* seen in the longer internode are gaps in the chloroplast layer and correspond to lines separating ascending and descending streams of protoplasm. Wall structure along these lines is shown in Figs. 6 and 7. Five or six "leaves" come from each node. × 6.

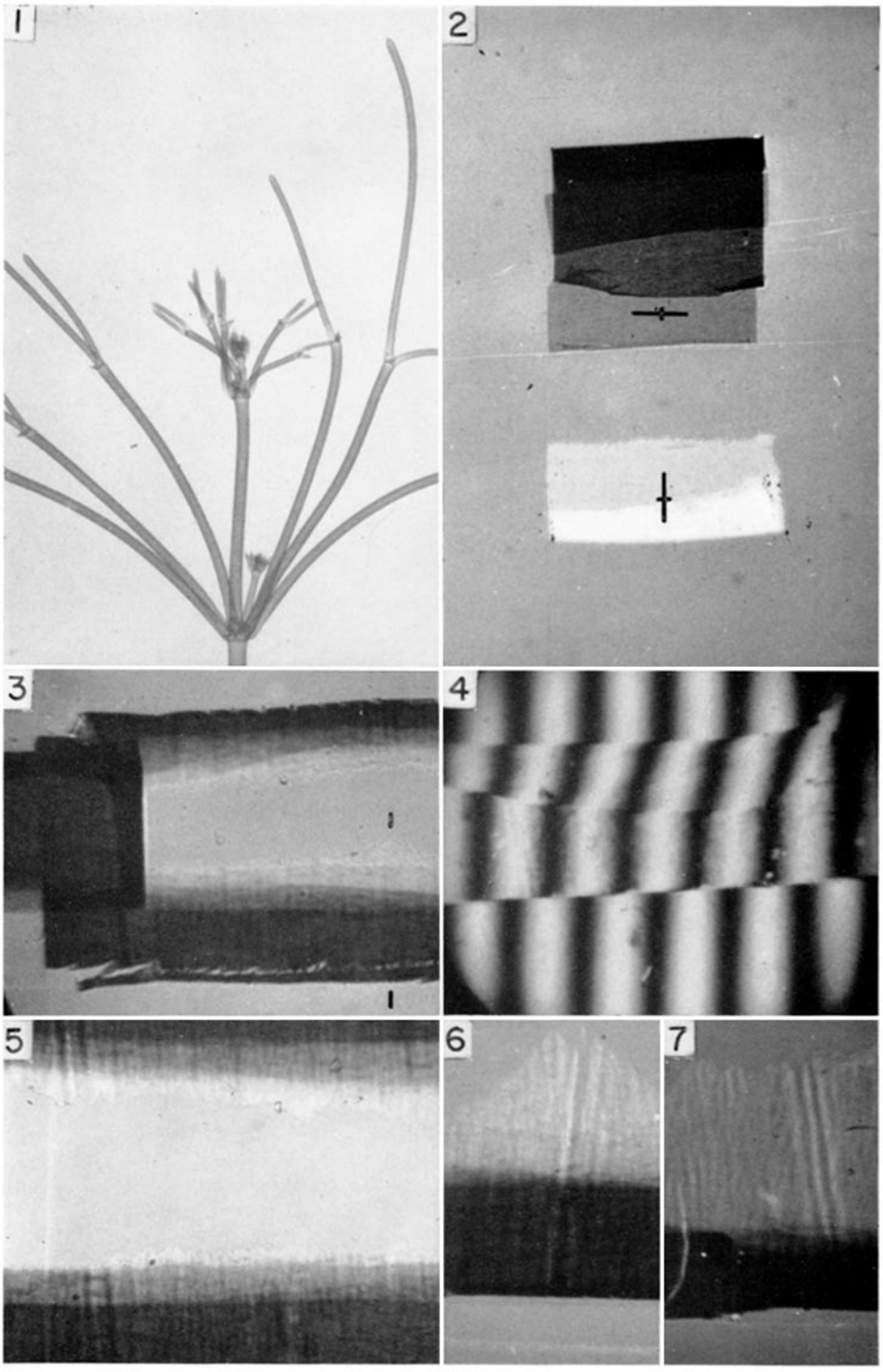
FIG. 2. A model made of folded leaf internode cell walls. The high index of refraction in the two parts of the model are at right angles as shown. The upper part has 4, 3, 2, and 1 layers of wall, from the top of the print downwards. The lower part has 1 and 2 layers proceeding from the center of the print downwards. The model is photographed at the 45° position in polarized light with the uppermost part (4 thicknesses) compensated (80 Å retardation). The range in intensity from brightest to darkest is from + 40 to − 80 Å. Negatives of such images were used for calibration of densitometric traces of torn wall edges. (See Text-fig. 4). × 100.

FIG. 3. A tear which has removed the center part of a small piece of wall; wedge-like tapers are at each side of the tear. The upper and lower extremities were trimmed with a razor blade. The two marks indicate the ends of a typical densitometric trace showing four different levels of brightness. Intensities are comparable with those in Fig. 2 as the orientation is similar. × 150.

FIG. 4. A torn edge of wall, as in the lower part of Fig. 3, photographed in the interference microscope. Fringes are displaced to the left as the specimen increases in optical thickness. The wedge-like character of the tear is clear. Densitometry was done as in Text-fig. 5. × 200.

FIG. 5. Two torn edges of wall showing, toward the center, areas lighter than the background and thus with axial orientation. Texture of the wall is evident. × 250.

FIGS. 6 and 7. Torn edges of wall showing the structure of the "striation," a linear area of wall separating ascending and descending streams of protoplasm. The striations consist of alternate fine linear areas of near-axial (bright) and near-transverse (dark) microfibrils. Persistence of the striation in the outer regions of the wall (particularly the near-transverse parts) in a wall showing multinet growth is remarkable. × 250.



(Green: Multinet growth in cell wall)