Plant Physiol. (1977) 60, 247-254

Cell Growth Pattern and Wall Microfibrillar Arrangement

EXPERIMENTS WITH *NITELLA*

Received for publication December 15, 1975 and in revised form June 14, 1976

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ABSTRACT

In cylindrical cells growing throughout their length, over-all transverse reinforcement of the wall by microfibrils is believed to be required for cell elongation. The multinet theory states that in such cells microfibrils are deposited at the inner surface of the wall with transverse orientation and are then passively reoriented toward the longitudinal direction by the predominant longitudinal strain (surface expension). In the present study young *Nitella* cells were physically forced to grow in highly abnormal patterns: in length only, in girth only, or with localized suppression of growth. Subsequent gradients of microfibrillar arrangement within the wall cross-section were measured with polarized light and interference microscopes. The novel wall structures produced were in all cases explainable by passive reorientation, *i.e.* by the multinet theory. The study also showed that orientation of synthesis remains insensitive to several of the physical manipulations that strongly influence the passive behavior of wall microfibrils. Only the localized complete suppression of surface growth led to the deposition of nontransverse cellulose. These results suggest that the presence of strain is needed for continued oriented synthesis, but that the directional aspect of strain is not an "instructional" agent continuously guiding the orientation of synthesis, once this orientation has been established.

Plant cell morphogenesis is governed to a large extent by the yielding properties of the cell wall. The driving force for cell enlargement is turgor pressure (25). This is nondirectional and would be expected to yield cells of spherical form. The most common departure from spherical form, cylindrical shape, can be accounted for by two alternate mechanisms: (*a*) nondirectional localized expansion as in the tip growth of root hairs and hyphae, (*b*) highly directional expansion of the whole side wall as in tissue cells and internodes of *Nitella* (37). In such cylindrical cells growing throughout their length one expects reinforcement in the transverse direction to prevent increase in girth. Appropriately aligned microfibrils have been generally found with certain outer epidermal walls being exceptional (7, 23, 28, 29).

Two modes of attainment of this over-all transverse arrangement have been proposed: "intussusception" wherein transverse microfibrils are added throughout the wall thickness, and "apposition" wherein cellulose synthesis is restricted to the wall inner surface. In the latter case one would expect a deterioration of alignment of any transversely arranged microfibrils because they are subject to longitudinal realignment by the growth of the wall. Surface structures corresponding to this passive reorganization of the primary wall were described by Roelofsen and Houwink (30); they termed the reorganization "multinet growth." Appropriate intermediate structures inside the *Nitella* wall were described by Green (11); cellulose synthesis localized to the wall inner surface was found by Green (9) and Ray (24). Recently, however, structures considered incompatible with multinet growth have been described (32).

Because one can evaluate passive microfibrillar gradients inside the *Nitella* wall we decided to physically impose unusual growth patterns on the *Nitella* cell and see if the corresponding unusual passive microfibrillar reorganization took place. This first aspect of the work is an unusually stringent test of multinet growth. In addition, these experiments can reveal the effects, if any, of unusual cell shapes and growth patterns upon the orientation of cellulose synthesis. Changes in this orientation would be apparent, assuming apposition, in the wall structure near the wall inner surface.

On this second question the experiments will test certain potential correlations between the orientation of recently synthesized wall and various other oriented features of the cell. An important intermediary role for microtubules on orientation control (16) is considered likely. If this is true, then any effective correlation will point to cell features that govern microtubule behavior.

A brief evaluation of potential influences on oriented wall synthesis follows.

a. Direction of Previously Deposited Cellulose. The possibility of direct coupling with previous synthesis has been excluded by experiments in *Nitella*. Transverse microfibril synthesis resumed after a colchicine treatment had disorganized wall synthesis (12).

b. Cell Shape. Synthesis could be oriented according to the direction of maximum curvature or possibly relative cell dimensions. *Nitella* cells that have acquired a round shape by a colchicine treatment resume transverse deposition when returned to a normal growth medium (P. A. Richmond, personal communication). Hence a role for curvature appears ruled out. Transverse wall structure in both young broad cells and elongate internodes of *Nitella* weighs against an overriding role for cell proportions (10).

c. Direction of Chloroplast Files and Striation Lines. Although there is no obvious causal mechanism for cellulose alignment by these structures, they are a possibility. They are oriented in the growth direction, roughly normal to the direction of wall synthesis.

d. Direction of Protoplasmic Streaming. This is also approximately axial. It seems to be an unlikely pertinent parameter because the streaming in *Nitella* is separated from the wall surface by a layer of stationary cytoplasm. In the round cells of "Cell Shape," streaming was highly abnormal yet typical wall synthesis resumed. Hence this possibility is counter-indicated.

e. Direction of Greatest Stress in Wall. In cylinders, the stress in the wall is unequally distributed, the transverse component being twice as great as the longitudinal one (3). Stress is force/

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area and is measured by the magnitude of the strain (deformation) it can cause in an isotropic body. In *Nitella*, the direction of maximum stress is transverse while that of maximum strain is axial. There is no way by which stress could be evaluated independently of strain; this seems to rule out the possibility of direct stress orientation of cellulose on *a priori* grounds.

f. Direction of Strain. Mechanical induction of new growth axes in *Nitella* (13) suggested a role for strain alignment but the strain pattern was not characterized. In *Nitella* lateral (leaf) development, the first appearance of transverse wall texture in the apical cell approximately coincides with a temporary transverse stretching of the cell surface (14). These results also suggest a role for strain at least in the initial alignment of cellulose synthesis.

Because strain appeared to be a potentially significant feature, special attention was paid to comparing a given strain pattern with the concurrent orientation of synthesis. Thus the effects of imposed novel strain patterns upon both the passive reorganization of outer parts of the wall and the orientation of active synthesis are to be considered. When the extension rate in each direction is expressed as a compound interest rate, the normal strain pattern has a 4.5 to 1 bias favoring elongation over increase in girth (*i.e.* the surface mainly "elongates"). The cells were subjected to four kinds of treatments: (*a*) suppression of all strain in the central portion of the cell, (*b*) exaggeration of transverse strain while suppressing longitudinal strain in a whole cell, (*c*) exaggeration of longitudinal strain while converting transverse strain to a contraction for a whole cell, and (*d*) as in *c* but with colchicine present to temporarily remove any stability associated with microtubules. In all cases turgor, and hence cytoplasm/wall contact, was normal or nearly so.

MATERIALS AND METHODS

The present study was carried out on *Nitella axillaris* Braun (also *N. flexilis* var. axillaris). Plants were grown in an autoclaved mixture containing 70 cc of garden soil plus 15 cc of peat moss in 6 liters of distilled H_2O. All the experiments were carried out on actively growing cells that measured between 2 and 4 mm long and were part of a plant that had at least three internodes below the cell in question.

The cells were subjected to four kinds of treatments, as mentioned in the introduction. For analysis, cells were placed in three categories: 1: control cells killed at the start of the experiment (initial controls); 2: equivalent cells which received the treatment (experimental cells); and 3: cells grown in parallel with the experimentals, but not receiving the physical treatment (grown controls).

TREATMENTS

a. Suppression of All Strains in Segment of Cell. This was achieved by enclosing approximately the central third of a young cell in a glass "box" made of coverslips, as seen in Figure 1, a and b. Cells were inserted in the gap between the coverslips serving as sides of the box, and the lid forced down by sliding the glass rod placed between the lid and the descending "fixed beam." This procedure securely enclosed part of the cell within the glass box. The boxes were suspended vertically in glass cylinders where the plants had been growing. The cells were allowed to grow for 4 or 5 days. After this period the cells were released from the boxes and the microfibrillar arrangement in the wall analyzed as described below.

It was essential to monitor any movement of the cell within the box. In this alga, internode cells longer than 1.5 mm have two helical striations which are visible on the otherwise green cell (8). In older cells these striations spiral sufficiently so that the two helices on the wall surface (front and back as seen from above) appear to cross, forming apparent X's. The pitch of these

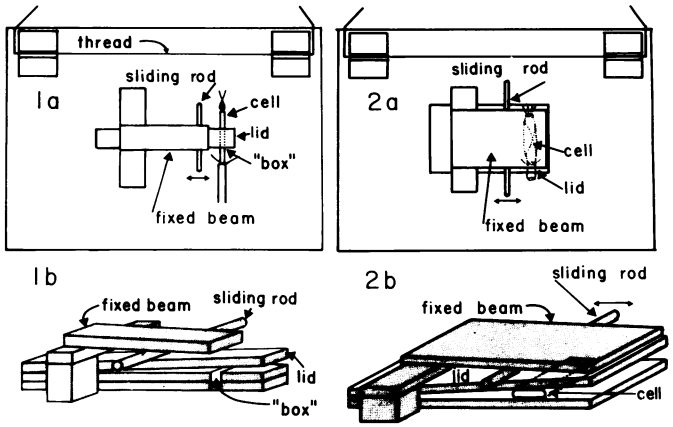

FIG. 1. Device for localized suppression of surface growth built on a microscope slide (50 × 75 mm). All parts are of glass, and all junctions were made with epoxy cement. a: the middle third of the experimental cell is gripped in a "box"; the whole assembly is then suspended vertically in a glass cylinder for subsequent cell and wall growth. b: detail. The young cell is inserted in open "box" as shown. The midregion of the cell is compressed (to fill the box region) when the lid is lowered by sliding the rod to the right.

FIG. 2. Device for compressing a young cell (including the nodes above and below) to allow growth in girth but not in length. a: general scheme with a cell in position. Note helical striation lines on the cell. b: detail. As in Fig. 1, the compression is increased as the rod is moved to the right. A highly compressed cell is shown.

lines changes as the cell grows. Both the pitch of the striation lines, and the distance between the enclosed X and the ends of the box were measured at the beginning and end of the experiment so that distinct clamping of the surface could be affirmed.

b. Promotion of Transverse Strain while Longitudinal Strain Was Suppressed. Here the entire cell was pressed between two coverslips allowing only sidewise expansion. The arrangement was somewhat similar to the box, above, but there were no sides to the box (see Fig. 2, a and b). When the rod was moved to the right, the lid compressed the cells between the coverslips. The experimental cell was flattened until the apparent diameter seen from above exceeded the original by 10%. It was empirically determined that this amount of compression allowed for lateral expansion without longitudinal growth (internode length remaining constant). Cells were grown as above.

c. Promotion of Longitudinal Strain while Converting Transverse Strain to Contraction. This was achieved by stretching the cell by means of the set up shown in Figure 3. A thread was tied to a lower node of the plant. A clamp (Fig. 3, b and c) attached to two buoyant corks gripped the upper portion of the experimental cell. The plant was first put into the cylinder as shown in Figure 3a. When the thread was pulled from the outside, the cork, which had been previously floating on the surface, was submerged and its buoyancy exerted tension between the points of attachment, including the experimental cell. The amount of tension could be controlled by varying the size and number of the submerged corks.

To exaggerate the relative effect of the tension, 4 bar (30 g/l) of mannitol were added to the medium. This reduced wall stresses generated within the cell, making the effect of the applied stress relatively greater.

Finally, a test was designed to see if wall synthesis pattern would be more effectively altered when the imposed strain pattern was combined with chemical treatment directed against microtubules. Clamped cells such as described under "c" were allowed to grow for 3 days in a medium containing 0.35% colchicine. During this period, unclamped growth controls acquired a swollen appearance in response to the treatment. At the end of the 3 days the cells were transferred to a normal growth

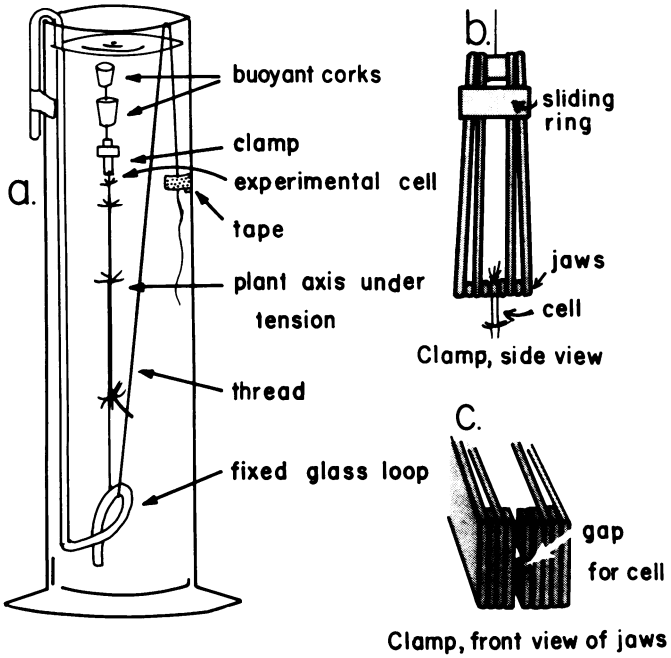

FIG. 3. Scheme for continuous stretching of growing cells. a: the thread tied to the base of the plant is pulled to submerge the corks which then put tension on the internodes below the clamp. Increase in cell length is often accompanied by decrease in cell diameter. b: detail of clamp which grips the upper part of the internode under study. Jaws are closed by lowering the sliding ring. All parts are made of glass coverslips. c: detail showing gap for the cell. It is smaller than the node above.

medium and allowed to grow for 3 more days, the experimental cells remaining under tension throughout. The rationale for this experiment was that perhaps some cell structure would respond to strain only in the absence of microtubules. Once reoriented it could serve as a guide for microtubule reassembly. Upon removal of the colchicine, resumed synthesis might take on a new configuration reflecting the imposed strain pattern.

OPTICAL ANALYSIS

The purpose of the analysis was to determine the orientation of cellulose microfibrils within cell walls grown as described. It is possible to deduce the orientation of microfibrils at different depths within the wall with the aid of two optical parameters, one measured in the polarized light microscope, the other in the interference microscope. For the mode of action of these microscopes see references 10 and 22.

The action of a piece of wall upon a beam of polarized light, measured as retardation, is a function of both the number and orientation of the birefringent microfibrils. When the retardation of a piece of wall is divided by its thickness, the resulting quotient gives a measure of the degree of orientation of the constitutent microfibrils, provided the percentage of crystalline material is the same in all samples. The higher the absolute value of this quotient, the better the alignment of the microfibrils.

When microfibrils are superimposed, parallel to each other and normal to the beam, their action on the beam is additive. When the crystalline microfibrils are longitudinal, the retardation and birefringence are positive by convention; if transverse, both are negative. When microfibrils are superimposed, but scattered at random, the effects on the beam cancel out and there is no net effect (zero retardation).

In order to study the orientation of microfibrils at different depths within the wall, a staircase was made from a single thickness of wall. To form the steps, the cells were first cut, cleaned, and opened, the inner surface against the slide (Fig. 4). Small cuts were made along one edge of the wall and alternate flaps of wall folded up. These flaps were caught with a very fine glass pin and pulled toward the opposite edge of the wall. This tearing operation produced ledges of varying thickness on each of the adjacent pieces of wall.

If we plot retardation *versus* thickness of successive steps, the slope of the resulting curve at any point will reflect the mean orientation of the microfibrils within that step. Because the staircase was constructed with the original wall inner surface adhering to the slide, the initial slope of the curve (lowest step) will reflect the arrangement of microfibrils at the innermost region of the wall (most recent synthesis). We plot negative retardation *versus* thickness, so when the initial slope is positive, the microfibrils are transverse. Continuous passive reorientation toward the longitudinal (multinet growth) would be reflected in a continuous fall in slope (see Fig. 7a, bottom). In a heterogeneous wall the slope for a given step will approximate the mean orientation within that step because the slope is a function only of the increments for retardation and thickness for that step. Continuous passive reorientation toward the longitudinal direction (multinet growth) would be reflected in a continuous fall in slope.

The staircase was constructed with the original wall inner surface adhering to the slide. Thus the initial slope of the curve will reflect the arrangement of microfibrils at the innermost region of the wall. If the initial slope is positive, the orientation is transverse.

It is difficult to determine the retardation of very thin ledges and a method was devised to reduce subjectivity and improve accuracy. The retardation of a ledge was approximately determined by rotating the 1/30 λ compensator. Then a series of photographs of the wall was taken at close consecutive settings of the compensator, bracketing the anticipated value. The negatives were projected on a flat surface. The light intensity of each ledge and that of the background were measured. These light intensity measurements were plotted, as the ordinate, against setting of the compensator (abscissa). The resulting curve pre-

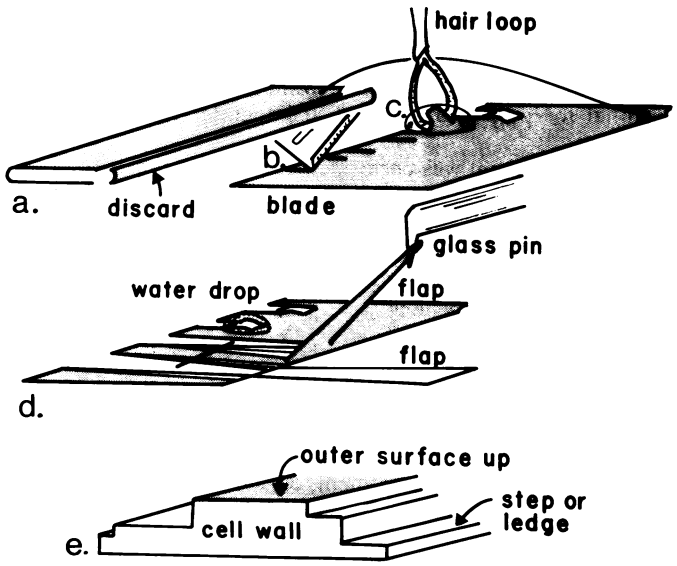

FIG. 4. Preparation of "ledges" or steps within the primary wall. Shading is on wall outer surface only. a: cleaned flattened cell is cut along one margin. Cell wall is dry. b: opened wall (outer surface up) is cut locally with a razor blade. c: resulting alternate flaps are raised, in a drop of water, with a hair loop, and folded over. d: moistened folded flaps are then caught by a glass pin (made on a microforge) and pulled across the wall. e: highly enlarged view of the resulting ledges. The lowest step contained only the innermost part of the wall. Section is along the solid line in d.

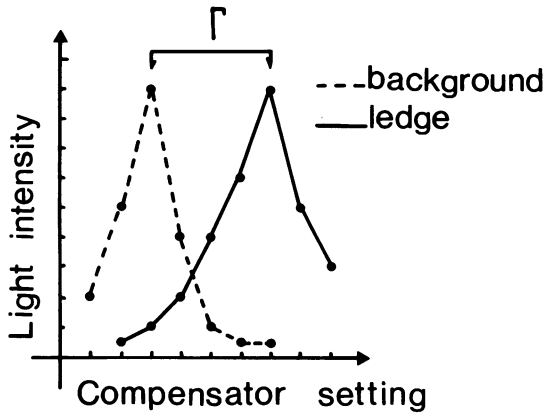

FIG. 5. Scheme for accuracy in measuring small retardations in polarized light. Rotating the compensator successively brings the background and the ledge to a minimum intensity. The position of the minimum can be judged from a series of negatives taken at successive compensator settings. As shown, the maximal brightness on the negative, as measured with a photometer, gives the value for the original minimum. Retardation of a ledge or wall is the difference, on the abscissa, between the peak for the background and that for the specimen.

sented a peak at the point of maximum compensation. The value of retardation for each wall locus was determined by the difference between its peak and the peak of the background curve (Fig. 5). For visualization of the ledges see Figure 6.

The measurement of thickness was carried out in an interference microscope whose field was set for parallel fringes. Optical thickness is proportional to the displacement of the fringes in the microscope. This displacement was measured on enlargements of the microscopic image (Fig. 6).

This analysis was preferred to the electron microscope analysis of tangential sections or replicas. The polarizing microscope automatically yields a mean statistical measure of microfibril orientation within the thickness of wall analyzed. The electron microscope gives an idea of what the orientation is like only at the surface of the restricted surface area being observed. Further, surface antifacts of tearing and preparation might dominate the electron micrograph image, while they would be inconsequential for polarized light which integrates through the wall fragment.

Each experiment was performed at least five times, on five or six cells each time. From each batch, the cells that showed the greatest departure from the normal pattern were analyzed as described above. Experience showed that the cells showing intermediate responses had walls of intermediate character, so analysis was directed mainly to cells showing the greatest response.

RESULTS

The results from the imposed strain pattern experiments can be analyzed in terms of (*a*) the passive properties of the wall and (*b*) the effect of the treatment on the active deposition of microfibrils. The results are given in Figure 7.

The growth pattern for each cell is given by the cross accompanying each graph. Each arm of the cross represents the growth rate in that direction. Rate = $(\ln X_2 - \ln X_1)/(\text{time}_2 - \text{time}_1)$, where X is the vertical or horizontal dimensions at two times. In normal growth, the vertical dominates by about a factor of 4.

Each curve starts at the origin; at zero thickness there is zero action on polarized light. Curves were fitted by eye because different treatments give qualitatively different curves, sufficient for interpretation.

a. Suppression of All Strain in Segment of Cell. In this case one would expect no change in the structure of the pre-existing wall because it was not deformed. Indeed, this is evident in the right-hand side of Figure 7c, dashed line, which resembles an initial control curve.

The curve is displaced from the origin by wall synthesis during the experiment. The initial portion of the curve is nearly flat and lies on the abscissa. This indicates there is very poor orientation of the microfibrils at the innermost portion of the wall. This suggests that in the absence of strain, something like a secondary wall was deposited. In *Nitella* the secondary wall, deposited after growth has ceased, has a random microfibril orientation (10). The unclamped part of the same wall grew normally, with typical transversely oriented synthesis (solid curves in Fig. 7c).

b. Promotion of Transverse Strain while Longitudinal Strain Was Suppressed. In these cells there was virtually no change in length while there was a 2- to 3-fold increase in girth. See crosses in Figure 7d.

These walls were extremely birefringent. Analysis of the wall showed good transverse alignment at the inner surface and further improvement of this alignment toward the outer part of the wall. This is seen in the continuous increase in slope of this plot in Figure 7d. The microfibrils are simply aligned in the direction of the predominant strain, as predicted by the multinet growth theory, even though this strain direction is transverse rather than longitudinal.

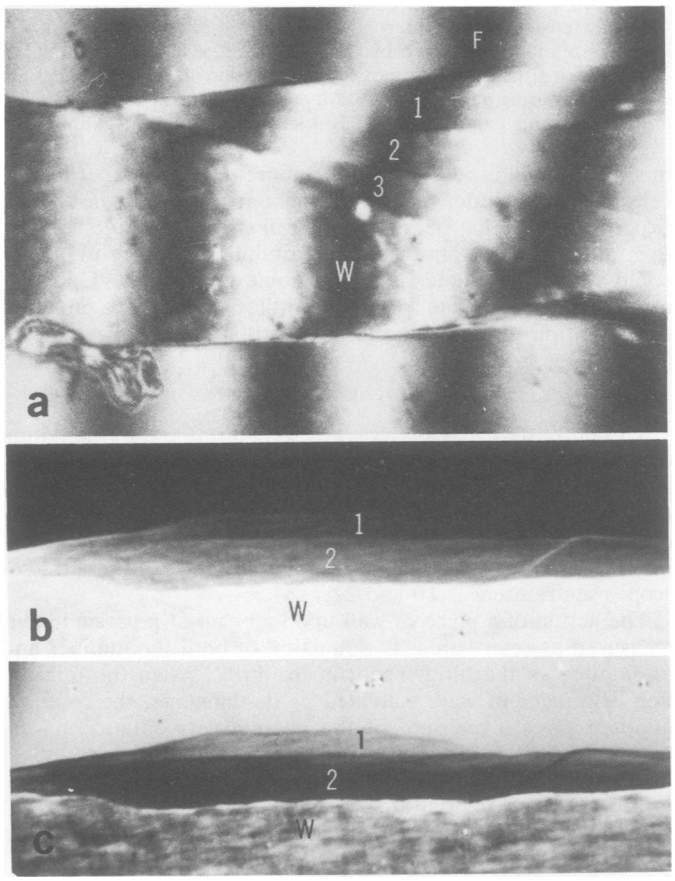

FIG. 6. a: interference microscope picture showing the progressive deflection, to the left, of background fringes (F) in three ledges and in the whole wall (W). The distance between two background fringes of the wall is one wavelength. Thickness of the wall is measured by fringe deflection and expressed as a fraction of a wavelength (optical path difference). b: a similar piece of wall as seen in polarized light. Increasing retardation of the two successive ledges and whole wall is seen as increased brightness. c: as in b but with the intermediate ledge compensated (made dark).

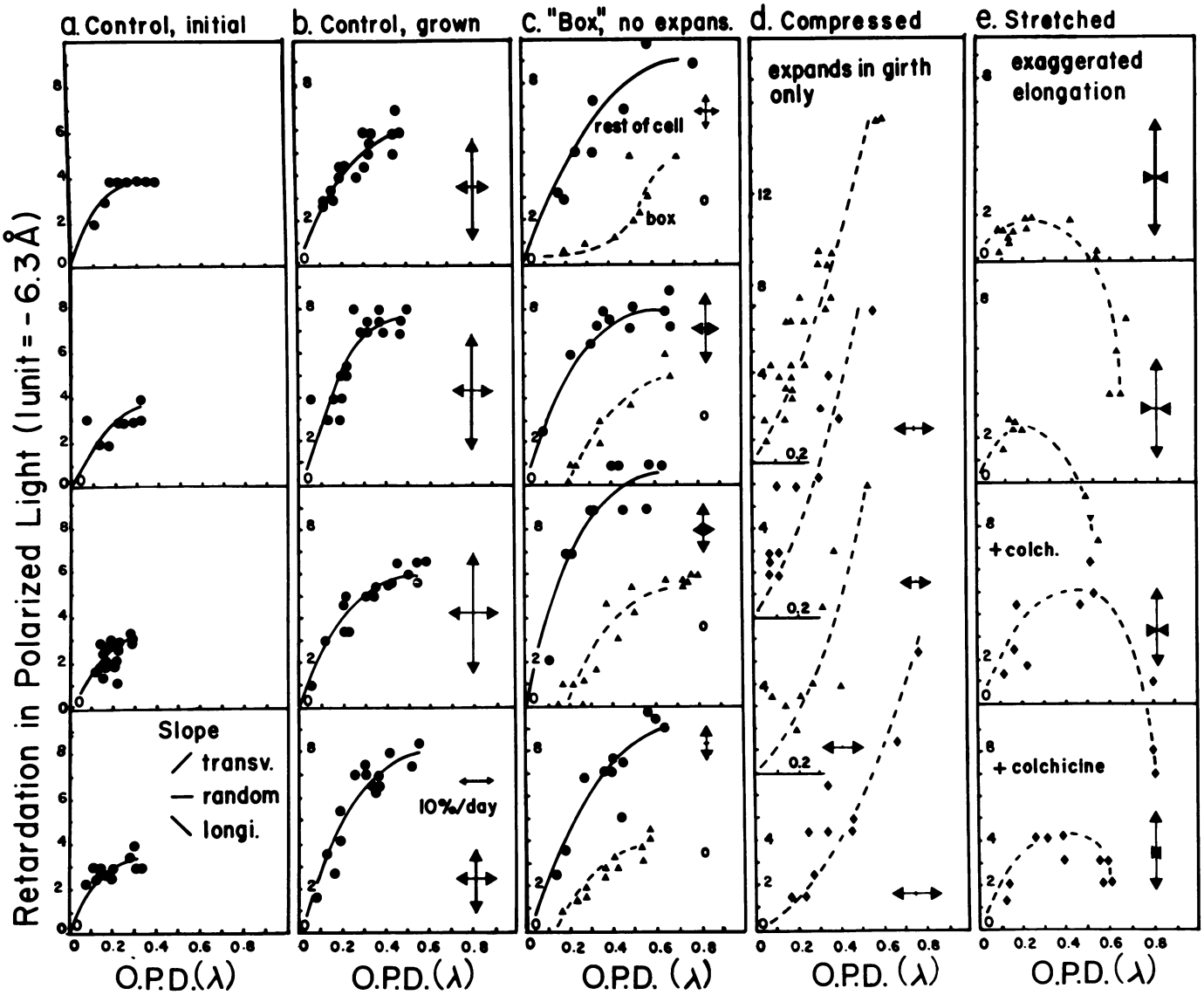

FIG. 7. Graphs portraying the internal arrangement of microfibrils in control and treated cell walls. In all cases distance from the wall inner surface is measured with the interference microscope in units of optical path difference (O.P.D.). The ordinate is action on polarized light for transversely oriented cellulose (negative birefringence). At any particular distance from the inner surface microfibrillar order is revealed by the slope of the curve. Positive slope is transverse order, horizontal (zero) slope is random, negative slope shows longitudinal order. See column a. Because qualitative features of the curves are diagnostic, curves were fitted by eye. (All curves must start at the origin.) Growth pattern of the particular cell (or cell portion) graphed is given by the crosses. The vertical arrow gives the longitudinal growth rate (over the past 4 or 5 days); the horizontal arrow is proportional to the rate of increase in girth. Calibration at the bottom of column b. Walls from untreated cells have solid curves. Walls from treated cells have broken curves. a: initial state of the cell walls. They are thin and have a positive slope near the origin (hence transverse microfibrils at the wall inner surface). b: untreated walls after 5 days. They have elongated, are thicker, and still have good transverse order at the inner surface. The curves tend to flatten at the outer surface. This shows near random microfibrils there. The continuous fall in slope indicates continuous passive realignment. c: the triangles (dashed curve) characterize the part of the wall held in a "box" to suppress any expansion. Note that the right portion of these graphs resembles the initial controls. The initial slope is low or zero indicating a near random arrangement for wall synthesized during the experiment. The free portions of the same cell (solid curve) show behavior similar to controls (b). This curve shows a localized physical influence on cell wall synthesis orientation. d: cells compressed so as to grow only in girth have good transverse order at the wall inner surface (positive slope). This shows that cells do not necessarily deposit microfibrils at right angles to the growth direction—as might be an explanation for normal (and ethylene-induced) synthesis. Extreme increase in slope reflects further passive alignment in the transverse direction—as would be predicted from the multinet growth theory. e: stretched cells have an exaggerated preponderence of the longitudinal component of growth because the transverse component is a contraction. This has no appreciable effect on the orientation of synthesis because initial slopes are comparable with controls. The effect on passive realignment is to exaggerate it. The slope falls unusually rapidly, large portions of the outer wall show longitudinal microfibril orientation. This result also supports the multinet theory. Colchicine treatment during stretch did not prevent normally oriented synthesis after drug removal. This indicates that stability of synthesis orientation does not require continuous presence of microtubules.

To assess the effect of the predominant (and unusual) transverse strain on the alignment of the newly deposited microfibrils, one compares the initial slope of this curve with that from a grown control. The initial slope of the curve of the experimental cell appears not to be significantly different from that of the grown control. Thus, in contrast to a above, the normal deposition of microfibrils was unaffected by the treatment, a 90° change in the direction of predominant strain.

As explained under "Materials and Methods," these cells were prevented from growing lengthwise by appressing them

between two pieces of coverslip. This left only the sides of the cells free to expand, while the parts that were against the glass were practically motionless. When the walls of these cells were observed under polarized light, they showed cracks that were more prominent on the strained portion than on the motionless part. Interference microscope analysis of these walls revealed that the cracks affected only the outer layers of wall.

Preliminary analysis of the recently deposited wall from the expanding and the fixed regions showed no difference in the arrangement of the microfibrils. This suggests that any tendency for random deposition in the nonexpanding part (see a above) was over-ridden, presumably by the presence of strain in regions adjacent to the clamped portion.

c. Promotion of Longitudinal Strain while Transverse Extension Was Turned into Contraction. Here the relative dominance of the longitudinal component of growth was exaggerated by enhancing this component directly (with applied tension). This led to actual contraction in the transverse direction.

The wall analysis is given in Figure 7e. Here the slope of the plot is constant near the origin, but falls rapidly in the outer part of the wall. This decrease is so pronounced that the curve drops below the abscissa, giving over-all positive birefringence (longitudinal order).

Again, the properties of the wall meet the expectations of the "multinet growth hypothesis" since the outer microfibrils continuously reorient according to the predominant (longitudinal) direction of growth. Exaggerating the directional quality of growth exaggerates the rate of reorientation (rate of change in slope).

No significant difference was detected between the initial slope of the curve and that of a grown control. This indicates that the oriented synthesis of microfibrils was insensitive to a drastic increase in the predominant strain, and/or a reversal in sign of the minor component. Some cells that did not change their initial diameter (*i.e.* did not contract) were analyzed and the results were essentially equivalent to those in Figure 7e.

The results of the above experiments indicate that while strain is *needed* for continued oriented synthesis (experiment a), the directionality of this strain does not bear on the directionality of the wall synthesis (experiments b and c). If strain is present, transverse synthesis continues. In contrast, passive realignment is remarkably sensitive to the directionality of the cell's strain pattern.

d. Colchicine Treatment Experiment. The wall analysis for this experiment is presented in Figure 7e. The left-hand portion of the plot reflects the most recent wall synthesis, which occurred after the cells were transferred to a normal growth medium. The positive slope indicates that there has been deposition of transversely oriented microfibrils after the cells were returned to a normal growth medium. This took place after the cell presumably had undergone disruption of its microtubules in the presence of extreme longitudinal strain. This indicates that the cell component which persists as a frame of reference during colchicine treatment is not influenced by longitudinal strain.

DISCUSSION

Multinet Theory. Our results will first be discussed in terms of their bearing on the two main parts of the multinet theory: (*a*) that microfibril synthesis occurs only at the wall inner surface and (*b*) that microfibrils, once deposited, passively realign according to their strain environment. Our finding that a random wall was deposited internally to the pre-established wall (experiment a) supports the first part. Our finding that over a wide range of physical treatments the internal configuration of the wall microfibrils does change in accord with the direction of the strain pattern, however abnormal, supports the second part.

As initially phrased, the multinet theory dealt mainly with transverse wall synthesis at the wall inner surface. It seems unreasonable to view evidence for other patterns of synthesis as being "against" the multinet theory because the two key suppositions (above) ignore the particular orientation of synthesis. Other systems clearly show a crossed-lamellar (or criss-cross) pattern of the microfibrils in the wall. This has been seen in outer epidermal walls (5) and very clearly in certain algae (21) and elsewhere (32). Preston has indicated that in *Chaetomorpha*, despite complexity of the wall, the two main features of the multinet theory appear to hold within a highly twisting growth pattern (21, 22).

The main kind of structural evidence that could rule against the first feature of the multinet theory would be the finding of structures in the interior of the wall that could not be reasonably derived, by passive displacement and realignment, from microfibrillar patterns at the site of synthesis (however complex these patterns might be). To our knowledge, no such structures have been proven.

The passive reorientation observed in the present study suggests that microfibrils can rotate their long axis. Such rotation is hard to reconcile with views that the cell wall may contain one, or just a few, microfibrils as suggested by the scarcity of ends of microfibrils in wall replicas. Perhaps ends (tapering and hard to see?) are present at synthesis or are produced later by breakage. An alternate explanation involves reorientation, with localized intramicrofibril bending, to produce a "trellis" pattern (1). This view challenges the bulk rotation aspect of the multinet theory, but not the view that passive deformation influences microfibril pattern. In brief, the view of primary wall cellulose pattern as involving two main physical aspects: synthesis at the wall inner surface and subsequent passive alignment is supported in detail by our results. A less clear picture must be presented for the physical factors governing the directional character of microfibrils at the time of synthesis.

Role for Microtubules at Site of Synthesis? The strong correlation between microfibrillar alignment and microtubule orientation (*e.g.* refs. 18, 27, and 31), plus the colchicine sensitivity of cellulose alignment (*e.g.* refs. 16 and 20), strongly suggest the immediate involvement of microtubules in cellulose orientation in certain cell types (4). Heath (15) has proposed a model wherein microtubules guide the movement of cellulose synthetic enzymes in the preferred direction. Against microtubular involvement are certain observations where oriented cellulose synthesis occurs in the absence of microtubules (34). See arguments of O'Brien (19) and Pickett-Heaps (20). Brown and Montezinos (2) postulate periodic reassembly of enzyme complexes to explain alternation in cellulose pattern in *Oocystis*. In *Nitella*, colchicine sensitivity is clearly present. The resumption of transverse cellulose synthesis after removal of the drug shows the pre-existing cellulose is not a critical template for cellulose orientation (12) and that some feature of the cell serves to properly "direct" microtubule reassembly, assuming microtubules are essential for ordered synthesis.

What Ultimate Feature of Cell Governs Oriented Synthesis? Assuming that microtubules are essential intermediates in oriented synthesis, they would merely serve as agents coupling some other oriented cell feature to oriented microfibril synthesis. Of the possible antecedent influences listed in the introduction, it was felt that strain patterns could be involved. While strain is required for normal oriented synthesis (experiment a) the direction of maximum (or minimum) strain does not correlate with the orientation of synthesis (experiments b and c). Even with the microtubules presumably eliminated from involvement temporarily (experiment d), strain had no obvious influence. These conclusions force us to re-examine the cell for other possible features that could persist through all our experiments. Cell form, curvature, chloroplast files, and streaming direction all seem ruled out by preliminary work of P. A. Richmond (personal communication). He has produced a film, taken

in polarized light, clearly showing recovery of ordered synthesis and directional growth by a colchicine-treated *Nitella* cell that had become perfectly round. Its originally longitudinal streaming pattern had been converted to one where flow was concentric around two poles. The poles were not at the ends of the cell, but were midway up each original striation. The chloroplast files were in similar arrangement. Thus transverse wall synthesis resumed despite the streaming pattern being highly abnormal. One oriented feature of the *Nitella* cell that we can still propose as persisting through all treatments known to us is the striation line itself. Even in a spherical cell, these two lines would run from node to node (Fig. 2a). By elimination, therefore, one can suggest that in *Nitella* the striation lines are logical candidates for wall synthesis organizers. The lines may be microtubule initiation centers analogous to the phragmoplast.

Stability or Lability of Oriented Synthesis in Growing Cells. Our work shows that transverse synthesis persists through severe physical alteration of the course of growth and resumes after combined chemical/physical treatment designed to disturb synthesis. This indicates that oriented synthesis is not maintained by subtle forces, but rather is somehow "locked in" against considerable perturbation. That transverse synthesis is not always so "stabilized" in this manner is shown by the fact that regions of *Nitella* wall that have transverse structure (*e.g.* basal region of the leaf apical cell) can subsequently give rise to a whole whorl of cylindrical branches, each with transverse structure. Such natural over-riding of an established microfibrillar pattern is indicated in the formation of lateral roots, where the new axis is formed *de novo*. Also, in the fern *Onoclea*, the distinctive cellulose orientation at the stem tip is locally reorganized to give the equally distinctive pattern for the leaf tip (17). The imposition of new specific directons of synthesis by physical treatments applied to the whole cell did not occur in *Nitella*. Success may involve more severe, or more localized physical changes, or may require that the cell be in some special "labile" state, as it presumably is earlier in development.

Need for Strain to Prevent Random Synthesis. The localized suppression of expansion did alter synthesis orientation, but apparently into a pattern already "standard" for the cell, namely that for its secondary wall. This experiment shows that primary growth and secondary wall formation can go on concurrently in the same cell. Parallel behavior has been described for wood fibers. They show intrusive tip growth at the ends, secondary wall formation in the midregion (36). Our work seems to be the first to show that changes in wall synthesis pattern can be experimentally changed by localized physical treatment. The need for strain for normal oriented synthesis suggests that some continued "dilution" of cytoplasm which accompanies expansion is necessary. Plasmolysis, which elimintes both strain and turgor, leads to random deposition in *Chaetomorpha* (22).

Redirection of Oriented Synthesis "at Will?" Experimental control of microfibril synthesis orientation is available in several systems. Unfortunately, the new pattern induced is either a randomized one (colchicine treatment) or, if not, a pattern that the cell would be expected to produce eventually. For example, an orientation response is brought on by ethylene (6, 26, 33, 35), where the synthesis changes from transverse to longitudinal. This also may be a pattern that the cell otherwise produces since longitudinal deposition has been suggested for the "corners" of parenchyma cells (22, 37) and is reported for the outer epidermal wall (5). That the ethylene induced reorientation could be a response to a new strain direction (as might be brought on by extreme wall softening) is somewhat counter-indicated by our work. Experiment b showed that *Nitella* at least does not deposit microfibrils normal to the direction of maximum strain.

A clear case of the artificial induction of a novel organized cell wall pattern is the physical production of laterals (with transverse wall structure) from the midregion of *Nitella* internodes (12). They grow from perforations in a jacket surrounding the cell; the details of the induced growth behavior were not well characterized. Presumably the treatments were more complex than those imposed here, or perhaps the behavior of the striation lines was critical. At any event the successful mechanical induction of a lateral, with its appropriate wall ultrastructure, remains in need of biophysical explanation. The effective processes presumably are those which govern the initiation of laterals in all plants where the functional correlation between microfibril pattern and growth direction obtains.

In summary, the present work shows that the imposition of unusual growth (strain) patterns on individual *Nitella* cells leads to changes in internal wall microfibrillar arrangements which are compatible with the predictions of multinet growth. These same treatments have no simple effects on the orientation of synthesis. In the absence of strain, even in a localized region within a cell, orientation of synthesis is lost. In the presence of strain, however, synthesis of the normal transverse orientation proceeds, apparently independent of the direction of strain. In contrast to orientations in the wall interior, the directional synthetic activity at the wall inner surface is relatively well stabilized against physical perturbation.

Acknowledgment – The photographic acid of K. Brooks is gratefully acknowledged.

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