

# Cell Growth and the Structure and Mechanical Properties of the Wall in Internodal Cells of *Nitella opaca*

## III. SPIRAL GROWTH AND CELL WALL STRUCTURE

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### SUMMARY

The internodal cells of the alga *Nitella opaca* L, which are in the form of long thin cylinders, exhibit the phenomenon of spiral growth, i.e. as the cells elongate they also twist about their longitudinal axis. It has been shown in an earlier paper (Probine and Preston, 1962) that the cell wall is mechanically anisotropic. In this paper the moduli necessary to describe the elastic behaviour of a material possessing this sort of symmetry are considered. It is pointed out that if the *Nitella* cell is regarded as a thin-walled cylinder built of a material possessing orthorhombic elastic symmetry, then there can be a coupling between shear and extension which will produce a torsional twist as the cylinder is pressurized.

It is suggested that this is the basic mechanism of spiral growth. Experimental evidence is presented which supports this view.

### INTRODUCTION

THE internodal cells of the alga *Nitella*, which take the form of long thin cylinders, exhibit the phenomenon of spiral growth, that is to say that as the cells elongate they also twist about their own axes. Since the cell is a hollow tube filled with liquid, or semi-liquid, cytoplasm with a central vacuole, the conclusion can hardly be avoided that these peculiar effects of growth must be due to some feature of the wall. There are a number of theories of extension growth in which it is suggested that the initial event in extension growth is a mechanical deformation of the wall by turgor pressure. If this view is correct, one would expect that spiral growth might have its origin in the mechanical properties of the cell wall, and complete understanding of the phenomenon might throw some light on the whole process of cell enlargement.

A description of the growth behaviour of *Nitella axillaris* Braun has been

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furnished by Green (1954). By placing a series of markers on the cell wall and observing their relative positions at various stages of growth it was shown that young cells exhibit a right-handed axial rotation as they elongate, while at a later stage (usually at a length of approximately 1 cm.), there is a reversal of rotation. Green has also shown that growth is evenly distributed throughout the length of the internodal cell. In this paper an attempt is made to show that spiral growth (in another species of *Nitella*, *N. opaca*) can be understood in terms of the structure and mechanical properties of the cell wall already described (Probine and Preston, 1961, 1962).

The phenomenon of spiral growth is also exhibited by the sporangiophore of *Phycomyces*, and most of the early work was carried out on this organism. The sporangiophore grows in the form of a thin-walled cylinder, the tapering tip of which carries the developing sporangium as an inflated ball. Growth occurs only in the apical 2 mm. or so. It has been demonstrated by Oort (1931), Oort and Roelofsen (1932), and Castle (1937, 1942) that markers placed in the zone of growth not only move upwards, but at the same time are displaced transversely indicating that there is a rotational growth of the cell about its own axis. Before the sporangium has begun to appear, the cell twists in a left-handed (*S*) direction (i.e. in the direction of left-handed screw). During the swelling of the sporangium the cell ceases to elongate and to rotate. Once the sporangium is fully swollen the structure rests for a time and then begins to elongate and twist in the opposite sense from the original direction (i.e. there is now a right-handed (*Z*) rotation). This continues for a period, but the rate of twisting gradually diminishes, falls to zero, and then develops in the left-handed sense.

Various theories have been put forward in explanation of this phenomenon. Castle (1936) suggested that twisting might be due to the anisotropic response of such a wall to the hydrostatic pressure within the cell due to turgor. No details of the process were given, however. Heyn (1936) suggested that, during elongation, plastic stretching of the wall occurs by failure of the wall along planes of greatest weakness in the crystallites. Since these planes are at an angle to the cell axis, spiral growth results. Heyn (1939) also proposed an alternative theory. He suggested that chitin molecules are originally deposited in the direction of the long axis parallel to the direction of protoplasmic streaming. As they arrange themselves into a crystal lattice, the forces of crystallization cause an oblique orientation of the chitin chains and the combination of 'rotating forces and of extending forces of elongation then results in spiral growth'.

Preston (1948) and Middlebrook and Preston (1952 *a, b*) have interpreted the behaviour of the wall in terms of its submicroscopic structure. They made careful X-ray and polarizing microscope measurements and concluded that chitin chains in the growth zone were oriented at about  $14^\circ$  to the transverse direction. A model of the wall, in the growth zone, was proposed in which the wall was thought of as being composed of a number of spiral springs (chitin chains) which are extended during the growth process. It can

be shown that when a 'spiral' spring (helical is more correct) is axially extended there is a rotation of the free end. Extension of the spirally arranged long chain molecules of chitin by the hydrostatic pressure within the cell would, by analogy, also cause a rotation of the free end of the cell. The theory was successful in accounting for the fact that elongation and rotation always go together. Further, such quantitative checks as could be applied gave values of the correct order of magnitude. To account for the reversal of spiral growth, it was assumed that at this stage of growth there was a transition from an almost parallel texture with low angular dispersion of microfibrils to one with a higher angular dispersion. If this change was sufficient the 'effective' elastic constants of the 'spiral spring' would be changed and there would be a change in the direction of rotation of the end.

Frey-Wyssling (1952) has criticized this theory on the grounds that the spiral spring model is not consistent with the structure of the primary wall of *Phycomyces*, viz., lamellae of crossed interwoven microfibrils with two distinct main directions. He suggested that growth is by intussusception and that the small area where the primary wall is loosened, and into which new fibrils are woven, moves around the wall. It is not immediately obvious, in the absence of a more complete description of the chitin chain arrangement and of details of the fibril loosening process, that such a growth mechanism would necessarily lead to rotational growth. It is possible to imagine circumstances in which such a process would lead only to a circular movement of the top of the sporangiophore, and not to rotational growth of the cell about its own longitudinal axis.

Although these theories of spiral growth propose rather different mechanisms, they have this in common; they all ascribe the mechanism of spiral growth to some sort of mechanical force (arising within the wall structure) which causes a lateral displacement of the wall elements. It seems to be generally agreed that, in the phenomenon of spiral growth of single cells, we have one case at least where mechanical forces control or modify growth.

#### *Outline of the present theory*

The structure and mechanical properties of the *Nitella* wall have been described by Probine and Preston (1961, 1962). Features relative to the present discussion are summarized below:

(a) There is a net preferred orientation of the cellulose microfibrils, the mean direction of which (referred to the cell axis) varies with the age of the cell.<sup>1</sup> In mature walls the mean orientation is almost transverse to the cell axis, but in very young cells (about 1 mm. long) it is inclined at about 10° to the transverse direction.

(b) The microfibrils are arranged in discrete layers with pectic substances and, probably, other non-crystalline components providing an amorphous filling between successive microfibrillar reinforced layers.

<sup>1</sup> The quantity actually measured is the major extinction position (m.e.p.) of the wall when it is viewed between crossed nicols in a polarizing microscope.

(c) The wall is elastically anisotropic, the elastic properties being generally consistent with what one might expect on the basis of their wall structure; viz., a high tensile modulus and low creep rate in the direction of the preferred orientation of crystalline microfibrils.

The measurements referred to above have shown that the wall has different elastic properties in the longitudinal and transverse directions. Further, it has been shown that the wall is laminated and, on wetting, there is a very much larger dimensional change in the radial direction than in either of the other two principal directions. It is, therefore, to be expected that the wall will have different elastic properties in the radial direction to those it possesses in either the longitudinal, or transverse, direction. For these reasons it will be assumed that the wall material possesses orthorhombic elastic symmetry, i.e. three mutually perpendicular axes of elastic symmetry.

The remainder of this section will be devoted to a consideration of the number of elastic moduli necessary to describe the elastic behaviour of a material possessing this sort of symmetry. It is pointed out that in the *Nitella* wall the structure is such that a coupling between shear and extension can arise and it is this which is thought to be the origin of spiral growth.

The most general expression for Hooke's Law relating strains in an elastic solid to stresses is:

$$\begin{aligned}x_x &= S_{11} X_x + S_{21} Y_y + S_{31} Z_z + S_{41} Y_z + S_{51} Z_x + S_{61} X_y \\y_y &= S_{12} X_x + S_{22} Y_y + S_{32} Z_z + S_{42} Y_z + S_{52} Z_x + S_{62} X_y \\z_z &= S_{13} X_x + S_{23} Y_y + S_{33} Z_z + S_{43} Y_z + S_{53} Z_x + S_{63} X_y \\y_z &= S_{14} X_x + S_{24} Y_y + S_{34} Z_z + S_{44} Y_z + S_{54} Z_x + S_{64} X_y \\z_x &= S_{15} X_x + S_{25} Y_y + S_{35} Z_z + S_{45} Y_z + S_{55} Z_x + S_{65} X_y \\x_y &= S_{16} X_x + S_{26} Y_y + S_{36} Z_z + S_{46} Y_z + S_{56} Z_x + S_{66} X_y.\end{aligned}\tag{1}$$

In these equations  $x, y, z$  are three perpendicular coordinate axes;  $x_x, y_y$ , and  $z_z$  are extensional strains;  $y_z, z_x$ , and  $x_y$  are shear strains;  $X_x, Y_y, Z_z$  are extensional stresses;  $Y_z, Z_x$ , and  $X_y$  are shear stresses; the moduli  $S_{ik}$  where  $i$  and  $k$  may take any values from 1 to 6 are constants of the material. The general properties of  $S_{ik}$  may be summed up as follows:

$$(a) S_{ik} = S_{ki}.\tag{2}$$

(b) If  $i = k = 1, 2$ , or  $3$ ,  $S_{ik}$  relates an extensional strain to an extensional stress in the same direction and

$$S_{ik} = 1/E_k,$$

where  $E_k$  is Young's modulus for the direction  $k$ .

(c) If  $i = 1, 2$ , or  $3$ ,  $k = 1, 2$ , or  $3$  ( $i \neq k$ ),  $S_{ik}$  relates an extensional strain in one direction to an extensional stress in a perpendicular direction and

$$S_{ik} = -\sigma_{ik}/E_i = -\sigma_{ki}/E_k \text{ (by equation 2),}$$

where  $\sigma_{ik}$  = Poisson's ratio =  $\frac{\text{contraction in } k \text{ direction}}{\text{extension in } i \text{ direction}}$  for a tensile stress in  $i$  direction.

(d) If  $i = k = 4, 5$ , or  $6$ ,  $S_{ik}$  relates a shearing strain to a shearing stress in the same plane and

$$S_{ik} = 1/G,$$

where  $G$  is a rigidity modulus.

$$\text{If } i = k = 4, \quad S_{ik} = 1/G_{yz},$$

$$i = k = 5, \quad S_{ik} = 1/G_{xz},$$

$$i = k = 6, \quad S_{ik} = 1/G_{xy},$$

where the suffixes to the  $G$ 's specify the direction of shear stress.

(e) If  $i = 4, 5$ , or  $6$ ,  $k = 1, 2$ , or  $3$ ,  $S_{ik}$  relates an extensional strain to a shear stress and vice versa.

(f) If  $i = 4, 5$ , or  $6$ ,  $k = 4, 5$ , or  $6$  ( $i \neq k$ ),  $S_{ik}$  relates a shear strain in one plane to shear stress in a perpendicular plane.

Because  $S_{ik} = S_{ki}$  the number of independent moduli is reduced from 36 to 21. Further, if the material is symmetrical in structure the number of independent moduli is reduced still further.

It has been assumed that the symmetry of the *Nitella* wall approximates to that of an orthorhombic crystal. The number of independent non-zero moduli is, therefore, reduced to nine. They are:

$$S_{11}, S_{21}, S_{31}, S_{22}, S_{32}, S_{33}, S_{44}, S_{55}, S_{66},$$

If the longitudinal ( $L$ ) direction (parallel to the axis of the cell) is identified with the  $x$  axis, the transverse ( $T$ ) direction with the  $y$  axis, and the radial ( $R$ ) direction with the  $z$  axis, then:

$$\begin{aligned} S_{11} &= \frac{1}{E_L}; S_{21} = -\frac{\sigma_{TL}}{E_T} = -\frac{\sigma_{LT}}{E_L}; S_{44} = \frac{1}{G_{RT}} \\ S_{22} &= \frac{1}{E_T}; S_{32} = -\frac{\sigma_{RT}}{E_R} = -\frac{\sigma_{TR}}{E_T}; S_{55} = \frac{1}{G_{RL}} \\ S_{33} &= \frac{1}{E_R}; S_{31} = -\frac{\sigma_{RL}}{E_R} = -\frac{\sigma_{LR}}{E_L}; S_{66} = \frac{1}{G_{LT}} \end{aligned} \quad (3)$$

where, for example,

$E_L$  = Young's modulus in  $L$  direction,

$\sigma_{RT} = \frac{\text{contraction in } T \text{ direction}}{\text{extension in } R \text{ direction}}$  for tension in  $R$  direction,

$G_{RT}$  = rigidity modulus for shear stresses in  $R$  and  $T$  directions.

This presentation is quite standard and is similar to that given by Hearmon (1948).

The above treatment applies to the special case in which the reference directions relate to the symmetry of the material. It has been shown that, in general, the m.e.p. is not quite transverse in *Nitella* (Probine and Preston, 1958). If we identify the axes of elastic symmetry of the wall material with the directions of the major and minor extinction positions, displacement of the m.e.p. from the transverse direction means that the effect on the elastic moduli of a rotation from the principal axes must be considered.

The general formulae for the effect of rotation from the principal axes on the elastic moduli of an orthorhombic crystal were given by Hearmon (1948). For this purpose they will not be given in full. It is sufficient to note that for a rotation  $\theta$  in the  $xy$  plane, from  $x$  towards  $y$ , about an axis  $z$ , 13 moduli are now required. Four new moduli have appeared, viz.,  $S'_{61}$ ,  $S'_{62}$ ,  $S'_{63}$ , and  $S'_{54}$ , where the dashes indicate elastic moduli in the new orientation. They are not independent moduli, but are expressible in terms of the nine fundamental moduli listed above, and the angle of rotation. To quote Hearmon: 'The existence of  $S'_{61}$ ,  $S'_{62}$ , and  $S'_{63}$ , means that shear stresses in the  $x$  and  $y$  directions produce pure extensions in the  $x$ ,  $y$ , and  $z$  directions and, conversely; the existence of  $S'_{54}$  means that shear stresses in the  $z$  and  $x$  directions produce shear strain in the  $zy$  plane and conversely.'

It is this coupling between shear and extension which is thought to be the basic mechanism in spiral growth.

The moduli  $S'_{16}$ ,  $S'_{26}$ , and  $S'_{54}$ , change sign as  $\theta$ , the angle between the transverse plane and the m.e.p., changes.

For example:

$$\begin{aligned} S_{16} &= -\frac{1}{2}(S_{11}-S_{22})\sin 2\theta + \frac{1}{4}(S_{11}+S_{22}-2S_{12}-S_{66})\sin 4\theta \\ &= -\frac{1}{2}\left(\frac{1}{E_L}-\frac{1}{E_T}\right)\sin 2\theta + \frac{1}{4}\left(\frac{1}{E_L}+\frac{1}{E_T}+\frac{2\sigma_{TL}}{E_T}-\frac{1}{G_{LT}}\right)\sin 4\theta. \end{aligned}$$

These coupling coefficients are zero when  $\theta = n \cdot \frac{1}{2}\pi$  (where  $n = 0$ , or any integer). The conditions for isotropy include

$$S_{11} = S_{22} \quad \text{and} \quad S_{11} - S_{12} - \frac{1}{2}S_{66} = 0.$$

From the point of view of the present discussion the *Nitella* cell can be considered as a thin-walled pressurized cylinder, which is built of a material possessing orthorhombic elastic symmetry.<sup>1</sup> Further, the axes of elastic symmetry (identified with the major and minor extinction positions) do not in general coincide with the principal axes of stress. There will therefore, be a coupling between shear and extension which will mean that a pressurized cylinder of the type described will not only exhibit longitudinal and transverse deformation, but will also exhibit a torsional twist. Further, since the

<sup>1</sup> Note: For completeness full three-dimensional orthorhombic structure has been attributed to the wall. In so far as the cell wall approximates to a *thin-walled* tube, the meaningful observable quantities are the stresses and strains which can occur in the two-dimensional structure which may conveniently be developed on to a plane by a cut along a generator of the cylindrical cell wall. There are three stresses and three strains related by the moduli  $S_{11}$ ,  $S_{22}$ ,  $S_{16}$ ,  $S_{26}$ ,  $S_{66}$ . Unless the cell is considered to have a thick wall, the introduction of further moduli can serve no useful purpose.

coupling moduli change sign as  $\theta$  changes sign, the direction of twist will change sign, as the m.e.p. changes from a left-hand to a right-hand spiral. When the m.e.p. is strictly transverse, the coupling moduli are zero and there is no tendency to twist in this case.

*Torsional behaviour as turgor pressure changes*

No attempt is made here to develop an analytical expression to describe the strain movements of a cylinder of the type described. The effect can be demonstrated experimentally however, and this has been done for *Nitella opaca*. It has been confirmed (Probine, 1959), that *Nitella opaca* has a very similar spiral growth behaviour to *Nitella axillaris* Braun.

The cell to be tested was suspended vertically from a fixed support and allowed to hang free in a beaker of distilled water. A light pointer was attached to the lower end of the cell to indicate rotation about the cell axis. The test cell was intact and undisturbed, the mounting to the support and to the pointer being made via the empty cells above and below it (Fig. 1). The cell with attached apparatus was then transferred to a mannitol (or sucrose) solution of strength just sufficient to plasmolyse the cell. As the turgor pressure dropped, the free end of the cell rotated about the cell axis. The direction of twist was reversed when the cell was again placed in water and the turgor pressure increased.

The magnitude and direction of rotation, as pressure increases from a state of plasmolysis to full turgor, is shown plotted against the length of the cell in Fig. 2. The points are considerably scattered, but one feature emerges quite strongly; for cell lengths less than about 12 mm. there are 16 points which indicate a right-hand rotation and none which indicate left-hand rotation, while for lengths greater than this arbitrary figure there are 13 indicating left-hand rotation and 2 indicating right-hand rotation. The very great scatter of points is not difficult to understand. The measurements are not easy to make, particularly since one is dealing with fairly delicate cells. There are, therefore, quite large measurement errors. Again, it is to be expected that there will be some variation from cell to cell (particularly when grown under different conditions) of those mechanical features of the wall which control magnitude of strain, viz. elastic moduli, wall thickness, turgor pressure, angle of m.e.p., length-diameter ratio, &c.

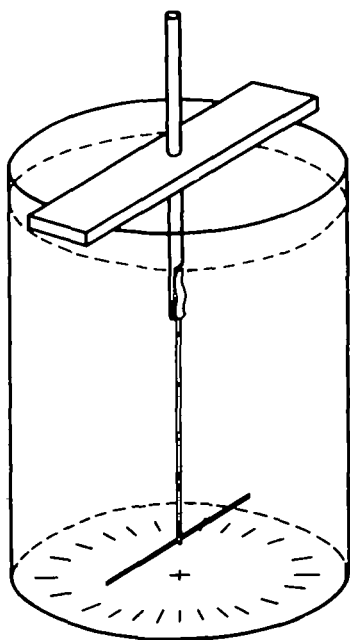


FIG. 1. Sketch showing *Nitella* internode suspended in mannitol solution with light pointer attached. Attachments made to cells above and below internode under test.

Because the very great scatter of points tends to obscure the main features, the data have been replotted in Fig. 3*a*. In this figure *direction* only is shown, and cell length is plotted on a logarithmic scale. This scale has been chosen merely to avoid crowding at the lower end, and not because it has any special significance. This method of plotting the results brings out the very notable

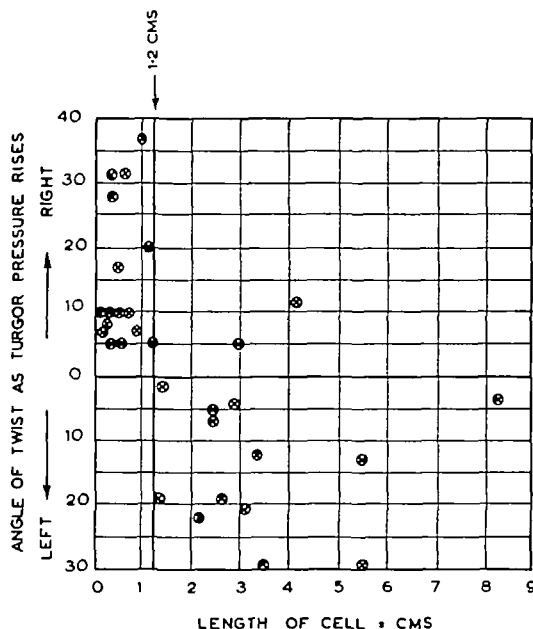


FIG. 2. Angle of twist of internodal cell of *Nitella* as turgor pressure rises from zero to full turgor plotted as a function of cell length.

behaviour of the cells more clearly. The following observations may be made:

(a) The cells show an easily discernible torsion when the internal pressure is increased from zero turgor pressure to full turgor.

(b) The direction of rotation is consistent with the direction of spiralling observed in spiral growth. All young cells appear to twist in a right-handed (Z) sense while most of the older cells twist to the left. There is no inconsistency in the behaviour of the three cells which show a right-hand twist at lengths of up to 4 cm. One cell of the *Nitella opaca* internodes during growth spiralled to the right up to a length of 27 mm., although the majority reversed at lengths far short of this. This distribution of directions is not one which would have arisen by pure chance with any reasonable degree of probability.

In Fig. 3*b* the direction of twist has been plotted against the angle of the m.e.p. (with due regard as to whether the m.e.p. is directed so as to make a left or a right-hand spiral around the wall). Those cells in which the m.e.p.



makes a left-hand spiral around the cylindrical wall exhibit a right-hand twist, while the twist is opposite for cells where m.e.p. makes a right-hand spiral. There is one exception; one cell with a right-hand m.e.p. also showed a right-hand rotation. (This result has been rechecked as far as was possible from the figures and there was no inconsistency such as would suggest an error.) The change-over from right- to left-hand twist does not seem to occur

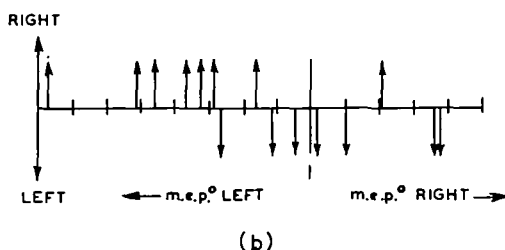
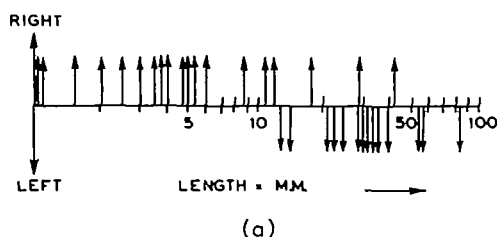


FIG. 3a. Direction of twist of internodal cell of *Nitella* as turgor pressure rises from zero to full turgor plotted as a function of length. (Logarithmic length scale chosen for convenience only.)

FIG. 3b. Direction of twist plotted as a function of the major extinction position (m.e.p.) of the cell wall.

when the m.e.p. is zero, but at a point where it makes a small left-hand spiral (about  $2^\circ$ ). It might well be that the axis of elastic symmetry does not exactly coincide with the m.e.p.

An indirect check on this 'change-over' point has been obtained in the following way. It appears from Green's work, and from the measurements on spiral growth made during this investigation, that reversal of spiralling quite frequently (but by no means always) takes place at a length of *about* 1 cm. Further, the measurements of direction of twist suggest that at about 12 mm. there is a critical point (perhaps in the form of a lower limit). From the records it has been possible to calculate the mean angle at which the cytoplasm streams in a cell 1 cm. long. The mean angle is about  $14^\circ$  (for the cell data given by Green (1954) in his Fig. 4, the angle at 1 cm. is  $11^\circ$ ). If a straight line of best fit is fitted to the points previously obtained by Probine and

Preston (1958) relating streaming angle to the angle of the m.e.p., the m.e.p. angle, corresponding to a chloroplast angle of  $14^\circ$ , is  $93^\circ$ . In terms of the scale used in Fig. 3*b*, this corresponds to helix angle of  $3^\circ$  in the left-hand sense. Too much weight cannot be given to this value, but it is nevertheless in very good agreement with the change-over point ( $2^\circ$ ) as indicated in Fig. 3*b*.

#### DISCUSSION

It has therefore been established that when the turgor pressure is changed in these cylindrical cells the cell twists about its own longitudinal axis, and this is interpreted as being due to a coupling between shear and extension arising out of the anisotropy of the wall.

Consideration has been given to the possibility that this 'pressure-change torsion' might be due to some mechanism unrelated to the anisotropy of the wall. For example, it might be due to a protoplasm/wall interaction which occurs as the protoplasm contracts away from the wall during plasmolysis. It is unlikely, however, that the protoplasm has the requisite mechanical properties. Further, even if such a possibility were entertained there seems to be no obvious explanation of reversal of twist, since the only (known) directional characteristic of protoplasm in *Nitella* (streaming) is always in the right-hand sense. The same changes in twist occur if the cell is kept in distilled water and the wall ruptured to lower the pressure. It seems likely, therefore, that the twisting of the cell is due to the elastic anisotropy of the wall itself.

The question now arises as to whether 'pressure-change torsion' and spiral growth are linked. The good agreement between the direction of twist and the direction of spiral growth at the various stages of growth is certainly such as to suggest that both phenomena have a common origin. Independent evidence that this is the case has been obtained by Frei and Preston (1961), working with the filamentous alga, *Chaetomorpha*. This alga also exhibits the phenomena of spiral growth, and it has been shown that the direction of twist (as the pressure rises) is in the same direction as the direction of spiral growth. The wall structure of *Chaetomorpha* is quite different from that of *Nitella*. It is possible that the crossed-fibrillar wall structure which is possessed by *Chaetomorpha* could be regarded as having the elastic symmetry of a monoclinic crystal (the angle between the crossed fibrils is rather less than  $80^\circ$ ). This type of crystal requires 13 independent elastic moduli completely to specify its elastic behaviour. These 13 moduli include as independent moduli the coupling moduli which appear in the orthorhombic case only when there is a rotation from the principal axes. It is to be expected, therefore, on the basis of the ideas expressed earlier in this paper, that *Chaetomorpha* would exhibit a 'pressure-change torsion' effect and the phenomena of spiral growth. The results of Frei and Preston (1961) confirm that it exhibits both.

The wall structure of the growth zone of sporangiophores in *Phycomyces* seems to be, at least superficially, similar to that of *Nitella*, and it is not

unreasonable to propose a similar mechanism of spiral growth in this case. A difficulty arises, however, owing to the fact that the direction of spiral growth in *Phycomyces* reverses without any (known) change in wall structure (e.g. direction of m.e.p.). The sign of shear/extension coupling moduli can be changed without changing the sign of  $\theta$  by choosing suitable values of the elastic moduli. Whether such suitable values of the elastic moduli are physically realizable, however, has not been confirmed.

Before concluding, attention should be drawn to the fact that certain tacit assumptions have been made in applying the general statement of Hooke's law to cell-wall material. They are set out below;

1. The material is homogeneous; i.e. it has identical physical properties at all locations (in a given direction).
2. The material is perfectly elastic.
3. Each component of stress at every point is a linear function of the corresponding component of strain at that point.
4. The strain is infinitesimal.

Assumptions (1) and (2) are certainly not strictly true. The crystalline microfibrils will have a comparatively high tensile modulus compared with the amorphous material in which they are embedded. Again, earlier observations reported by Probine and Preston (1962) have shown that the wall is not perfectly elastic. It is not thought, however, that these departures from an ideal material will affect the argument in principle.

In developing this theory of spiral growth use has been made of the observation by Probine and Preston (1958) that the m.e.p. of the *Nitella* wall is not strictly transverse. Green and Chapman (1955) concluded that 'the cellulose microfibrils are well oriented and run strictly in the transverse direction. This orientation is found in all portions of the wall and in all stages of growth.' Subsequently Green (1958) modified this view and pointed out that the arrangement of microfibrils on the inner surface of the wall varied with the age of the cell. Following the paper by Probine and Preston (1958), Green (1959) re-examined the wall structure of *Nitella axillaris* Braun and concluded that if there was a helical structure it was not such as could be distinguished by a study of the major extinction position. He published quintuple-exposure photographs of internodes taken between crossed nicols such that each specimen was photographed with its longitudinal axis parallel to the direction of the polarizer and at  $5^\circ$  and  $10^\circ$  either side of this position. The specimen was cut so that one observed alternate areas of single and double wall. It was claimed that if *Nitella axillaris* Braun and *Nitella opaca* were of similar structure one would expect, on the basis of Probine and Preston's work, that single layers would extinguish at one or other of the ends of the series (i.e.  $10^\circ$  off the central position). We do not agree with this interpretation. Green's examples were for cells whose streaming angles ranged between  $13^\circ$  and  $21^\circ$ . According to the figures published for *Nitella opaca* the most probable m.e.p.'s for such cells would be between  $3^\circ$  and  $6^\circ$  and the scatter is such that they could easily be lower. We do not agree

therefore that the published photographs prove the point since they could not be expected to show any dramatic evidence of helical structure. Further, a helical wall structure can be demonstrated directly. It is possible to tear the cell wall in such a way that a long strip of wall material unwinds from the inside of the wall in the form of a continuous ribbon. This is taken as strong evidence for the existence of a helical structure in the form of a flat helix.

The existence of a helical structure is not in doubt for cells of the *Nitella opaca* type. The situation for *Nitella axillaris* is not clear but it is now accepted that the fibrils are not strictly transverse. The difference if it exists is probably one of degree. Further, the difference of opinion over the position of the m.e.p. does not affect the correlation between 'pressure-change torsion' and spiral growth which applies not only to *Nitella opaca* but to *Chaetomorpha* as well.

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