THE STRUCTURE AND PLASTIC PROPERTIES OF THE CELL WALL OF *NITELLA* IN RELATION TO EXTENSION GROWTH

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Summary

The internodal cells of *Nitella opaca* L. have been used in earlier studies to assess the part which mechanical properties of the wall may play in the control of cell growth (Probine and Preston 1962). The wall is mechanically anisotropic in both its plastic and elastic properties, and it is shown in this paper by an approximate theoretical treatment that a mat of cellulose microfibrils, embedded in a plastic matrix and having a distribution in the plane of the wall like that observed in *Nitella*, would lead to longitudinal and transverse plastic extensions in the ratio observed in the growing cell. Factors which would affect cell shape are discussed.

The pattern of growth of the *Nitella* internode is discussed, and it is shown that, if the wall is formed by the "multi-net" process, a steady-state microfibril distribution can be maintained, as would seem to be required by the observation that there is a constant ratio between increase in length and increase in diameter during extension growth. There is direct experimental evidence that the wall is formed by this process.

I. INTRODUCTION

The plant cell wall is a complex material with complex plastic and elastic properties. Since extension growth involves changing the shape and size of the cell, these plastic and elastic properties must surely have some bearing on the course of extension growth, either directly or indirectly (Probine 1965). In this paper we propose to consider the relationship between the plastic properties of the wall and the growth and form of the cell.

Although it is believed that the work which follows is of general applicability, the actual experimental and theoretical work is based on the structure and physical properties of the giant alga *Nitella opaca* L. (Fig. 1). Studies of the cell walls of giant alga have contributed a great deal to the general body of knowledge on cell wall structure. These organisms have been selected firstly because their cell wall structure is interesting in itself, and secondly, because of their size they can be used in studies which would be very difficult to carry out on smaller cells. The internodal cell of *Nitella*, in particular, is very suitable for the sort of study outlined here for the following reasons:

(1) Being a single cell, extension growth takes place free from tissue interactions, and secondly, it does not need to be isolated from other tissue by a physical or chemical technique, which might alter its properties.

(2) It is large enough to be easily handled and physical properties, such as plasticity and elasticity, can be *directly* determined on the cell wall material.

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(3) During extension growth the wall grows uniformly over its whole surface (Green 1954).

The cell wall of *Nitella* consists of a non-crystalline matrix of pectic substances, hemicellulose, protein, etc. reinforced with microfibrils of crystalline cellulose (Probine and Preston 1961). Averaged over the whole thickness of the wall the microfibrils have a net preferred orientation, the mean direction of which, referred to the cell axis, varies with the age of the cell. In mature walls the mean orientation is almost transverse to the cell axis, but in young cells (about 1 mm long) it is inclined

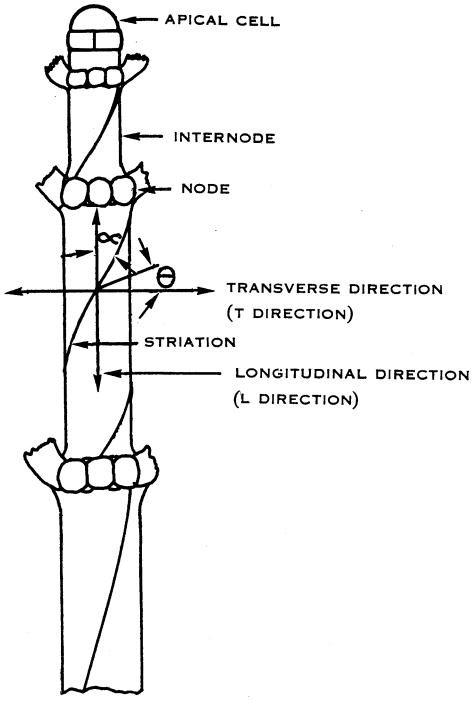

Fig. 1.—Diagram of a *Nitella* shoot. The angle between the direction of protoplasmic streaming and the longitudinal axis is designated by the angle α. The direction between any microfibril and the transverse axis is designated by the angle θ.

at about 10° to the transverse direction (Probine and Preston 1958). The microfibrils are not perfectly aligned along the preferred direction, but show considerable scatter about the mean direction.

The microfibrils have an extremely high modulus of elasticity (i.e. capacity to resist stretching). Treloar (1960) has calculated the tensile modulus of cellulose and obtained a value of $6{\cdot}9\times10^{11}$ dyne/cm^2, which is in quite good agreement with the best experimental value of $9{\cdot}0\times10^{11}$ dyne/cm^2. For comparison the corresponding value for mild steel is 18×10^{11} dyne/cm^2.

The fact that cellulose microfibrils are such effective reinforcing cell wall components, which are oriented in a preferred direction, is likely to have great significance in any discussion on the way in which the wall deforms during growth. It requires no great insight to conclude that a wall which is reinforced with transversely oriented microfibrils is likely to resist deformation in the transverse

direction to a greater extent than in the longitudinal direction. This assumption was checked by Probine and Preston (1962). They showed that the cell wall material was more plastic in the direction of greatest extension growth, and was less plastic in the direction of minimum extension growth. The directions of maximum and minimum wall plasticity coincided with the directions of minimum and maximum reinforcing with cellulose microfibrils.

There is, therefore, a correlation between structure, wall plasticity, and extension growth, and interrelations between them will be considered in more detail below. Various aspects of the problem will be considered in the following order:

(1) The pattern of growth of the *Nitella* internodal cell.
(2) The process of wall formation in relation to the observed pattern of growth.
(3) The relationship between the structure of the wall and its plastic properties.
(4) The relationship between the plastic properties of the wall and the growth and form of the cell.

II. Pattern of Growth of the Nitella Internode

A great deal is known about the growth pattern of very young internodes, mainly from the work of Green (1954, 1958, 1960, etc.), but their small size has prevented *direct* observation of the plastic and elastic properties of the wall material from such cells. Since information on these physical properties is available only within the range of 1 mm to a maturity length that varies between 40 and 60 mm, discussion of the growth pattern is therefore restricted to this range of cell length.

(*a*) *Rate of Growth*

In considering the process of enlargement it is of interest to know whether growth is occurring at a constant rate. A sensitive indicator of the growth process is the change in length per unit length per unit of time; one asks whether $(1/L).(\mathrm{d}L/\mathrm{d}t)$ remains constant. If

$$(1/L).(\mathrm{d}L/\mathrm{d}t) = r,$$

where r is a constant, then

$$L = L_0 \mathrm{e}^{rt},$$

where L_0 = initial length, and L = length after time t. This is the equation which describes the growth of the cell during the "log phase". If $\log L$ is plotted against t, then one obtains a linear relationship between them if r is constant. Departure from linearity implies that r is varying. It is easier to see changes in r, however, if the slope of the curve is plotted as a function of $\log L$, and this has been done in Figure 2 where $(1/L).(\mathrm{d}L/\mathrm{d}t)$ has been plotted as a function of $\log_{10}L$. Over the range of cell lengths considered, the growth rate falls off as the cells approach their final length. However, the growth rate is substantially constant over the greater part of the range considered here, which suggests that those factors which are significant in controlling cell extension also remain substantially constant.

(*b*) *Ratio between Cell Length and Cell Diameter*

Green and Chapman (1955) have pointed out that the relationship between length and diameter in *Nitella* is of the form

$$L = aD^m,$$

where a and m are constants, from which it follows that

$$(\mathrm{d}L/L) = m(\mathrm{d}D/D). \tag{1}$$

In other words, in each growth event there is a constant ratio between the percentage increase in the longitudinal direction and the percentage increase in diameter.

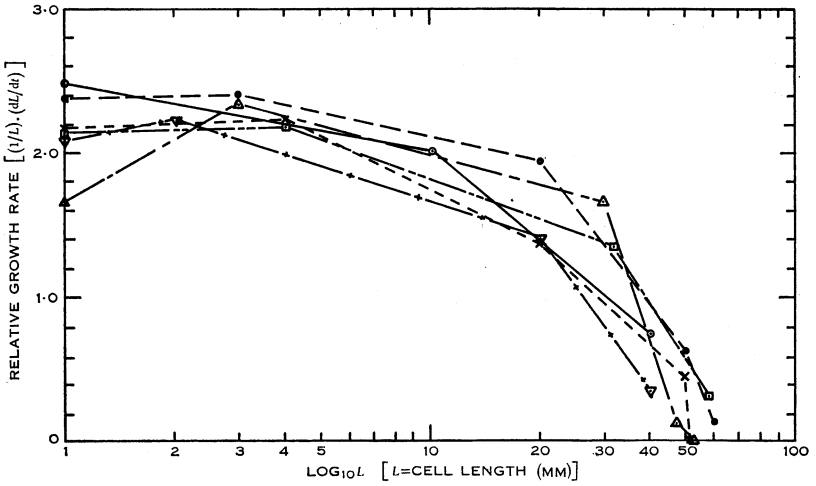

Fig. 2.—Growth rate (relative rate of elongation per plastochron) plotted as a function of cell length L (log scale)—data extracted from Green (1958).

If the plants are grown in controlled conditions so that growth is uniform, the length and diameter of successive internodal cells can be plotted against each other on a log/log scale, and this has been done to obtain the data for Figure 3. In this

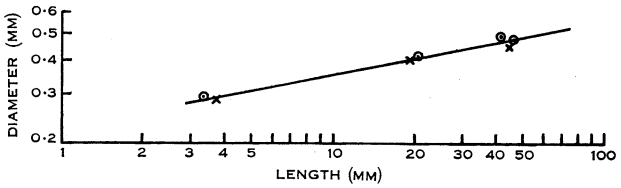

Fig. 3.—Length and diameter of *Nitella* internodes plotted on log/log scale.

case the constant $m = 5$, i.e. the cell is growing proportionately more in length than diameter and a constant ratio between them seems to hold during most of the period considered here.

(c) *Change in Birefringence of Cell Wall*

Since we are interested in the effect of wall structure on growth, one must be aware of structural changes in the wall which take place during growth. Green (1958) has made a study of the way in which the birefringence of the wall changes during growth. The cell wall material is optically anisotropic, and is therefore doubly refracting. If n_γ is the larger refractive index and n_α is the smaller refractive index, then the birefringence is $n_\gamma - n_\alpha$. The optical path difference, R, of the two waves which pass through the specimen is easily measured with a polarizing microscope and compensator. Thus

$$R = T(n_\gamma - n_\alpha),$$

where T is the thickness. Therefore

$$\text{Birefringence} = R/T.$$

In Figure 4, Green's measured R/T is plotted as a function of $\log_{10}L$. Since we may reasonably assume that birefringence is a measure of the distribution of microfibrils in the plane of wall, changes in the value of R/T, would indicate either that there had been a change in microfibril distribution or in the amount of microfibrillar material.

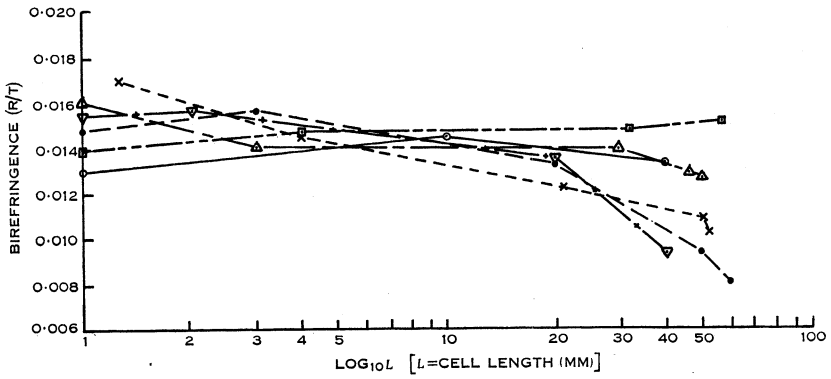

Fig. 4.—Birefringence of cell wall of *Nitella* internodes plotted as a function of cell length L (log scale)—same cells as those in Figure 2.

Although it tends to fall slightly towards the end of the growth period, the value of R/T is reasonably constant for the greater part of the range considered, and it would appear that there are no very notable structural changes during the growth period considered.

(d) *X-ray Diffraction of Cell Wall*

The interpretation of polarizing microscope evidence is not unambiguous, however. It is therefore necessary to support such measurements by other evidence. Because cellulose microfibrils are crystalline, their orientation in the plane of the wall can be studied by X-ray diffraction. A typical curve of microfibril distribution

in the plane of the wall of a young cell, obtained from the distribution of X-ray intensity around the $10\bar{1}$ arc, is shown in the heavy solid line in Figure 5.

If one examines the microfibril distribution, by X-ray diffraction, in a series of cells ranging from young to mature, there is evidence that the microfibrils are not as well oriented in the preferred direction in mature cells as in young cells. It is not easy to put the change in microfibril distribution in quantitative terms because of scatter from amorphous material which tends to be more troublesome in the older cells. The best that can be said at present, is that the change in microfibril distribution appears to be small, and is only noticeable in cells which have ceased to extend or are very near the end of extension growth.

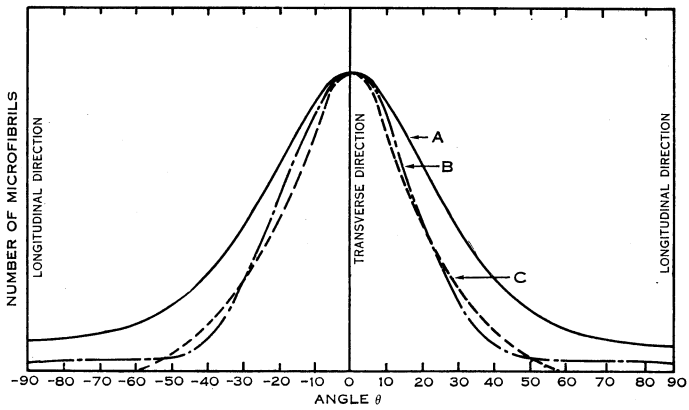

Fig. 5.—*A*, distribution of microfibrils in the plane of the wall, $F(\theta)$, determined by X-ray diffraction. *B*, distribution of microfibrils in the "new growth", $g(\theta)$, required to maintain a steady-state distribution—calculated from $F(\theta)$. *C*, distribution of microfibrils on inner wall—from replicas of young cells examined in the electron microscope.

(*e*) *Electron Microscopy of Cell Wall*

The electron microscope can also provide relevant evidence on microfibril orientation. Green (1958) and Probine and Preston (1961) reported that in cells approaching the end of their elongation, microfibrils could be observed on the inner surface of the wall with directions quite removed from the transverse. Probine and Preston suggested that at this stage a crossed-fibrillar structure was being laid down and that this differed only in the perfection of its orientation from that already described for some other algae (Preston and Astbury 1937; Preston and Cronshaw 1958; Frei and Preston 1961).

Probine, Dyer, and Jones (unpublished data) have shown recently that a thin sheet of cell wall material can be stripped from the inner surface of mature *Nitella* internodal cells. When the complete cell wall is fixed in osmium, dehydrated in alcohol, embedded in Araldite, and post-stained with Millonig's lead stain, this inner region of the cell wall is revealed, in section, as a series of light and dark bands. The dark bands appear to consist of dark-staining fibrils, which in longitudinal–radial section produce a "herring-bone" pattern (Plate 1).

EXTENSION GROWTH OF *NITELLA*

Section of portion of cell wall and cytoplasm showing characteristic layering on the inside of the cell wall of a mature internodal cell. The "herring bone" pattern appears to be due to dark-staining fibrils, which may be in a crossed-fibrillar configuration.

In young cells this characteristically banded wall is absent, and it first appears late in the development of the cell. In Figure 6, log L is plotted as a function of internode number. The number of layers (one bright and one dark band constitute a "layer") at each length is shown on the curve. It will be seen that the number of layers increases rapidly towards the end of elongation. There is some evidence, as yet incomplete, that the layers consist of microfibrils arranged in a cross-fibrillar pattern. If this is so, however, the orientation must be very imperfect as a thin sheet of wall consisting of several of these layers shows no obvious preferred orientation when examined by X-ray diffraction. The X-ray diagram is typical of cellulose I.

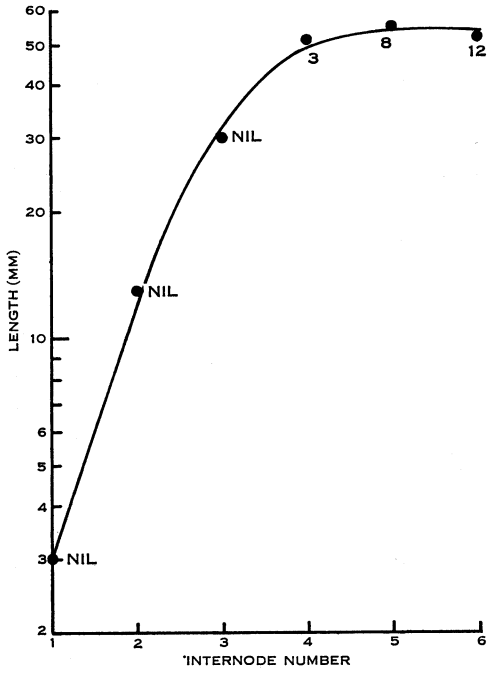

Fig. 6.—Length of each internode cell (on log scale) plotted as a function of the internode number. The number of layers of the type illustrated in Plate 1 is indicated against each point plotted. The layering which is characteristic of the mature internodes, is absent in young internodes.

It is tentatively suggested that these layers are the result of a change in the type of wall deposition—perhaps analogous to the switch from primary wall to S1-type wall in tracheids. Whatever their significance, however, there is no doubt that a change in the disposition of microfibrils occurs as the cell wall approaches maturity and the change will have some affect on the physical properties. Since it comes late in cell development, it is not directly relevant to the main phase of cell extension; but it may well be a significant factor in the change in physical properties which occur as cell extension ends (Probine and Preston 1962).

III. "Steady-state" Wall Formation

If there were any large change in the overall distribution of microfibrils during growth of the cell, one would expect this to affect the relative rates of increase of diameter (D) and length (L). The fact that there is a constant ratio between the

percentage increase in length and the percentage increase in diameter (eqn. 1), i.e. m is a constant in the equation

$$(\mathrm{d}L/L) = m(\mathrm{d}D/D),$$

suggests that the wall structure must be substantially the same after an incremental change in length, as it was before the increase in length. In terms of the overall distribution of microfibrils, it suggests that a "steady-state" distribution is maintained over the greater part of the range of lengths considered here, and that this is so is borne out by the observations on wall structure reviewed above.

The process of wall formation must, therefore, be such that a steady-state microfibril distribution can be maintained. Green (1960) has shown that the optical properties of the *Nitella* cell wall are consistent with the "multi-net" theory of growth (Roelofsen and Houwink 1953). This theory argues that new sets of microfibrils are successively laid down on the inner face of the cell in a nearly transverse direction, and that as growth proceeds and the cell increases in length, each microfibril is pulled more and more towards the longitudinal direction. As the area of the wall increases, therefore, any given group of microfibrils will tend towards a more nearly axial orientation, appear to migrate towards the outside of the wall, and suffer a reduction in fibril density.

One can therefore imagine a state of affairs in which the overall distribution of microfibrils does not appreciably change as the cell grows. New growth provides the nearly transverse microfibrils to replace those that have been drawn into a more longitudinal direction, and those at greater angle to the transverse direction do not increase in density because they are continually spread more sparsely over the increasing area of the cell wall. It is interesting, therefore, to see whether the pattern of new growth that occurs on the inner wall of the cell could lead, merely by the reorientation processes (multi-net growth), to the steady state that is observed. For those who do not wish to follow the mathematical theory, it may be omitted. The general argument is taken up again after equation 13.

It is convenient to describe the fibril distribution by a function $F(\theta)$ such that $F(\theta).\delta\theta.\delta n$ is the number of fibrils that cross an elementary length δn drawn at right angles to them and make angles ranging between θ and $\theta+\delta\theta$ to the transverse* axis of the cell. It is observed that as the cell increases in diameter, there is a proportional increase in wall thickness. It is, therefore, required that $F(\theta)$ should vary during growth in proportion to the diameter of the cell.

When a cell increases in length from L to $L+\Delta L$ and in diameter from D to $D+\Delta D$ (and it will be assumed that ΔL and ΔD are very small), all longitudinal and transverse elements of the cell wall increase in these proportions. A fibril that originally made an angle θ with the transverse direction would, after the growth, make an angle $\theta+\Delta\theta$ where

$$\tan(\theta+\Delta\theta)/\tan\theta = [(L+\Delta L)/L].[D/(D+\Delta D)].$$

* One could equally well adopt the convention of measuring θ with respect to the longitudinal axis of the cell. Since microfibrils are laid down in the transverse direction (approximately) and are reoriented towards the longitudinal direction as the cell elongates, we have adopted the convention in this paper of measuring θ from the transverse direction.

This statement ignores any tendency of the cell to twist as it grows, but this treatment is thought sufficient for the present purpose. The angular change $\Delta\theta$ is therefore given by

$$\Delta\theta = \tfrac{1}{2}(\Delta L/L - \Delta D/D)\sin 2\theta. \tag{2}$$

The fibrils that lie in any direction after the growth are not therefore the same fibrils as those that had this direction before the growth occurred. They may have acquired this direction through being reoriented in the above manner, or they may be new fibrils created in the new material laid down on the inner wall.

Consider a set of fibrils that before the growth have directions between θ and $\theta+\delta\theta$. Their number per unit distance at right angles to their length is $F(\theta).\delta\theta$. If the fibrils have an average length l, the total number in the whole cell is

$$F(\theta).\delta\theta.\pi DL/l. \tag{3}$$

This number, which does not include any new fibrils laid down, does not change when the cell increases in length and diameter. The fibrils are assumed to be inextensible so that the length l is unchanged. The area of the cell wall has increased, however, drawing the fibrils apart, so after this growth the number per unit distance at right angles to their length is

$$F(\theta).\delta\theta.\pi DL/\pi(D+\Delta D).(L+\Delta L)$$

$$= F(\theta).\delta\theta(1-\Delta L/L-\Delta D/D). \tag{4}$$

The fibrils now lie in new directions. Those that originally had a direction θ now have a direction $\theta+\Delta\theta$. Those that had a direction $\theta+\delta\theta$ now have a direction $\theta+\delta\theta+\Delta_1\theta$, where $\Delta_1\theta$ is, according to (2),

$$\Delta_1\theta = \tfrac{1}{2}(\Delta L/L-\Delta D/D)\sin 2(\theta+\delta\theta). \tag{5}$$

Thus the new range of directions is

$$\delta\theta+\Delta_1\theta-\Delta\theta = \delta\theta[1+(\Delta L/L-\Delta D/D)\cos 2\theta]. \tag{6}$$

Now we shall be concerned with these new directions and shall consider the changes in fibril density that have occurred there, so for brevity it is convenient to describe the new directions as θ_1 and $\theta_1+\delta\theta_1$. That is

$$\theta_1 = \theta+\Delta\theta, \tag{7}$$

$$\delta\theta_1 = \delta\theta[1+(\Delta L/L-\Delta D/D)\cos 2\theta]. \tag{8}$$

Expression (4) for the density of fibrils that have been reoriented to these directions may then be rewritten, using equations (5), (7), and (8), as

$$F(\theta_1-\Delta\theta).\delta\theta_1[1-(\Delta L/L-\Delta D/D)\cos 2\theta_1].(1-\Delta L/L-\Delta D/D),$$

which is

$$\delta\theta_1[F(\theta_1)-\tfrac{1}{2}(\Delta L/L-\Delta D/D)\sin 2\theta_1.\mathrm{d}F(\theta_1)/\mathrm{d}\theta_1]$$

$$\times[1-\Delta L/L-\Delta D/D-(\Delta L/L-\Delta D/D)\cos 2\theta_1]. \tag{9}$$

The density of fibrils created in new growth may be written as

$$g(\theta_1).\delta\theta_1, \tag{10}$$

where $g(\theta_1)$ is the function that describes the amount of new growth. The sum of expressions (9) and (10) is the new density of fibrils in the range θ_1 to $(\theta_1+\delta\theta_1)$. But before the growth, the density of fibrils in this range was

$$F(\theta_1)\delta\theta_1. \tag{11}$$

According to our definition of the steady state we expect to find that the density has increased in proportion to the diameter and is

$$F(\theta_1).\delta\theta_1.[1+(\Delta D/D)], \tag{12}$$

in which case we shall equate the sum of expressions (9) and (10) to expression (12). In making the equation it is convenient to assume some ratio m for the increases in length and diameter, or

$$\Delta L/L = m(\Delta D/D).$$

The equation becomes, after some reduction,

$$\frac{g(\theta_1)}{\Delta L/L} = \frac{1}{m}\left\{F(\theta_1)[m+2+(m-1)\cos 2\theta_1]+\tfrac{1}{2}\left[\frac{\mathrm{d}F(\theta_1)}{\mathrm{d}\theta_1}(m-1)\sin 2\theta_1\right]\right\}. \tag{13}$$

There is therefore a distribution of microfibrils in the "new growth" on the inner wall, such that the reorientation processes (multi-net growth) lead to an overall distribution which remains constant during extension growth.

The growth distribution $g(\theta_1)$ was calculated from the above equation using the overall distribution of microfibrils, determined by X-ray diffraction. An electron micrograph of the inner face of *Nitella* cells was then made to find the actual angular distribution in the new growth. The three curves are shown plotted in Figure 5. They are plotted to the same maximum so that their variations with angle can be compared. The agreement between the observed angular distribution in the new growth and the theoretical prediction of it, is at least as good as the approximate theory and the experimental determination justifies.

We therefore have a mechanism of wall formation whereby the anisotropy of the wall can remain constant during elongation.

IV. Plasticity of the Cell Wall

Growth implies a permanent enlargement of the cell and hence a plastic yielding of the cell wall. In a theoretical treatment of wall plasticity one would wish to show that, for known plastic properties of the matrix, a mat of interlacing microfibrils, having a distribution with angle like that observed in *Nitella*, should inevitably lead to longitudinal and transverse plastic extensions in the ratio observed in the growing cell. Since a crystalline microfibril may be supposed to be incapable of plastic extension, all parts of the microfibril cannot remain in the same relationship to the matrix after extension, as before extension. In effect, therefore, the microfibril must have moved relatively to the mean position of the material round it,

and consequently will have experienced some frictional forces which will presumably set up tensions in the microfibrils to resist the deformation. It is necessary to consider how the frictional force on an elementary length of microfibril may depend on its velocity relative to the mean position of the material that surrounds it.

At all times during the plastic extension, the mat of fibres is subject to longitudinal and transverse tensions in the ratio 1 : 2, because the cell is cylindrical. From this ratio of stress, one may predict a ratio of plastic yield, but this prediction will depend very much on the model one proposes for the mechanical system.

It is sufficient for the present purpose to ignore the slight tendency of a *Nitella* cell to twist as it grows. Such a twist may be expected to develop for mechanical reasons if the density distribution of fibrils is slightly asymmetric about the transverse direction. There is evidence for such asymmetry, but for the present purpose only the symmetrical part of the density distribution will be considered. Again, for the benefit of those who do not wish to follow the theoretical treatment, the discussion is resumed in the next section.

To relate the pattern of growth of the cell to the density distribution of fibrils in its wall one may proceed as follows. Consider any direction at angle θ to the transverse axis. An interval on the cell wall, of unit length in this direction, has component lengths $\cos\theta$ in the transverse (y) direction, and $\sin\theta$ in the longitudinal (x) direction. If the cell increases its length by a factor $(1+\epsilon_1)$ and its diameter by a factor $(1+\epsilon_2)$ all longitudinal and transverse intervals on the cell wall increase in those ratios, so the component lengths of the unit interval originally at direction θ become

$$(1+\epsilon_1)\sin\theta,$$

and

$$(1+\epsilon_2)\cos\theta.$$

Assuming ϵ_1 and ϵ_2 to be small, the new length is therefore

$$= [(1+\epsilon_1)^2\sin^2\theta+(1+\epsilon_2)^2\cos^2\theta]^{\frac{1}{2}}$$

$$= 1+\epsilon_1\sin^2\theta+\epsilon_2\cos^2\theta. \tag{14}$$

This is the factor by which the element of wall originally in the direction θ has expanded. If the strains ϵ_1 and ϵ_2 are changing, then the rates of change of strain in the two principal directions are

$$v_1 = \mathrm{d}\epsilon_1/\mathrm{d}t,\ v_2 = \mathrm{d}\epsilon_2/\mathrm{d}t,$$

and elements of the cell wall measured in direction θ show a rate of extension

$$v = v_1\sin^2\theta+v_2\cos^2\theta. \tag{15}$$

The frictional force, and therefore the mean tension developed in the microfibril, will depend on the law relating the velocity (rate of shear strain) to the shear stress, for the matrix material. One may classify plastic flow into four main types

and the treatment will depend on the particular law one assumes. We may have

(1) Newtonian flow: $v = (1/\eta)P$;
(2) non-Newtonian flow: $v = (1/a)P^r$;
(3) Bingham flow: $v = (1/\eta)(P-P_0)$; or
(4) quasi-plastic flow: $v = (1/a)(P-P_0)^r$.

In these equations v is the rate of shear strain, η is the viscosity, P is the stress, and a, P_0, and r are constants. Alternatively, if a displacement involves the breakage of a number of bonds, the total energy is proportional to the displacement, and the frictional force is independent of the velocity (since the work is merely proportional to the distance moved). This is analogous to solid friction.

(*a*) *Non-Newtonian Flow*

The authors have considered first the case in which the matrix exhibits non-Newtonian Flow. In this case the mean tension developed in a microfibril may be written as

$$Av^n, \tag{16}$$

where the coefficient A is a frictional constant which takes into account the "viscosity" constant a and the length and diameter of the microfibrils, and $n = 1/r$. Now in terms of the distribution density $F(\theta)$ one can say that the number of fibres per unit distance normal to their length, and with directions between θ and $\theta+\delta\theta$ is $F(\theta).\delta\theta$. The number crossing a unit length drawn in the longitudinal direction is $F(\theta).\delta\theta.\cos\theta$. Each fibre exerts a tensional force whose component at right angles to this unit longitudinal length is $Av^n\cos\theta$. Thus the total tensional force across the unit longitudinal length, due to these fibres is

$$F(\theta)\delta\theta.\cos\theta.Av^n\cos\theta.$$

From all the fibres that are present, the total tensional force across the unit longitudinal length is

$$P_2 = A\int_{-\frac{1}{2}\pi}^{\frac{1}{2}\pi} F(\theta).v^n.\cos^2\theta.\mathrm{d}\theta. \tag{17}$$

This is the wall tension in the transverse direction. By a similar argument one finds that the wall tension in the longitudinal direction is

$$P_1 = A\int_{-\frac{1}{2}\pi}^{\frac{1}{2}\pi} F(\theta).v^n.\sin^2\theta.\mathrm{d}\theta. \tag{18}$$

But the *Nitella* cell is cylindrical and it is readily seen that an internal pressure must produce a tension in the transverse direction that is twice as great as the tension in a longitudinal direction. The rates of extension of the wall must therefore be such that $P_2 = 2P_1$. One has therefore to determine what ratio between the extension rates v_1 in the longitudinal direction and v_2 in the transverse direction will provide tensions that satisfy equations (17) and (18). The result depends of course upon the index n that is assumed for the power law of friction.

In the case of solid friction, n is zero. If any expansion occurs in the direction of a fibre, the tension is prescribed and independent of the rate. In the observed growth pattern of a *Nitella* cell it can be readily shown that all intervals on the cell wall increase in length, whatever their orientation, so as a first approach one may assume that all fibres exhibit their constant tension. Then for the distribution density that is observed experimentally and whose curve is given in Figure 5, it is found from expressions (2) and (3), setting n equal to zero, that P_1 and P_2 have the calculated ratio, $P_2/P_1 = 3{\cdot}23$; this is too large a ratio.

In the case of fluid friction (Newtonian fluid), n is unity and equations (17) and (18) lead to the result that if $P_2 = 2P_1$ then $v_1/v_2 = 2{\cdot}6$. This is appreciably less than the observed ratio in growth which averages $5{\cdot}0$ for the species used by us.

If one adopts a fractional index for the index n (non-Newtonian fluid), the solution has to be sought by trial and error. The ratio P_2/P_1 was calculated for various values of the index n and it was found that $P_2/P_1 = 2$ for an index $n = 0{\cdot}66$.

(*b*) *Quasi-plastic Flow*

We do not believe, however, that a simple power law represents the true behaviour of the matrix. Figure 10 of Probine and Preston's (1962) paper shows the way in which rate of plastic deformation depends on the applied stress. It will be noticed that the rate of deformation is very low until the stress exceeds a certain minimum value (yield point) and then increases rapidly. It would appear, therefore, that the behaviour of the matrix is more nearly that of a quasi-plastic material for which

$$v = (1/a)(P-P_0)^r.$$

We may therefore rewrite equation (16) as:

$$\text{mean tension developed in fibril} = Av^n+P_0,$$

where $n = 1/r$, and it follows by similar reasoning to that used before that:

$$\frac{P_2}{P_1} = \frac{\int_{-\frac{1}{2}\pi}^{\frac{1}{2}\pi} F(\theta).(Av^n+P_0)\cos^2\theta.\mathrm{d}\theta}{\int_{-\frac{1}{2}\pi}^{\frac{1}{2}\pi} F(\theta).(Av^n+P_0)\sin^2\theta.\mathrm{d}\theta}. \tag{19}$$

If we assume that $\Delta L/L = 5\Delta D/D$, i.e. $v_1 = 5v_2$ (as before), then

$$\frac{P_1}{P_2} = \frac{Av_2^n\int_{-\frac{1}{2}\pi}^{\frac{1}{2}\pi} F(\theta)(5\sin^2\theta+\cos^2\theta)^n\cos^2\theta.\mathrm{d}\theta + P_0\int_{-\frac{1}{2}\pi}^{\frac{1}{2}\pi} F(\theta)\cos^2\theta.\mathrm{d}\theta}{Av_2^n\int_{-\frac{1}{2}\pi}^{\frac{1}{2}\pi} F(\theta)(5\sin^2\theta+\cos^2\theta)^n\sin^2\theta.\mathrm{d}\theta + P_0\int_{-\frac{1}{2}\pi}^{\frac{1}{2}\pi} F(\theta)\sin^2\theta.\mathrm{d}\theta} \tag{20}$$

There are two terms, a "velocity-dependent" term, and a "velocity-independent" term. The value of the index n, for which $P_2/P_1 = 2$, will depend on the magnitude of the constant P_0. Figure 10 of Probine and Preston (1962) suggests that the magnitude of the velocity-independent term, which depends on the value of P_0, is about one-third of the stress in the longitudinal direction at normal turgor.

If the constants are chosen so that

$$P_0 \int_{-\frac{1}{2}\pi}^{\frac{1}{2}\pi} F(\theta)\sin^2\theta.\mathrm{d}\theta = 0{\cdot}4Av_2^n \int_{-\frac{1}{2}\pi}^{\frac{1}{2}\pi} F(\theta)(5\sin^2\theta + \cos^2\theta)^n\sin^2\theta.\mathrm{d}\theta,$$

then n can be determined by calculating P_2/P_1 for various values of n, and choosing the one for which $P_2/P_1 = 2$. In this case, $n = 1$. When $n = 1$, the relationship between rate of flow and shear stress for the matrix is that of a Bingham fluid $v = (1/\eta).(P-P_0)$, which is a special case of a "quasi-plastic" fluid.

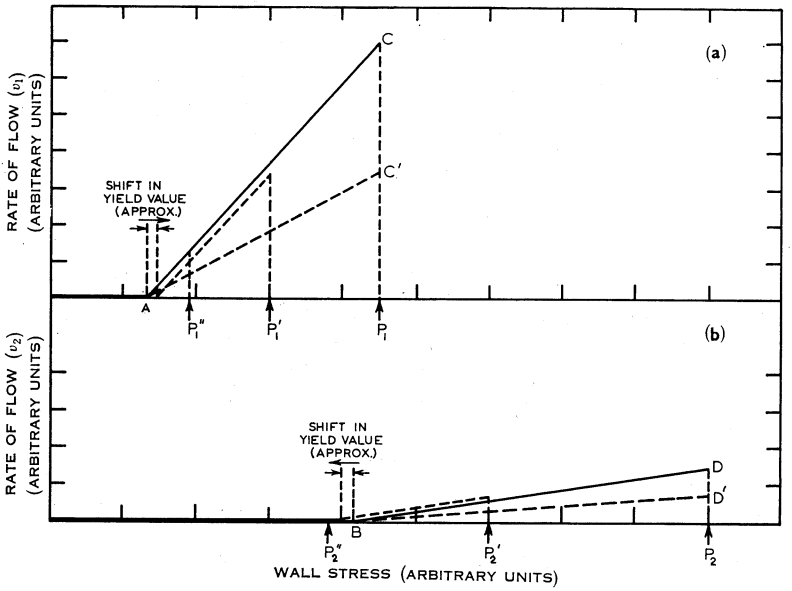

Fig. 7.—Calculated rate of plastic flow in the longitudinal direction (a) and transverse direction (b) as a function of the wall stress due to turgor pressure. Stress in the longitudinal direction at normal turgor is designated P_1 and in transverse direction by P_2. Because the cell is cylindrical $P_2 = 2P_1$.

So that the form of the equations for P_1 and P_2 ($n = 1$) can be visualized, they are plotted in Figure 7. It will be seen that the rate of plastic flow in the longitudinal direction, as a function of applied stress due to turgor pressure [Fig. 7(a)] is of similar form to the experimental curves of Figure 10 of Probine and Preston (1962).

V. Factors which Influence Cell Shape

It is interesting now to consider the factors which the theory suggests may affect the shape of the cell. The rate of plastic flow in the longitudinal direction as the result of applied stress due to turgor is shown in Figure 7(a). The corresponding curve due to transverse stress is shown in Figure 7(b). The stress is plotted on the same scale in each case, but because the cell is cylindrical the maximum stress in transverse direction, P_2, is twice that in the longitudinal direction, P_1. Equation (20) suggests that the stress at which the material begins to yield depends

on the magnitude of the constant P_0, which is a property of the matrix, and also on the distribution of the microfibrils $F(\theta)$. The wall first begins to yield at stress A for longitudinal strips and at stress B for transverse strips. Once the material begins to flow, it flows at different rates in the two directions because of the distribution of microfibrils, $F(\theta)$.

The theoretical work suggests, therefore, that the distribution of microfibrils $F(\theta)$ in the plane of the wall is the basic cause of the anisotropic plastic properties, and therefore it will profoundly affect the shape of the cell (i.e. by modifying the growth ratio v_1/v_2). The theory therefore accords with the experimental findings of Probine and Preston (1962) on the plastic behaviour of longitudinal and transverse strips of cell wall material. The plastic behaviour of longitudinal strips as determined experimentally is shown in Figure 10 of Probine and Preston (1962). Plastic flow of transverse strips was found to be very small and the theory indicates that it should indeed be small.

Since $F(\theta)$ itself is dependent on $g(\theta)$—the distribution of microfibrils in the "new growth"—$g(\theta)$ would appear to be the primary variable in determining cell shape. We have not yet carried out the exercise of following the changes in cell shape which follow a change in $g(\theta)$ but it would be a most interesting exercise to do this, and it is one of the ultimate aims of this sort of analysis.

A decrease in turgor pressure, which would bring the wall stress from P_2 to P_2' and from P_1 to P_1' (in Fig. 7), will cause the ratio v_1/v_2 to change, since the stress in the transverse direction exceeds the yield stress by a much smaller proportion of the total stress in that direction, than the stress in the longitudinal direction exceeds the yield stress in that direction. Indeed, if we consider an extreme case in which the turgor pressure is reduced so that the wall stress falls to P_2'' and P_1'' there is then no yield in the transverse direction and the cell increases in length only. Actually, of course, an increase in length only, would cause $F(\theta)$ to change until the yield points and rates of growth had again reached a steady state. For example, the change in yield stress which follows the reduction in turgor to P_2' and P_1' is illustrated in Figure 7. The net result, however, is a change in cell shape (i.e. in v_1/v_2).

It is interesting to note that in all of these cases there is a certain amount of "feedback". For example, a lowering of the turgor pressure causes a change in the ratio of longitudinal to transverse expansion, and this in turn produces a change in the distribution $F(\theta)$ which changes v_1 and v_2 in such a way as to counteract the change in cell shape. Again, a change in $g(\theta)$, which has the immediate affect of, say, narrowing the distribution $F(\theta)$, will cause a change in the transverse to longitudinal expansion which will tend to broaden $F(\theta)$ and so partly compensate for the change in $g(\theta)$.

A change in $F(\theta)$ due to a change in microfibril disposition near the end of growth will change both the yield stresses and the rates of flow in each direction. If the switch in wall deposition is to a cross-fibrillar structure, or to something approaching a random distribution of microfibrils, the stresses at which yield occurs (in the longitudinal and transverse directions) will tend to approach each other. The data in Figure 10 of Probine and Preston (1962) are not really complete enough to make a firm statement on the point, but there is an indication that the yield stress

in the longitudinal direction is higher for low growth rate cells than for high growth rate cells, as would be expected. Such a change would affect the ratio v_1/v_2, but since it occurs only at the end of cell expansion it would have little effect on cell shape.

A change in relative proportions of cellulose microfibrils and matrix in the cell wall will change the yield points and hence the ratio of longitudinal to transverse expansion.

In the foregoing we have assumed that the matrix does not yield until the stress exceeds a certain miminum value. This is suggested by the data in Figure 10 of Probine and Preston (1962) and also by the work of Cleland (1959) who has shown that the effect of auxin on the irreversible expansion of *Avena* coleoptiles depends on the osmotic value of the solution, and that plastic extension only occurs when the turgor pressure exceeds some critical value. The fact that there is a critical stress which must be exceeded suggests perhaps that what is involved is the rupture of a certain class of chemical bonds (perhaps hydrogen bonds, multivalent cations linking carboxyl groups, etc.). Until the stress is high enough to break these bonds the wall does not yield. We offer no direct experimental evidence on this point. It is worth noting, however, that if the viscosity of the matrix increases, say, there is no change in the ratio v_1/v_2 and therefore in cell shape. The slope of the flow curve is changed from AC to AC′ and BD to BD′ (in Fig. 7). If the change in the matrix (say, a change in chemical composition) is such as to change its yield stress (P_0), then a change in cell shape will result.

The above treatment is an approximate one. We have not considered the possibility that the frictional constant A may vary with the orientation of the microfibril, either because of the variation in microfibril density, or because the matrix may be anisotropic in its properties. Again, we have not considered the effect which flow in one direction may have on the flow in another. We have also assumed that the stress is borne chiefly by the microfibrils, and in this connection the frictional constant A depends not only on the viscosity of the matrix, but also on the length/diameter ratio of the microfibrils. Microfibrils must necessarily be long, therefore, if they are to influence cell shape in the way we have suggested.

As far as the development of the microfibril pattern and its effect on cell shape is concerned, one may imagine that growth proceeds as follows:

(1) The apical cell is hemispherical and the microfibrils are laid down at random (Green 1958) so that it tends to enlarge uniformly. A single isolated cell with such a structure would enlarge isodiametrically. The apical cell is, however, the terminal cell of a filament of cells so that other factors, such as the cross-wall at its base, will affect its shape.

(2) In the most recently formed internodal cells the laying down of random microfibrils gives way to microfibrils with a preferred orientation, approximately at right angles to the cell axis (Green 1958; Probine and Preston 1961). The new pattern of wall structure presumably modifies the plastic properties of the wall so that it tends to increase in length more rapidly than in diameter. If the microfibril distribution in the new growth remains constant, a steady state is reached so that the overall microfibril

distribution and the ratio $(\Delta L/L) : (\Delta D/D)$ remains substantially constant. A slight asymmetry in microfibril distribution about the transverse axis causes the cell to twist as it grows, and leads to spiral growth (Probine 1963*a*, 1963*b*).

(3) As the cell approaches maturity the viscosity of the matrix increases and the growth rate begins to decrease. About this time the pattern of wall deposition in the new growth changes to an imperfect crossed-fibrillar or random distribution (Green 1958; Probine and Preston 1961; Probine, Dyer, and Jones, unpublished data). As the result of these changes, extension growth ceases, but wall deposition continues for a time.

One asks how the switch from random to oriented microfibrils comes about; what factors determine the orientation in the new growth; what causes the viscosity of the matrix to change; and what causes the switch to a new microfibril distribution as the cell reaches maturity? Ben-Hayyim and Ohad (1965) have recently suggested that the degree of orientation and the pattern of orientation may be due to the presence of different polysaccharides which influence the orientation of the cellulose microfibrils during formation and deposition. Preston (1962) has suggested that a catalytically active protein moves around the cell membrane and causes the microfibrils to be formed in predetermined directions. Ledbetter and Porter (1963) have suggested that the microtubules in the cytoplasm near the plasmalemma may be involved in cellulose orientation. Probine (1963*a*, 1963*b*) has pointed out that the three directions of orientation in *Valonia* may be a basic pattern of microfibril crystallization. In a given type of cell, or at a given stage of development, crystallization occurs in only one of these directions (perhaps for the sort of reason advanced by Ben-Hayyim and Ohad), and if this direction is approximately transverse to the cell axis, it leads to a multi-net structure typical of the primary wall. At a later stage of development, and due to a change in the metabolism of the cell, the pattern of crystallization changes leading to the crossed-fibril type of wall structure. Although the pattern of crystallization depends on the cell metabolism, the orientation of the basic pattern does not occur at random but seems to be determined by the protoplasm—perhaps by the surface properties of the plasmalemma.

At the moment, therefore, there is considerable speculation on the problem of cellulose orientation and the way it comes under genetic control. The aim of this paper, however, has been to indicate how the pattern of microfibril synthesis influences cell development once the pattern is established. We do not believe that growth is merely a question of the mechanics of deformation; but we do believe that the mechanics of wall deformation may be important in understanding the growth process.

VI. Conclusions

(1) It is shown that there is a distribution of microfibrils in the new growth on the inner wall, such that the reorientation processes in multi-net growth lead to an overall distribution which remains constant during extension growth. The distribution of microfibrils in the new growth calculated from the overall distribution agrees reasonably well with the observed distribution in electron micrographs of the inner wall of young cells.

(2) It is shown, by an approximate theoretical treatment, that, by making reasonable assumptions about the properties of the matrix (based on experiment), a mat of microfibrils, having a distribution with angle like that observed in *Nitella*, would lead to longitudinal and transverse plastic extensions in the ratio observed in the growing cell.

(3) It is suggested by the theory that overall distribution of microfibrils in the plane of the wall is the basic cause of anisotropic plastic properties. Since the overall distribution is itself determined by the distribution of microfibrils in the new growth, cell shape is primarily controlled by this variable, and it determines the shape which cells ultimately acquire. Other factors which may influence cell shape would seem to be turgor pressure, the relative proportions of cellulose microfibrils and matrix, and the yield stress of the matrix. The theory suggests that a change in the viscosity of the matrix will change the growth rate but does not influence cell shape.

(4) It is pointed out that there appears to be a certain amount of feedback built into the control of cell shape. A change which may cause the cell shape to change in a particular way is partially offset by a change in microfibril distribution which tends to produce a change in the opposite direction.

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