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CELL-WALL STRUCTURE AS RELATED TO SURFACE GROWTH

SOME SUPPLEMENTARY REMARKS ON MULTINET GROWTH

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Several years ago the author has proposed a theory to explain the microfibrillar texture observed electronmicroscopically in primary cell walls of growing tubular cells (HOUWINK and ROELOFSEN, 1954; ROELOFSEN 1950, 1951; ROELOFSEN and HOUWINK 1951, 1953). It was called multinet growth. This theory supposed that the major part of new microfibrils is continuously deposited transversely on the inside of the wall. As growth proceeds, the unidirectional extension of the wall causes a reorientation of the texture, first into a crossed fibrillar or random structure, finally into a more nearly longitudinal texture. This progressive reorientation does not occur in the inner layer but only in those layers which are displaced outward by the cell wall deposition on the inside. This will ultimately result in a longitudinal arrangement on the outside while the freshly deposited inner side continuously exhibits a more or less transverse orientation. It was also pointed out that such reorientation cannot occur in the whole primary cell wall but only in certain layers of it.

The theory was postulated especially for cells or parts of cells exposed to the atmosphere, viz. plant hairs, fungal hypha and aerenchyma cells.

These are simple cases because the cells are in no way influenced by adjacent cells. Moreover they have comparatively thick walls.

However, also the ultrastructural aspects of the cell walls of many other types of growing tubular cells were later found to be in line with multinet growth, viz. parenchyma cells of roots and coleoptiles, epidermal root cells, some cambium initials, the growing tips of differentiating xylem and phloem elements (BAYLEY, COLVIN, COOPER and MARTIN-SMITH, 1957; MÜHLETHALER, 1950; SETTERFIELD and BAYLEY, 1957; WARDROP, 1954, 1955, 1956; BÖHMER, 1957). Hence multinet growth seems to occur rather universally.

In some cases, however, structures were found that were considered to be at variance with multinet growth. Firstly, in some very thin cell walls fibril reorientation did not occur and since growth was believed to be localized in a number of thin regions or even perforations, which were supposed to change constantly their location, this type was called mosaic growth (FREY-WYSSLING and STECHER, 1951; STECHER, 1952).

A second "abnormal" phenomenon was the fact that on the outer side of growing parenchyma cells of coleoptiles and root tips there

appeared to occur along the cell edges, conspicuous longitudinal thickenings with longitudinal fibrillar structure (MÜHLETHALER, 1950; SETTERFIELD and BAYLEY, 1957; WARDROP, 1955). It was believed that these impeded growth and that these cells lengthened at their tips only (MÜHLETHALER, 1950).

Another abnormal case had been presented already a long time ago by the collenchyma cells of *Heracleum* stems which were known to exhibit longitudinal fibril orientation in all stages of growth in all parts of the cell wall (MAJUMDAR and PRESTON, 1941).

Furthermore in the thick outer walls of epidermis cells of *Avena* coleoptiles the expected transverse inner layer was usually lacking, although it regularly occurred in epidermal cells of the onion root. In the former case the fibrils were longitudinally oriented throughout the whole thickness of the wall and moreover there were indications of cell wall material being deposited in all layers, even underneath the cuticle (BAYLEY, COLVIN, COOPER and MARTIN-SMITH, 1957). Cell wall deposition within the wall was also suggested for young sklerenchyma cells of *Asparagus* shoots (STERLING and SPIT, 1957) and for the growing cell wall of *Nitella* (GREEN and CHAPMAN, 1955).

However, several of the structures, supposed to contradict multinet growth, to our mind are fully in line with it or are variants of it. Therefore it seems appropriate now, to publish what to our mind might be the explanation of the deviations found. In addition some remarks are made which might contribute to a more complete and more universal understanding of the mechanism underlying multinet growth, the conditions which realize it and those which prevent it.

1. *Effect of uniform distribution of wall tension throughout the wall thickness.*

As was remarked already some years ago (ROELOFSEN, 1950, 1951) no major reorientation of fibrils in a particular cell wall layer can occur in lengthening tubular cells so long as this layer takes its full share in counteracting turgor pressure. In that case transversal wall tension in the layer is twice the axial tension (CASTLE, 1937; DIEHL, GORTER, VAN ITERSON and KLEINHOONTE, 1939; VAN ITERSON, 1937) and hence the transversely oriented fibrils are subject to a stress which on the average is considerably higher than the average force which can be exerted on them by fibrils that might change their orientation in a more axial one.

In this way the striking retention of transverse orientation in *Tradescantia* staminal hair cells, which under certain conditions lengthen quickly without cell wall deposition, was explained already in 1937 by VAN ITERSON. Undoubtedly the same phenomenon occurs during the rapid enormous lengthening of grass stamens which also stay negatively birefringent (FREY-WYSSLING and SCHOCH-BODMER, 1938), as also during the lengthening without wall deposition observed by BONNER (1935) with coleoptiles at low temperatures.

This recognition readily explained the universal occurrence of transverse orientation in all growing tubular cells and at the same

time the immediate axial reorientation of the fibrillar structure when these cells were plasmolyzed and subsequently stretched a mere 8 % as was demonstrated by BONNER (1935).

The logical consequence of this reasoning when applied to multinet growth is that those wall layers which during multinet growth do reorient, are no more subject to a predominant transverse tension but to predominant axial tension. If only tensions due to internal pressure are to be considered, this on its turn involves that some other cell wall layer, probably the inner transversely oriented one, has taken over the burden of counteracting turgor pressure or at least of the transverse component of the wall tension due to it.

One thing which follows from this is that if no such taking over occurs, in other words, when all layers in the wall remain under the unequal tension that must occur in the wall as a whole, no one layer will be reoriented even if it is extended considerably and, as demonstrated by the objects mentioned, even in case there is no deposition of new cell wall material in it at all.

This in our view is what is likely to happen in very thin cell walls with so-called mosaic growth. Here there is no question of wall layers which are displaced outwardly since the cell wall material is deposited throughout the thickness of these tenuous walls. The wall consists so to say of the innermost layer of the multinet model. We see no reason to suppose why growth should be restricted to the loose areas and perforations found by those who coined the same mosaic growth, nor is there any evidence that these change their location. Part of them look like artefacts due to the preparation and there are also primary pit fields among them, which of course are stationary. Autoradiographs of growing cells did not confirm the supposition that the pit fields are regions of growth, but indicate an even distribution of growth (SETTERFIELD and BAYLEY, 1957; WARDROP, 1956), as is in line with multinet growth. Hence we do not advocate the term mosaic growth. It implies a hypothesis which does not seem necessary and was not made likely. Perhaps the term "thin-wall type of growth" will do better.

Uniform distribution of wall tension throughout the thickness of the wall, might also explain the ultrastructure of the thick, but presumably chemically different walls of *Nitella*, which according to GREEN and CHAPMAN (1955) have a transverse structure throughout the whole thickness. We are inclined to consider also this type as a variant of multinet growth since the senior author informed us recently that new cell wall material is deposited on the inner side only. Certainly it is not at variance with typical multinet growth occurring elsewhere. Evidently, the observed ultrastructure of the *Nitella* cell wall is no reason to accept with Green and Chapman that cell wall material must be deposited within the wall during growth. This assumption is superfluous if all layers are supposed to be subject to the described differential tension.

Of course the fact that another explanation is possible does not disprove the original explanation of these authors, but we wish to

remark that more conditions than just cell wall deposition in all wall layers must be fulfilled to retain transverse orientation in any layer which is lengthening but is not under differential tension. All adhaesion points between the fibrils of such a layer must be loosened and the deposition of cell wall material must exactly compensate lenghtening, so that the layer retains its thickness. Otherwise reorientation will still occur. Hence, the explanation of the authors requires several assumptions.

2. *Deviations due to additional wall tensions.*

Another logical consequence of the described influence of the wall tension, also pointed out by VAN ITERSON (DIEHL *et al.*, 1939) is that if a cell wall is subject to some tension in addition to that caused by internal pressure, this will influence its ultrastructure.

It is known e.g. that like many other epidermi, the outer epidermal wall in *Avena* coleoptiles is subject to longitudinal tension, exerted by the parenchyma; in fact the epidermis inhibits coleoptile growth. Possibly there is also additional transversal tension in this wall, but if the resultant axial tension in the outer cell wall would be greater than the resultant transversal tension, this would imply that all layers in this wall, included the innermost one, will be axially oriented, independent of the direction the new fibrils have when they are deposited onto the inner side. That is what in fact has been observed in this particular epidermal wall. Of course conditions might be different in other epidermal walls like that of onion root, which show the ideal multinet type of ultrastructure. This clearly depends on the magnitude and the distribution of the tissue tensions.

Since cells within growing tissues may be subject to all kinds of wall tensions in addition to that caused by internal pressure, all kinds of deviations of the ideal model of multinet growth and of the "thin-wall type" of structure may be expected.

The collenchyma cells of *Heracleum* for instance which, as remarked, have predominant longitudinal structure in all stages of growth, might be subject to a continuous longitudinal tension. But in principle this structure might also be the result of elimination, or at least reduction, of the transverse wall tension by the internal pressure of adjacent cells. We believe that the latter possibility is less likely because in meristematic tissues surrounded by an extensible epidermis, it is not very probable that the mutual pressure of the young thin walled cells will eliminate the normal differential tension in the cell walls. The predominance of transverse structures in both meristematic cells and *e.g.* young hairs, in fact indicates that similar conditions prevail in both.

However, such cells as cambium initials in tree trunks might very well be subject to considerable unidirectional tensions. To our mind the finding of a typical multinet type of wall structure in at least one case (WARDROP, 1954) is more a matter of surprise than of anticipation, especially if one realizes that these cells mainly lengthen at their tips

and that their radial walls broaden. It is not surprising that different types of "abnormal" structures have been found in cambial tissues (BOSSHARD, 1952) as well as perhaps the "thin-wall type" (SVENSSON, 1956).

Another case in which quite abnormal wall tensions may be expected, is the growing tip of cells that lengthen by intrusive growth, such as phloem fibres, wood fibres and tracheids. The tips presumably dissolve or split the middle lamella between adjacent cells and pushing these further apart, grow into the conical space formed. One cannot foresee what will occur with wall tension here. Possibly the result will be different in different cases. What seems a multinet type of structure was found in the tips of some special parenchyma cells, of phloem cells and perhaps of xylem cells of *Avena* coleoptiles (MÜHLETHALER, 1950). In tips of differentiating wood fibres and tracheids a very conspicuous axial structure seems to occur at least on the outer face (WARDROP, 1954), but it is not known what structure occurs at the inner side.

Once it was stated (FREY-WYSSLING, 1953; MÜHLETHALER, 1950) that growing tips are perforated and that the protoplasm protruded, but evidence was lacking. There is no reason to suppose that there is any essential difference in the mechanism of surface growth at a tip as compared with another enlarging cell wall area, although the effect of surface growth on the orientation of the microfibrils in the tip is more variable than with a flat area (HOUWINK and ROELOFSEN, 1954; DE WOLFF and HOUWINK, 1954).

In our opinion the ultrastructure of the outer surfaces of growing cortical fibres of *Asparagus* (STERLING and SPIT, 1957) is typical of the "thin-wall type" in the initials and of the multinet type in older cells. It might be that the frequent absence of the anticipated transverse structure on the inner side of the wall is caused by additional axial wall tension. On the basis of the evidence presented it does not appear necessary to accept the more exceptional mechanism of growth suggested by Sterling, which involves absence of adhaesion points between the cellulose fibrils and unexpected mechanical properties of the non-cellulosic wal constituents.

Finally we remark that since edge thickenings of thin tubular cells will bear the greater part of the axial wall tension, the axial fibrillar structure throughout their thickness, except in their innermost layer, is to be expected. Even if in some cases also the inner surface would exhibit axial fibrillar structure this would be conceivable.

3. *Behaviour of microfibrils during cell lengthening*

That microfibrils in growing primary walls do reorient is not merely indicated by what seems to happen during multinet growth, but has actually been demonstrated in two cases (HOUWINK and ROELOFSEN, 1954). Since the cell diameter did not diminish the observed reorientation implied slipping of fibrils past one another. This phenomenon must also be accepted to occur during lengthening of structures with either transverse or longitudinal orientation.

It seems appropriate to realize in some more detail what will occur with the microfibrils during extension and reorientation of the texture, since the latest conceptions on these points are fifteen to twenty years old and were based on fringe micelles being the building stones of the wall. In the meantime electron micrographs have shown that cellulose consists of very long individual microfibrils of uniform thickness, which essentially are bundles of partly crystallized chain molecules. These microfibrils may aggregate on their part to bundles, but never anastomose. So there are no true junctions in them but only points and regions of adhaesion where there is contact between overcrossing or parallel microfibrils. Most likely, the adhaesion is due to hydrogen bridges between hydroxyl groups of the superficial cellulose molecules of the microfibrils (probably hemicellulose may act as intermediate).

Let us imagine a mesh of cellulose or chitin microfibrils which are predominantly oriented transversely in the primary wall of a tubular cell. A minor part of the fibrils runs more or less obliquely, overcrossing the other fibrils. On the average about 10 % of the space is occupied by cellulose; the mean fibril distance is three times their thickness (PRESTON and WARDROP, 1949). Evidently, the fibrils are embedded in a mass of non-cellulosic material, which is known to be amorphous.

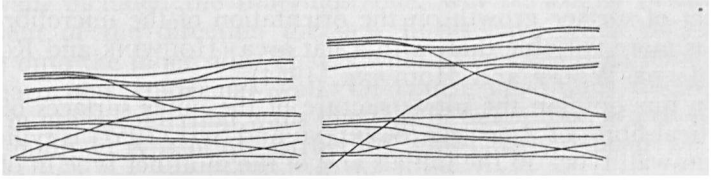

Fig. 1. Scheme of the axial extension of a predominantly transverse fibrillar structure.

This will reduce the points of contact between the cellulose fibrils considerably, but they are very long and in fact there are many phenomena which leave no doubt that there must be many points and regions of adhaesion between the fibrils. Locally they even form part of bundles of fibrils or are twisted around another fibril. This can be seen on any good electron-micrograph but it must be remembered that on these the structure appears much denser than it is in reality.

Those fibrils which run more or less parallel and hence usually transversely, have many and long adhaesion regions, will be fixed relatively strongly and are continuously subject to a tension along their axis, which constitutes the transversal tension due to turgor pressure. We suppose for the moment that there are no other layers.

Those fibrils which run more or less obliquely, either over their full length or partly so, will overcross the transverse fibrils and will also have many adhaesion regions, but relatively short and weak ones and hence these fibrils will be fixed much less strongly than the trans-

verse ones. If this is the sole wall layer, they too have to bear a tension, the sum of the axial components of which constitutes the axial tension in the wall, known to be half the transversal one.

Now we suppose that the cell is lengthening without deposition of wall material, as has been actually observed in the cases mentioned in section 1. This means that the turgor pressure produces a higher axial wall tension than the more or less obliquely oriented fibrils can bear. The axial wall extension involves a widening of the mesh and a loosening of the adhaesion points along the greatest part of the obliquely oriented fibrils, which will start to slip past the transversely oriented ones. Most of the latter are fixed much stronger, so that they can never be disconnected by the pulling force of any obliquely oriented fibril. Neither can there be any question of pulling the whole transversely oriented mesh out of its direction, for this would imply that the axial wall tension would be higher than the transversal one, which is impossible.

The conclusion is that during lengthening as a result of turgor pressure the predominant transverse orientation will theoretically be retained infinitely, the layer thinning out progressively, the mean distance between the transversely oriented "rings" of fibrils becoming greater and the mean fibril tension higher. Before the cell bursts along an axial split, equilibrium is usually reached because a progressively higher axial tension can be borne by the increasing number of loosened fibrils, the more so since these acquire a more nearly axial position. Perhaps the non cellulose part of the wall also takes its share of the wall tension.

Usually there will be one or more other layers, situated more peripherically, with more nearly axial structures, which will more easily and sooner bear the axial tension than the inner one. As was made clear already, the fibrillar texture in these must have been reoriented as a whole at the moment the axial tension in these particular layers exceeded the transversal one. A correspondingly greater part of the total transversal tension in the wall must have been taken over by the inner layer. Here transverse wall tension will be more than twice the axial tension, which provides additional assurance for the retainment of its transverse structure during lengthening.

We suppose that in cell walls with multinet structure, the fibrillar "rings" in the layers which are displaced outward loose their tension because the adhaesion regions between the fibrils are weakened progressively by the action of non-cellulosic materials. Probably this does not occur in *Nitella*.

It is readily seen that if the turgor pressure would be removed, even very small axial tensions will extent the wall and will soon reorient the whole transverse mesh. However, this can only occur under artificial conditions.

4. *Is surface growth intussusception or apposition?*

In the preceeding section the deposition of cell wall material was left out of consideration. In principle the mechanism of extension is

not changed by simultaneous fibril deposition. New fibrils are inserted in transverse position in the extending mesh on the inner surface of the wall and will soon be fixed onto existing ones.

The question whether this is intussusception or apposition is not purely academic which appears from the following example. A particular experimental result with *Avena* coleoptiles might be taken to indicate that growth was dependent on and brought about by intussusception of wall material, but this, so the authors reasoned, was unlikely since the wall showed a multinet type of structure and hence new wall material is added by apposition. Therefore another explanation for the observed relation between growth and wall deposition was sought.

It is clear that with multinet growth the overall result of cell wall deposition as a matter of fact is apposition; the innermost layer is the youngest one and more nearly peripherical layers are progressively older. However, the process of microfibril deposition occurring in the inner layer to our mind is not only apposition but certainly partly intussusception. If one realizes that only 10 % of the space is occupied by microfibrils, new fibrils which are laid down, will often find themselves lying between previously deposited ones, which is intussusception. Elsewhere the new fibril overcrosses older fibrils; this is apposition. Hence it might very well be that growth of a cell wall showing multinet structure under certain conditions is dependent on deposition of cellulose. Also the deposition of non-cellulose material between the fibrils seems partly intussusception, partly apposition.

5. *Dislocation of cell wall material due to turgor pressure.*

So far the constitution of growing cell walls has been calculated on the basis of mere suppositions concerning their water content. These varied between 66 and 92 % on the same object by the same author. Own analyses of disintegrated and washed maize mesocotile cell walls, which had been freed of adhering water, had the following result: water 60 %, hemicelluloses and pectin 20 %, cellulose 12 %, protein 3 % and lipids 2 %. Hence it is clear that the main part of the growing cell wall is a noncellulosic amorphous matrix.

Considering firstly, that it is safe to accept that the cellulose fibrils are the only elements with tensile strength and secondly that, based on the figures mentioned, the non-cellulose contains 72 % water (water in cellulose is negligible), the supposition that this matrix of non-cellulose is soft and plastic, seems likely.

Considering furthermore that the cellulose fibrils apparently may be loosened locally and may slip past one another, it is also likely that if a growing cell wall of e.g. a parenchyma cell, is subjected to a local pressure, normal to its surface, the non-cellulose matrix together with loose fibrils or fibril bundles, will start to flow within the plane of the wall.

In turgid meristematic tissues the cell walls are, as a matter of fact, subject to such pressures and hence the question arises whether there are structural aspects indicating such dislocations.

A turgid parenchymatic tissue may be compared, in fact has often been compared, with a foam. In the walls of both cells and air bubbles, the pressure in question is greatest on the faces and least in the edges since polyhedral units with internal pressure tend to become globular. Hence in foams the liquid is squeezed from the faces towards the edges and runs off through these.

We believe that the conspicuous cell edge thickenings as observed in growing parenchymatic cells are the result of such dislocations of the non-cellulosic matrix together with those fibrils which are fixed insufficiently to withstand the forces exerted by the flow of the plastic matrix. In a tubular parenchymatic cell with multinet structure these fibrils are obviously to be found in the outermost layers since those of the inner layer are well fixed (see previous sections). Moreover these are nearest to the middle lamella which is the layer richest in non-cellulose and hence is most subject to flow. Thirdly their orientation is more nearly normal to the direction of flow, which involves that they are subjected to greater forces than any other fibrils.

The structure of the edge thickenings as revealed in electron micrographs (BAYLEY, COLVIN, COOPER and MARTIN-SMITH, 1957; MÜHLETHALER, 1950) is fully in line with this concept of the accumulation of the outermost fibrils of the cell faces in them. It explains both the thickness of these edges and their longitudinal fibrillar structure, although it must be realized that cell extension will further orient the fibrils once these have nestled themselves in the thickenings. The separation of the thickening from the cell center by a transversely oriented layer now is also clear.

Once the thickenings were considered to be secondary cell wall depositions, but on the contrary, they contain the most primary microfibrils of the primary wall. These will be localized in the center of the thickenings, their edges containing younger fibrils, for it is evident that the accumulation process will continue as long as cell wall material, comprising both cellulose and non-cellulose, is deposited on the cell faces.

One of the conditions determining the rate of flow of cell wall material to the cell edges will obviously be the pressure gradient. This will increase as soon as the intercellular spaces are filled with air. We do not know whether this occurs during growth. In any case, the outer cell edges of epidermal cells abut on the atmosphere from the very beginning and hence these edges may be expected to be considerably thicker than any other ones. Also because they probably will accumulate the loose fibrils from more than half of the surface of the radial walls of the epidermal cells, since the pressure within the inner cell edge will certainly be higher than in the atmosphere so long as there is no intercellular space.

Probably this accumulation process accounts both for the considerable thickness of the outer epidermal wall as compared with other walls in the growing organs studied so far and for the increase in thickness during cell extension of all its layers as was observed by BAYLEY et al. (1957) in *Avena* coleoptiles. This would eliminate the

necessity of assuming that cell wall material is being deposited within these layers during lengthening. It would also explain why, as has been remarked (SETTERFIELD and BAYLEY, 1957), the autoradiographs of epidermal cells supplied with isotope, did not reveal as great an accumulation of isotope in the outer wall as was expected; the accumulated fibrils are old ones.

Finally it should be remarked that the accumulation process removes the outermost fibrils of a cell wall and therefore tends to obliterate the traces of multinet growth, so that we arrive at the perhaps somewhat discouraging conclusion that even typical multinet growth might be invisible. This actually seems to occur in radial epidermal walls.

The theory rises the question how the dislocating fibrils pass the plasmodesmata. Are the latter disconnected and regenerated easily, or are the fibrils pulled around the primary pit fields? Certain electronmicrographs clearly suggest the latter process, which in the author's opinion is a support for the theory, but all the same it still remains a hypothesis.

Naturally the concept is compatible with the possibility that edge thickenings and thick outer epidermal walls may partly arise from another process, viz. a locally increased synthetic activity of the protoplasm. This actually applies for outer epidermal walls, as could be deduced from autoradiographs (WARDROP, 1956; GORHAM and COLVIN, 1957; SETTERFIELD and BAYLEY, 1957; BÖHMER, 1957).

6. *A hypothesis on the biosynthesis of cellulose microfibrils.*

The mechanism of synthesis of cellulose molecules is still unknown, although it is likely that some hexosephosphate containing compound is the immediate presursor (SCHRAMM, GROMET and HESTRIN, 1957).

Electronmicrographs of the formation of microfibrils in young cultures of *Acetobacter xylinum* showed that they appear only outside of the cell wall proper. Originally it seemed as if they originated by aggregation or coagulation within an amorphous mass, supposed to contain cellulose-molecules (MÜHLETHALER, 1949) but in a recent investigation (COLVIN, BAYLEY and BEER, 1957) the amorphous mass could not be detected; the fibrils appeared without any preliminary stage.

To our mind the former process of formation as a matter of fact is very unlikely. Firstly because in coagulating cellulose solutions smooth individualized fibrils of constant thickness have never been observed, there always appear anastomosing threads of varying thickness. Secondly because native cellulose has another crystalline structure (cellulose I) than coagulated cellulose (cellulose II).

Hence it is likely that the protoplasm or at least an enzyme plays a role in the formation of the microfibrils. It is difficult and to our mind unattractive to imagine either ditchlike templates in the outer protoplasm for moulding cellulose or a kind of contractile vacuole with one or more pores spouting out the fibrils. Moreover the cellulose

fibrils in cultures of *Acetobacter xylinum* are produced without any contact with the protoplasm. Therefore we suggest the hypothesis that an enzyme is responsible for the formation of the microfibrils.

If there would be two enzymes, one polymerizing glucose from the precursor to cellulose molecules, the other coagulating the molecules in a special way, it would still be difficult to understand why the fibrils never anastomose, not even when produced by a homogenate of cellulose synthesizing bacteria in which presumably the two enzymes, if previously occurring in some structural unit, will be more or less separated. One would expect in that case a mixture of fibrils with coagulated cellulose, but instead only completely normal fibrils were found (COLVIN, 1957).

Therefore we suppose that there is one enzyme which synthesizes at the same time the molecules and the microfibrils. We imagine the enzyme molecule or possibly some of them, being located on the top of a micro-fibril and attaching the glucose from the precursor to the molecule ends protruding at the tip.

Recently it was indeed found that the mean fibril length in young cultures of *Acetobacter xylinum* increased with time (COLVIN *et al.*, 1957). This is no proof of tipgrowth but fully in line with it. Another indication is that such a mechanism would at the same time explain two points.

Firstly why microfibrils do not anastomose and do not vary much in thickness. The enzyme molecule(s) being adsorbed onto the tip, might have a restricted mobility which need not imply that the fibril must have a circular diameter (there are indications of flatness).

Secondly such a mechanism might perhaps explain why only a cellulose I crystal structure is formed and no cellulose II. Here we have short protruding ends of cellulose molecules which enable the glucose monomers to arrange themselves one by one into the crystal lattice, a process which is quite different from the rapid coagulation of long molecules from a supersaturated solution or from swollen amorphous cellulose, which at normal temperature always gives rise to cellulose II, even if the molecules are previously oriented as occurs when spinning rayon fibres and when native cellulose fibres are mercerized.

A consequence of this hypothesis is that in the crystal lattice of cellulose the molecules cannot alternate in direction as is evident for coagulated cellulose and as is commonly also accepted for cellulose I. All molecules in one fibril must be oriented in the same direction, viz. with their non-reducing ends towards its growing tip. Otherwise two enzymes must be active on the tip and there must be two precursors. The one enzyme hypothesis involves that the microfibril can only grow at one tip and since so far all polysaccharides appear to be synthesized at the non-reducing molecule end, we accept this for cellulose too. The X-ray evidence on cellulose is not at variance with this requirement of our hypothesis (VAN DER WIJK and MEYER, 1947). Only, we must presume that the fibrils in native fibres alternate in the direction of their reducing tips, otherwise the formation of cellulose II during mercerization would not be clear.

It seems to us that also some structural aspects of the cell wall such as the intertwining of the fibrils can be more easily understood on the basis of tipgrowth of fibrils. Perhaps it will once be possible with electron microscopes with very high resolving power, to verify whether in cellulose-synthesizing preparations one of the tips of the fibrils is indeed protein covered.

It should be remarked that in some organisms amorphous cellulose and in others cellulose II seems to occur (KREGER (in press); MYERS, PRESTON and RIPLEY, 1956; NICOLAI and PRESTON, 1952). Other enzymes might be active in these cases. Without the hypothesis of fibril forming enzymes these differences would be greater mysteries.

7. *Mechanism of spiral growth*

The conception on the mechanism of extension of a fibrillar structure normal to the predominant fibrillar orientation, as discussed in section 3, enables us to propose a hypothesis on the mechanism of spiral growth which is more in line with facts known at this moment than the theory proposed some years ago by the author (ROELOFSEN, 1950; ROELOFSEN and HOUWINK, 1951).

The spiral growth of *Phycomyces* sporangiophores and of *Tradescantia* staminal hairs was supposed to be due to the extension of a fibrillar texture having an average orientation according to a helix with a pitch about equal to the angle between the cell axis and the direction of growth.

Although in *Tradescantia* hairs the presence of this supposed helical structure was conclusively proven, it was not so with *Phycomyces*. Here polarization microscopy revealed a predominant transverse structure in most cases. In *Nitella* cells, which also exhibit spiral growth, such a structure was even found in all cases (GREEN and CHAPMAN, 1955). However, since at least the sporangiophores twist when the internal pressure is changed, there must be some kind of helical structure in the wall, which apparently is most often not noticeable.

We believe that the figure presented in section 3 may explain this paradox.

If there would occur a little more fibrils overcrossing the transverse fibrillar "rings" in one oblique direction than in the other one, this would cause a slight mutual shift between every two "rings" when these separate during extension of the wall and the summation of a huge number of similar oriented mutual shifts, might result in the twist observed during growth and when changing the internal pressure. Still, the *mean* fibril orientation might deviate so little from the transverse that it might be indetectable, but this need not be always the case, so that the oblique orientation as observed with *Tradescantia* would be conceivable as a quantitative difference only.

If this would be the true mechanism of spiral growth, the next question would be why one of the directions of overcrossing fibrils would come to predominate. A preferred clockwise or anticlockwise direction of growth of the fibrils (see section 6), combined with proto-

plasmic flow towards the growing apex, which would swing part of them while anchored at one end, into an oblique orientation (see ROELOFSEN, 1950), might be the answer. Of course the next question then would be about the reason for the preferred direction of fibril growth and this looking back for causation would continue.

REFERENCES

BAYLEY, S. T., J. R. COLVIN, F. P. COOPER and C. A. MARTIN-SMITH. 1957. J. Biophys. Bioch. Cyt. *3*:171.
BÖHMER, H., 1957, Planta *50*:461.
BONNER, J. 1935. Jahrb. wiss. Bot. *82*:377.
BOSSHARD, H. H. 1952. Ber. Schw. Bot. Ges. *62*:482.
CASTLE, E. S. 1937. J. Cell. a. Comp. Physiol. *10*:113.
COLVIN, J. R. 1957. Arch. o. Bioch. a. Biophys. *70*:294.
COLVIN, J. R., S. T. BAYLEY and M. BEER. 1957. Bioch. Bioph. Acta *23*:652.
DIEHL, J. M., C. J. GORTER, G. VAN ITERSON jr. and A. KLEINHOONTE. 1939. Rec. trav. bot. Neerl. *36*:711.
FREY-WYSSLING, A. 1953. Submicroscopic morphology of protoplasm, Elsevier Publ. Comp.
FREY-WYSSLING, A. and H. SCHOCH-BODMER. 1938. Planta *28*:257.
FREY-WYSSLING, A. and H. STECHER. 1951. Experientia *12*:420.
GORHAM, P. R. and J. R. COLVIN. 1957. Exper. Cell Res. *13*:187.
GREEN, P. B. and B. CHAPMAN. 1955. Am. J. o. Bot. *42*:685.
HOUWINK, A. L. and P. A. ROELOFSEN. 1954. Acta botanica Neerl. *3*:385.
ITERSON jr., G. VAN. 1937. Protoplasma *27*:190
MAJUMDAR, G. P. and R. D. PRESTON. 1941. Proc. Roy. Soc. Lond. *B130*:201.
MÜHLETHALER, K. 1949. Bioch. Bioph. Acta *3*:527.
MÜHLETHALER, K. 1950. Ber. Schw. Bot. Ges. *60*:614.
MYERS, A., R. D. PRESTON and G. W. RIPLEY. 1956. Proc. Roy. Soc. Lond. *B144*:440
NICOLAI, E. and R. D. PRESTON, 1952. Proc. Roy. Soc. Lond. *B140*:244.
PRESTON, R. D. and A. B. WARDROP. 1949. Bioch. Bioph. Acta *3*:549.
ROELOFSEN, P. A. 1950. Rec. trav. bot. Neerl. *42*:73.
ROELOFSEN, P. A. 1951. Bioch. Bioph. Acta *7*:43.
ROELOFSEN, P. A. and A. L. HOUWINK. 1951. Protoplasma *40*:1.
ROELOFSEN, P. A. and A. L. HOUWINK. 1953. Acta bot. Neerl. *2*:218.
SCHRAMM, M., Z. GROMET and S. HESTRIN. 1957. Bioch. J. *67*:669.
SETTERFIELD, G. and S. T. BAYLEY. 1957. Can. J. Bot. *35*:435.
STECHER, H. 1952. Mikroskopie *7*:30.
STERLING, C. and B. J. SPIT. 1957. Am. J. o. Bot. *44*:851.
SVENSSON, A. A. 1956. Arkiv för Kemi *10*:239.
WARDROP, A. B. 1954. Austr. J. o. Bot. *2*:165.
WARDROP, A. B. 1955. Austr. J. o. Bot. *3*:137.
WARDROP, A. B. 1956. Austr. J. o. Bot. *4*:193.
WIJK, A. J. A. VAN DER and K. H. MEYER. 1947. J. Polymer. Sc. *2*:583.
WOLFF, P. M. DE and A. L. HOUWINK. 1954. Acta bot. Neerl. *3*:396.