The Structure of the Wall of the Green Alga
*Valonia ventricosa*

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[Introduction]

Although for many years the study of cytology has tended to concentrate attention more and more on the protoplast as the fundamental unit of the plant, there can be no doubt that the membrane surrounding this unit plays a part of considerable importance in its life processes. The deposition of such a membrane, by a process which is as yet quite obscure, is obviously closely connected with protoplasmic activity, and a detailed investigation of its structure is bound to lead to a better understanding of this connexion. At the same time, the shape and size of a cell are clearly due in some degree to the action of forces external and internal on the membrane, so that a study of the structure of the plant cell wall should therefore also yield information of considerable importance in the solution of botanical problems concerned with cell elongation and growth. Comparatively recent investigations, carried out chiefly on plant fibres, have shown that the most important component of cell walls, from a structural point of view, is the polysaccharide cellulose. This substance is known to occur in varying proportions in the walls of almost all plant tissue and its structure has been worked out, chiefly by X-ray and chemical methods, with some degree of certainty. Although much remains to be discovered of the organization of cellulose in the wall, certain details are now quite clear. Celluloses obtained from many and varied plant sources have all proved to have essentially the same structure. They exist only in the form of chains of \( \beta \)-glucose residues, at least 500 Å long (Hengstenberg and Mark 1928), bound together laterally by secondary valences to form a three-dimensional lattice. The conception of a definite micelle, in the sense of Nägeli, is no longer widely held, although the lattice is not uniformly regular throughout the wall. The chains of cellulose are more probably bound together into ill-defined bundles separated by regions in which they are not so perfectly oriented.
The Wall of Valonia ventricosa

This conception of the existence of cellulose in long molecular chains has arisen from the examination of the secondary walls of plants, but as yet no direct experimental determinations have been possible of its structure in primary walls where it is known to occur (e.g. in Vicia faba, see Tupper-Carey and Priestley 1922). Recent work (Preston 1934) on the tracheids of the conifer, however, show that it is possible to carry over the idea of the long-chain structure of cellulose even to these delicate primary walls.

This widespread distribution of cellulose with essentially the same structure makes it possible to generalize results obtained on the wall of one type of cell to cover that of many other types, and it is from this point of view that the work described below will be of interest to botanists. It is possible to make observations on the large cells of Valonia which imperfections of technique make impossible with the minute cells of the higher plant. Moreover, accurate observation of the structure of the whole wall, which can be made only on large cells such as this alga affords, will probably yield results with an important bearing on the problems involved in the deposition of the cellulose wall at the protoplasmic surface.

Valonia is a member of the Siphonales (Fritsch 1935) characterized by its bubble-like cells, which in some species may be two or three centimetres long and which are found in the warmer seas, sometimes in apparently irregular clusters and sometimes in the form of neat palisades. Of the three species used in this investigation, V. ventricosa and V. macrophysa form usually spherical or pear-shaped cells which in the former generally occur singly, being larger than the proliferating cells of V. macrophysa; while V. utricularis proliferates freely giving close clusters of cells which are relatively smaller and frequently somewhat elongated. The bulk of the work to be described below has been carried out on V. ventricosa, although sufficient observations have been made on the other two species to show that their cell-wall structure is essentially similar.

A brief account of the morphology of the alga Valonia has been given in the first paper of this series (Astbury, Marwick and Bernal 1932), but it becomes necessary here to enlarge upon this outline. The following summary is based on Oltmanns (1922) and Fritsch (1935), to whom reference may be made for further details. The cell may be imagined as a large bubble, often approximately spherical, with a large vacuole and a thin lining of protoplasm. Imbedded in this lining are to be found numerous nuclei, while further away against the cell wall occur the chromatophores, in the form of plates of irregular outline often united to form a network and frequently containing pyrenoids. The protoplasmic lining is in turn completely surrounded by a comparatively thick wall, consisting chiefly of cellulose.
Valonia is a coenocytic organism with the protoplast contained in large vesicular cells, but minute cells are often formed as a result of accumulations of protoplasm in certain regions of the surface. A strongly curved wall, shaped like a watch-glass, is formed round such protoplasmic masses giving a cell with a characteristic appearance. This process gives two kinds of cells, larger ones which appear in the upper part of the cell, and smaller ones which occur particularly at the base. The latter grow out into short, lobed, but single-celled structures which form the holdfasts, while the larger cells on the upper part grow out into new bubble-like structures which resemble the parent in every way, including the power of forming new cells.

The reproduction of cells by the formation of zoospores has been closely observed and described by several investigators. The propagation of zoospores is made obvious several days before their ejection by the various localized changes occurring in the wall and protoplasm of the mother cell (Kuckuck 1902). The fertile plasma is not separated from the rest of the plasma by a wall as it is, for example, in Vaucheria, Bryopsis, etc., but the vacuole is in direct communication with the outside environment at the time of spore ejection. Previous to ejection, the wall is completely pierced, the opening being subsequently closed up and the mother cell regaining its original condition. There seems to be no evidence that this spore formation takes place under any particular area of wall. Certainly in the present research no disturbances in wall structure have been found such as one would expect a priori from such openings in the membrane. Either the cell under investigation had never produced zoospores or the perforations are closed up in such a way as to leave no trace of their existence.

In the course of the work to be described below it has become clear that the wall structure of the cells of Valonia is strikingly similar to that of the fibres of the higher plant. The wall is laid down in microscopically visible layers, which may be as many as thirty or forty in number, and the crossed cellulose chains previously described are found, as a result of taking numerous X-ray photographs of the same cell, to be portions of two complete sets of chains traversing the whole wall surface. Of these two sets one forms a left-hand spiral round the cell, while the other takes the form of meridians running from one pole of the spiral to the other. At the two poles of the spiral, therefore, the typical X-ray photograph of Valonia is no longer obtained, being replaced by a Debye-Scherrer ring diagram. Moreover, these two sets of chains correspond to the microscopically visible striations in the wall and occur in separate layers rather than in the same layer. The existence of the striations in different layers had been already
The Wall of Valonia ventricosa

indicated in the work of Correns (1892) and it has been verified during the present research. The view put forward by Sponsler (1931) that the chains are definitely oriented about their axis, with the planes of 6·1 A spacing always parallel to the wall, has been shown to be only roughly true. The chains do tend to lie in this position, but there is a considerable dispersion.

Apart from the disturbances due to the poles of the spiral, the only breaks in the regularity of the wall structure occur at well-defined places where “watch-glass” cells have been formed. At the holdfasts and at the scar left by the falling off of a bud cell the wall undergoes some modification. The region of the holdfasts show a series of raised circular rims some $\frac{1}{10}$ mm. diameter which present a crater-like appearance under the microscope when illuminated parallel to the wall surface. The two sets of striations on the wall inside each rim are continuous with those outside it, although a piece of wall containing a rim gives a Debye-Scherrer ring diagram in the X-ray microcamera (see Preston 1934). Bud scars have a similar appearance, though on a much larger scale.

**Striations and Wall Layers**

Although in the first paper of this series a conclusive demonstration was given of the correspondence between the directions of the striations and those of the cellulose chains, it may not be out of place here to enlarge upon this point. The results presented in this connexion will serve in particular to indicate the order of precision in the various interrelations between the cellulose chains, extinction positions, and striations.

Observations have been made on the striations on many pieces of Valonia wall taken from several cells, and in only one case was there observed an obvious discontinuity in direction unaccounted for by a fold in the wall (special reference to this exceptional specimen will be made later). The constancy in direction of the striations can readily be observed under the microscope in any piece of wall, although each striation is not equally visible at all points, and this necessarily implies that the angle between the striations must also be constant. While separate specimens have been obtained with interstriation angles varying from 60° to 80° or more, the angle in any one specimen varies in a strikingly gradual fashion. This will be clear from Table I, which gives a series of readings on a single small piece of Valonia wall. The results of many measurements, of which Table I is a representative sample, indicate that in general the striations travel in lines which are to a close approximation straight over a distance of several
millimetres. In particular, the area covered by the slit of the X-ray spectrometer (diameter 0·5 mm.) is uniform in this respect.

Table I

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This remarkably uniform nature of the striations alone would indicate that they are reflections of inner structural details of the wall, but a series of observations was carried out on the X-ray spectrometer and under the microscope in order to demonstrate the correlation still more completely. A method was used similar to the demonstration given in the previous paper. Small areas, about the size covered by the spectrometer slit, were marked out on a piece of wall and the directions of the cellulose chains were determined by the X-ray method. These were plotted on paper together with the corresponding striations and, wherever possible, the major extinction position. In some cases the region examined consisted of “mosaic” areas (Preston 1931) too small to allow the determination of a representative extinction position; and in others one of the two sets of striations was too indistinct for exact determination of its direction. In spite of this, a sufficiently large number of observations was made to show conclusively that the sets of striations are parallel to the cellulose chains, and that the major extinction position lies in the acute angle between them. It is true that in some cases there is a discrepancy of a few degrees, but we cannot expect exact agreement every time owing to the frequent indistinctness of one set of striations. Bearing this point in mind, the correspondence between the directions of cellulose chains and striations is found to be extremely close. Typical results are presented in figs. 1 a, b, from which several further conclusions may be drawn. In every case, the more easily visible set of striations corresponds to the set of cellulose chains giving the more intense diffraction spots. The striations not only indicate the directions of the two sets of cellulose chains, but they afford also a qualitative measure of their relative importance. Again, the figure shows conclusively that the major extinction position lies in the acute angle between the cellulose chains, and always closer to the more important set. This, of course, is what we should expect from the multi-ply structure.
The Wall of Valonia ventricosa

described below, a structure which is in effect a series of superposed birefringent plates with extinction positions not coincident. Attention may be drawn specially to fig. 1b, representing a set of observations on neighbouring areas of a piece of the wall. In area A the major extinction position lies about 15° to the left of the more important set of chains, while in C,

![Diagram A](image1)

![Diagram B](image2)

3 mm. away, it lies some 30° to the right. B, on the other hand, represents the only observed specimen in which an abrupt change occurred in the direction of the striations. At this point there existed a definite boundary between two areas, each with its own striations. From A to the "frontier" the striations behaved normally; but in this region they changed over abruptly to those in C, and the extinction position altered simultaneously in

![Diagram C](image3)

![Diagram D](image4)

Vol. CXXII—B.
Fig. 1b—Directions of cellulose chains, striations, and major extinction positions at various points on a single piece of Valonia wall (see key diagram). At B, the "frontier" region (see text), the two sets of striations and major extinction positions are drawn separately for clearness; at D and E the limits of variation of the major extinction position are as indicated. Cellulose chains ———; striations ———; major extinction ˙.; more important sets — o ——— — o ——— — o ——— — o ———.
a corresponding manner. The change in the direction of the striations corresponds to a similar change in that of the cellulose chains, and there can be no doubt that the direction of the extinction position at any point is determined partially by the direction of the chains.

Areas D and E are no less interesting from another point of view. Although each piece consisted of mosaic areas much smaller than the area included in the X-ray beam, and the major extinction position varied over a considerable angle from one mosaic area to the next, only the usual two sets of cellulose chains and striations could be detected. It is therefore obvious that the direction of the major extinction position is determined not only by the directions of the cellulose chains, but also by the proportions of the two sets present in the wall thickness. One small area in E showed a major extinction position exactly parallel to the more obvious set of striations (which was unusually pronounced compared with the second set in the area): this particular area, therefore, was structurally different from the rest of the wall in that one set of chains was almost entirely absent. The majority of the mosaic areas, however, undoubtedly arise from varying proportions of two sets of chains in the wall thickness, and this single case represents one of the limits of variation. Perhaps a point raised in the previous paper may again be emphasized. Any work on biological structures carried out under the polarizing microscope alone must be regarded with suspicion until confirmatory evidence has been obtained, such as is afforded by the X-ray method.

We have, therefore, a wall consisting of many microscopically visible layers and corresponding to a network of cellulose chains making an angle of some 80° with each other. Complete understanding of such a structure is obviously impossible without an investigation of that of the individual layers—to decide whether even the finest layer has a structure similar to that of the whole wall, or whether the wall is composed simply of more or less alternating layers each with only one direction of cellulose chains. The work of Correns (1892, quoted from van Iterson 1933) supports the latter alternative. Correns concluded from careful microscopical examination that the odd layers had one set of striations, while the even layers had the other. If this is true, then any one layer cannot have everywhere the same thickness. The directions of the extinction positions vary from point to point, a change which is necessarily connected with the relative amounts of the two sets of chains. If, then, the same number of layers of each kind are present in two neighbouring mosaic areas their relative thickness must vary.

This work of Correns has now been verified by physical means. As yet
no direct tests of the structure of a single lamella have been possible. Extremely thin lamellae can be stripped from fresh cells, but even these fail to show any indication of a structure different from that of the whole wall. It has not yet been found possible to strip off a layer with a single set of chains. On the other hand, indirect evidence does certainly support the work of Correns.

As shown by van Iterson, Jr. (1933), pieces of *Valonia* wall can be torn in such a way that the torn edge exhibits a fringe of fibrils. Here and there, the otherwise straight edge of the wall is interrupted by sets of these fibrils pulled out from the wall. van Iterson gives a drawing showing a small piece of wall standing out from a torn edge and with such a fringe of fibrils. This small piece shows only a single set of striations, which in the drawing are obviously the origin of the fibrils, and is therefore a single layer in the present sense. It has been found impossible to repeat this observation exactly. Many pieces of wall have been subjected to a treatment similar to that of van Iterson, but in no case was it found that the fibrils at the torn edge originated from the set of striations perpendicular to the edge. On entering the wall the fibrils obviously turned through a considerable angle, and finally were lost among the striations parallel to the torn edge. Fig. 2, Plate 1, makes this clear. It would seem that the fibrils perpendicular to the torn edge are the first to break, leaving the two pieces joined together by the lateral fibrils which are then pulled out before breaking.

In fig. 3 is given a diagrammatic representation of a second type of observation that may be made at a torn edge. Such an edge often shows
The Wall of Valonia ventricosa

a terraced effect due to the stripping off of various numbers of wall layers. The portion of wall illustrated is a particularly interesting example of such a phenomenon. Three distinct regions can be seen: $A$, which represents the whole wall thickness; $B$, where only a few layers are left; and $C$, which is probably a single layer. It is unfortunately impossible to present an actual photograph of this specimen, since attempts completely to flatten the wall for distinct focusing in the camera caused this part to break up into fibrils. The striations and extinction positions marked in the figure, however, make it quite clear that the removal of several wall layers has caused a change in the orientation of the major extinction position, and that in the region $C$ the layer consists of a single set of cellulose chains. There can be no doubt that the layers are not identical with one another, and we may fairly conclude that their structure is distinct from that of the whole wall in that each is built from one set of cellulose chains. The whole wall is composed of a series of superimposed layers each with its own cellulose chain direction.

Thus it would seem that, in *Valonia*, both striations and layering are definitely related to structural details in the wall. Now although the external form of the *Valonia* cell is widely different from that of the fibres of the higher plant, the structure of its wall is essentially the same. This will be clearly demonstrated below. The present results, therefore, give further support to the generally accepted view that, in general, whenever striations are visible on the walls of cells of the higher plant (e.g. phloem fibres, xylem fibres and tracheids, cotton hairs) they are not merely artefacts but correspond closely to the structure of the walls. This is undoubtedly true for the walls of certain conifer tracheids, since Frey-Wyssling (1930) has observed striations parallel to the major extinction position which in turn have been shown to be parallel to cellulose chains (Preston 1934).

The exact significance of the striations and layering of the plant cell wall has been the centre of considerable discussion for many years. Many cases of cell walls with crossed striations have been quoted, notably by Reimers (from Steinbrinck 1927, and Herzog and Janeke 1928). These observations refer almost exclusively to phloem fibres (e.g. of hemp, hop, ramie, flax), in which the wall layer showing one of the sets of striations usually predominates. The conception of Nägeli that the appearance of striations is caused by regions of high and low water-content has been rejected by Dippel (1879), Schmitz (1880), Strasburger (1898), and Krabbe (1887), who agreed that in phloem fibres the striations are merely distorted contact faces between adjacent "screw bands" in intimate contact. These authors also contested Nägeli's observation that two sets of striations can appear in one layer of the wall. Their view of the origin of striations has in turn been
rejected by Correns (1893) as physically impossible; he is of the same opinion as Nägeli. Wiesner (1892), again, put forward a third hypothesis in which the wall is composed of "Dermatosomes" which aggregate to form both fibrils, leading to striations, and layers. He considered these "Dermatosomes" to be separated by layers of "some protein or its derivative", a residue of the original protoplast of the cell; but repeated experiments by Correns have failed to show any trace of protein in the wall. The primary cell walls of plants certainly contain a protein complex (Tupper-Carey and Priestley 1923), but there seems to be no evidence for any considerable amount of protein in the secondary layers such as are under consideration here.

There can be little doubt that the effect of difference in water content on the visibility of layers and striations is only of secondary importance and is inseparably connected with a difference in chemical constitution. Hess, Ludtke, and others (van Iterson 1933) have been led, on the basis of swelling experiments, to the assumption of partitions of non-cellulosic substances between the wall layers and even the fibrils, and the fact that this conception fails to account for certain phenomena does not invalidate their argument outright. It is interesting in this respect to note that Farr and Eckerson (1934) have recently observed in the protoplasm minute bodies which they describe as cellulose particles surrounded by a layer of pectin, although the significance of this observation is perhaps open to question (Bailey and Kerr 1935). At the same time, the view that striations are due merely to the separation of fibrils by less perfectly oriented regions of the same composition cannot be entirely disregarded.

The Organization of the Wall as a Whole

None of the observations presented above suggests any fundamental difference between the cell wall of Valonia and that of the fibres of the higher plant, in spite of the difference in cell size, and the correspondence is again evident when we come to consider the details of the organization of the wall as a whole.

The modification of wall structure, which must occur at the tips of cells whose walls are wound with a molecular spiral, has hitherto been a point of mere conjecture and any investigation throwing light on this subject cannot fail to be of considerable value. The opportunity was taken, therefore, of carrying out a survey of the whole Valonia wall. The uncertain visibility of the wall striations made it impossible to follow microscopically
The Wall of Valonia ventricosa

their directions uninterruptedly round the cell, so the investigation had
to be carried out by X-rays.

A herbarium specimen of *V. ventricosa* collected at St Croix and sent to
us by Børge sen, to whom our thanks are due, was emptied of its contents
through a small perforation. Into this perforation a fine glass capillary
tube was inserted and fastened in place by a minute ring of cellulose cement,
whence by alternate emptying and filling of the cell with distilled water the
remains of the protoplast, etc., were finally ejected. Incrustations clinging
to the outside of the wall were removed by subsequent treatment with
N/20 HCl. When dry, the cell was sufficiently rigid to be mounted on the
X-ray spectrometer by clamping the capillary tube to a brass arm with
a universal joint. By careful adjustment any part of the wall could thus
be set perpendicular to the X-ray beam. In order to obtain reference lines
whereby the directions of the cellulose chains as given by the X-ray
photograph could be transferred to the cell itself, the following procedure
was adopted. The cell was mounted on a spindle by means of which it
could be rotated and raised through measured distances, and a series of
lines, some 3 mm. apart and forming complete circles round the cell, was
traced in Indian ink using a modification of the usual inking system of
barographs, etc. A pair of straight wires was then attached to the spectro-
meter so that they could be set parallel to that part of a line on the cell
nearest to the area under examination, and would cast a shadow on the
photographic film. In general, this area under examination was arranged
just to touch the spectrometer slit, while the photographic film was placed
as near to the other side of the cell as possible. It was then quite a simple
matter to differentiate between the diffraction spots produced by the two
opposite sides of the cell (see fig. 4).

In order to obtain a map of the whole wall a method was used similar
to the familiar “lines of force” method of mapping magnetic fields. The
chain directions were determined at an arbitrary point in the wall and were
then drawn upon the wall. Now previous experience had shown that the
direction of either set of chains was almost constant over a length of 1½ mm.
A second point was therefore chosen, 1½ mm. from the first in the direction
of one of the sets of chains, and the directions again determined. This
process was continued round the cell using only one set of cellulose chains.
In general no difficulty was experienced in determining which of the two
directions at a new point corresponded to the one being traced, any un-
certainty where it arose being entirely removed by the investigation of
intermediate points.

A starting point was chosen about midway between the base and tip
of the cell, where one set of chains was found to lie approximately along a line joining the tip and the base. On following this direction, the chains were found to form a great circle round the cell, passing amongst the holdfast scars and across the cell apex. Unfortunately, the value of this set of observations was somewhat reduced by very considerable dispersion of the X-ray diffraction spots in certain regions, particularly near the holdfast scars and the cell apex. Investigation of the second set of chains, however, confirmed this result in a very striking manner. At each point on this second track both directions of cellulose chains were marked in ink upon the wall, although only one of them was followed. The second chain direction was thus found to make a slow spiral round the cell, the turns of the spiral becoming smaller as the apex and base of the cell were approached until finally, both at the apex and the base, a point was reached where the X-ray photograph characteristic of Valonia was no longer obtained. Both points were strictly localized and gave a photograph consisting of a series of rings such as is obtained from a crystalline powder. It is important to note that these two “poles”, as they may be termed, were discovered not by accident.
or by a method of trial and error, but by painstakingly following the spiral set of chains round the wall. They would appear to be produced by the wall deposition mechanism of the plant rather than by any local, accidental change in environmental conditions. A model of the structure of the *Valonia* wall is shown in fig. 5, Plate 1, in which one "pole" can be seen towards the upper end. The X-ray photograph of this "pole" is reproduced in fig. 6, Plate 1. It may be pointed out that the second set of chains recorded at each point of the spiral may be linked up with chains immediately above and below and that the circles thus obtained form, so to speak, "meridians" uniting the two "poles". Whether it is an invariable rule that one set of chains always forms great circles uniting the tip and base of the cell, as in the present case, is not yet clear. A decision on this point is best made upon a long, narrow, cylindrical cell; and only one of this type was available. In this one specimen, however, one set of chains was observed to run approximately along a "meridian" at all points of the wall which were investigated. We may thus picture the *Valonia* cell wall as consisting of two crossed sets of cellulose chains, one running in great circles (possibly always from base to tip and back), and the other forming a slow spiral round the cell axis joining the two points of intersection of these great circles.

It has been pointed out above that at the base of the cell, near one of the "poles", there occurs some disturbance in the otherwise regular appearance of the wall surface. In this region, clusters of raised, rim-like structures may be observed which obviously mark the sites of previous rhizoids (holdfasts). In some few cases in the specimens available the rhizoids can still be seen attached to the cell in the form of long, narrow, and hollow cylinders widening into trumpet-shaped attachments at the point of connexion. Such a rhizoid may be seen in the photomicrograph of the surface of the basal region of a cell shown in fig. 7, Plate 2. The preparation shown in this figure was stained in methylene blue to bring out the fact, which is perhaps more obvious in cross-section, that the wall is much thinner inside the rims than outside. This no doubt explains the X-ray photograph obtained in these rhizoids. If we choose a rim of such a diameter as just to be included in the X-ray beam, then the X-ray diffraction pattern obtained appears to arise solely from the rim. Although the wall inside the rim seems to be identical in structure with that outside, and the striations on it are continuous with those on the rest of the wall, its thickness is so small compared with that of the rim itself that its X-ray photograph does not mask that of the rim. The X-ray microphotograph of such a rim was found to consist of a series of concentric rings, indicating
random arrangement of the cellulose particles. This is exactly what we should expect from consideration of cross-sections of the wall. Fig. 8, Plate 2, is a photomicrograph of such a cross-section. Here the remains of a rhizoid are seen clinging to the wall (on the left) and located immediately above a small cell cut off from the parent. The whole structure is filled with small granules which appear to be plastids surrounded by a comparatively thick layer of starch, and may perhaps play a part in the development of the rhizoid. It is clear from the photograph that the raised rim seen in surface view consists merely of a ring of the outer layers of the wall turned on edge, and the X-ray photograph is in effect that of a cross-section of a cylindrical holdfast.

The structure of the rhizoids as illustrated in figs. 7 and 8, Plate 2, and by the X-ray microphotograph, is in complete agreement with the descriptions given by other workers (Famintzin 1860; Börgesen 1905). It is quite clear that the cylindrical outgrowths originate as small cells cut off on the inside of the "main" vesicle by surrounding a small collection of the necessary plasma masses by a strongly curved subsidiary wall, before the deposition of the wall of the mother cell is complete. As more and more layers are deposited over this "watch-glass" wall, by the continuous deposition of new wall substance by the parent cell, it becomes eventually buried in the wall. It may well be that the wall on the outside of this small cell is then considerably thinner than that on the inner side. At the same time it must be noted that the inner wall borders, not on the open sea, but on a virtually incompressible interior supported by comparatively firm walls. It is not surprising therefore, that if the "watch-glass" cell begins to expand the expansion takes place towards the outside. This would explain the formation of both rhizoids and bud cells on the outside of the parent plant. Here, however, we meet with a difficulty. Whereas the bud cells are usually almost spherical in form, rhizoids are always produced as long, narrow cylinders. Hitherto no explanation of this divergent behaviour of essentially similar cells has been possible. Now that the wall structure of the plant has been determined, we find that the rhizoids arise from regions of the wall adjacent to the poles of the spiral, and it is not unreasonable to suppose that the difference in behaviour of "watch-glass" cells is connected with the difference in wall structure. Such a connexion could, of course, be traced only in the vaguest terms at present, and its investigation is a subject for further research.
Relation of Cellulose Chains to Wall Surface

It has been mentioned already that the conclusion arrived at by Sponsler (1931) that in Valonia the planes of 6.1 A. spacing lie parallel to the wall surface is only partly justified. In the course of the present research it became clear that any X-ray results obtained from blocks built up by superposing many pieces of the wall are liable to be misleading. It was thought advisable, therefore, to reinvestigate the question of the angular dispersion of the cellulose chains, using single pieces only. To this end a small area of wall was selected on which one set of striations predominated and whose X-ray photograph showed a preponderance of one set of chains over the other. Fig. 9, Plate 2, is an X-ray photograph of the area chosen: one set of reflexions is so much more intense than the other that for the purpose of studying its angular dispersion the weaker set may be disregarded.

The most direct method of demonstrating the angular dispersion of the cellulose chains is illustrated by the photograph shown in fig. 10, Plate 2, for which the flat piece of wall was mounted horizontally on the spectrometer with the main set of chains parallel to the X-ray beam. If now, as Sponsler suggested, the cellulose chains had been lying in only one orientation round their axis, not arcs, but spots as definite as those in fig. 9, would have appeared in the photograph. The photograph reveals in fact quite a considerable dispersion, for both of the inner sets of arcs (corresponding to planes of spacing 6.1 and 5.4 A.) can be traced round a complete circle. The intensity, however, certainly does decrease rapidly at fairly definite limits, and roughly speaking it may be said that the normal to the plane of spacing 6.1 A. is confined to about 60° on either side of the normal to the wall surface.

Discussion

The remarkably regular organization of the wall of such a large cell as that of Valonia is perhaps the most interesting result of this research. The fact that the cell has a structure fundamentally similar to that of the minute fibres of the higher plant serves once more to emphasize the essential unity underlying biological phenomena. It has long been a question whether coenocytic cells, such as we have under examination here, could be regarded as single units comparable with the protoplasts of higher plants containing but a single nucleus, but as regards the wall at least there can no longer be any doubt about this. The wall of Valonia would appear to be just as
uniform in structure as that of uninucleate cells and can be regarded only as that of a single cell.

The appearance of crossed striations is of course not a novel phenomenon in wall structure. It is widely recognized that in bast fibres the secondary wall is laid down in definite layers and that these layers can be striated in different directions. For example, fibres from hemp and hop plants have two secondary layers, the striations on both running round the cell in a right-hand spiral, with the spiral on the outer layer less steep than that on the inner. On the other hand, bast fibres from flax and oleander show definitely crossed striations, the spirals on the two layers being of opposite sign. Moreover, in both types of cell the view put forward by Nägeli that crossed striations can appear in a single wall-layer is no longer held. As has been shown above in the case of *Valonia*, striations in different directions invariably occur in different wall layers. Here, however, the similarity between *Valonia* and fibres effectively ceases. Whereas in bast fibres change in spiral sign occurs but two or three times and the structure of one layer is not repeated in a subsequent layer, *Valonia* has numerous layers which alternate regularly in striation direction in a very exact manner. This deposition of alternate layers, each with the same direction of molecular chains as the last layer but one, presents perhaps the most intricate problem in wall formation as yet encountered in botany. It seems impossible without serious modification to invoke the idea of pseudo-crystallization of new substance on an old wall such as is often put forward in discussions of wall deposition: at the least, it must be recognized that the growth mechanism involves a periodic halt in the effectiveness of an old wall in orienting new layers.

The existence of "mosaic" areas is to be explained on the lines already laid down. They arise as a result of variations from point to point in the proportions of the two sets of cellulose chains in the wall thickness. It seems reasonable to suppose that these variations are due rather to a varying thickness of the wall layers than to a fluctuation in their number. The mechanism underlying the formation of mosaic areas is no doubt to be sought in fracture of the wall during development, as already suggested by one of the present writers (Preston 1931).*

With regard to the geometrical form of the path followed by the spiral set of cellulose chains it would appear that this approximates most to an

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* This idea is supported by the fact that remnants of wall layers, presumably the original outer layers, are often found clinging to the plant when collected. This has been pointed out to us by Dr Steward, of Birkbeck College, London, who recently had the opportunity of studying *Valonia* in its native habitat.
equiangular spiral described on the surface of a spheroid. Fig. 11 illustrates a prolate spheroid, which is a reasonably fair description of many Valonia cells. If the spiral at any point \((\theta, \phi)\) of its path makes a constant angle \(\alpha\) with the meridian \((\theta = \text{constant})\) through that point, then

\[
\cot \alpha = \frac{\delta s}{y} \frac{\partial}{\partial \theta}
\]

or

\[
\theta \cot \alpha = \int \frac{ds}{y},
\]

the solution of which, if \(\theta = 0\) when \(\phi = \pi/2\), is:

\[
\theta \cot \alpha = -\frac{\sqrt{a^2-b^2}}{2b} \cos^{-1} \left[ \frac{b^2}{a^2} - \frac{a^2-b^2}{a^2} \cos 2\phi \right] - \frac{1}{2} \cosh^{-1} \left[ \frac{2b^2}{a^2 \cot^2 \phi + 1} \right].
\]

For the sphere this reduces to:

\[
\theta \cot \alpha = \log \tan \frac{\phi}{2},
\]

as may be readily derived directly.

* This general formula is given here in case experimental opportunity should arise later of testing it rigidly.
It should be noticed that $d\phi/d\theta = 0$, when $\phi = 0$ and $\pi$, and is a maximum when $\phi = \pi/2$, as was actually observed for the Valonia cell described above.

Owing to irregularities of growth it is hardly a practical proposition, however, to make a strict quantitative test of the equation, though it may be that careful examination of more abundant material than was available for these experiments would reveal specimens sufficiently perfect. At the moment we are justified in saying only that the angle $\alpha$ is roughly constant and not far removed from a right angle—the mean of the values given in Table I, for instance, is about $83^\circ$—and that the spiral reproduces the main features of the path of a point moving on the surface of a spheroid so as to make a constant angle with the meridians.

The approximate constancy of angle between meridians and spiral that is maintained through alternate layers, and the fact of alternating deposition itself, seems best explained for the time being in terms of a rhythmic orienting mechanism embodied in the polynuclear protoplasmic lining. Recent observations on the cytoplasm of algae, those of Chadefaud (1933) for instance, are strongly suggestive of such a mechanism, and the following extract from Chadefaud’s paper, “Existence d’une structure infra-visible orientée du cytoplasme chez les Algues”, is very much to the point:

“L’existence d’une structure orientée du cytoplasme se traduit d’une façon encore plus intéressante dans les grandes cellules allongées du tissu central de Chorda filum. Le cytoplasme de ces cellules possède deux séries de lignes directrices, à peu près orthogonales, et fortement inclinées par rapport à l’axe longitudinal de la cellule. L’une de ces directions est prédominante: elle oriente la plus grande partie des phéoplastes, des chondriosomes et des amas de physodes, et tous les noyaux, qui sont étirés en fuseau. Quelques phéoplastes seulement sont orientés selon l’autre direction. Or, il est très curieux de remarquer que ces deux directions de la structure cytoplasmique coïncident avec celles des deux systèmes de fines stries que présente la membrane celluloso-pectique, et que révèlent de façon très nette les ponctuations en X de cette membrane. On trouve ainsi une relation évidente entre la structure cytoplasmique et celle de la membrane cellulaire.”

The occasional appearance of a third orientation lying between the two predominating sets of cellulose chains is possibly a manifestation of attempts to set up a spiral of opposite sign and may represent still another link with the structure of the fibres, in which the occurrence of spiral reversals is fairly common.

van Iterson (1936) considers that the approximate orthogonal relation between the cellulose crystallites in adjacent layers of the wall of Valonia
The Wall of Valonia ventricosa

is simply a consequence of an alternation of wall stretching and protoplasmic streaming, the direction of easiest stretch being at right angles to the length of the crystallites already laid down. The periodic stretching would be caused by the strong increase each day in the turgor pressure in the cell, for example, and it is supposed that the stresses so set up determine the direction of flow of the protoplasm as it deposits the next layer with the crystallites lying along this direction of flow. The concept perhaps marks an advance in the sense that it offers something rather more concrete to work on, but it still leaves vague the initiation of the process and does not explain how stretching can take place in a multi-ply structure first in one direction and then in a direction at right angles. The idea may be valid for a wall consisting of two layers only, but it is not easy to see how the mechanism continues to operate with such angular regularity beyond this stage. In any case, the impression gained from the studies reported above is that the average angle of crossing is definitely less than a right angle, though to be sure it might be possible to trace this deviation to some secondary source.

Finally, reverting once more to the question of the approximate orientation parallel to the cell wall of the planes of spacing 6.1 A, a recent paper by Sisson (1936) is very illuminating. From an X-ray study of the various types of crystallite orientation that can be brought about artificially in membranes of bacterial cellulose Sisson concludes that in general whenever a sample is constricted in one direction, whether by drying or by pressure, then the cellulose crystallites have an inherent tendency to set themselves with the planes of spacing 6.1 A normal to the direction of constriction. It would appear, therefore, that to explain in Valonia—or in any cellulose wall for that matter—the observed selective orientation of the crystallites about their long directions it is probably unnecessary to invoke anything more complicated than the simple act of drying.

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Summary

The cell wall of *Valonia ventricosa* has been studied in detail by means of X-ray diffraction photographs and the polarizing microscope.

It is found to consist of layers in which the cellulose chains in any one layer are inclined to those in the preceding and subsequent layers at an angle which is on the average rather less than a right angle.

The chains of one set of layers form a system of meridians to the wall, while those of the other set build a system of spirals closing down on the two “poles” defined by the meridians.

The two sets of striations on the layers of the wall correspond closely to the meridian and spiral directions of cellulose chains, while the extinction directions, being defined both by the directions and by the relative proportions of the two sets of cellulose chains, lie in variable positions between.

The development of the rhizoids has been investigated and found to be associated with regions of the wall adjacent to the poles of the spiral.

The plane of spacing 6.1 Å of the cellulose crystallites is, roughly speaking, confined within an angle of about 60° to the wall surface.

It is suggested that the path of the cellulose spiral is that of a logarithmic (equiangular) spiral described on the surface of a sphere or prolate spheroid.

The relation is traced between the structure of the walls of fibres of higher plants and that of the cell wall of *Valonia*.

References

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**Description of Plates**

**Plate 1**

**Fig. 2**—A typical fringe of fibrils at a torn edge of *Valonia* wall. Note that on entering the wall the fibrils turn round and finally disappear among the striations almost parallel to the edge.

**Fig. 5**—Model of the wall structure of *V. ventricosa* showing the spiral organization of one set of cellulose chains. The spiral can be seen closing in towards the point marked on the model, which thus represents one “pole”.

**Fig. 6**—X-ray photograph of the wall of *V. ventricosa* at a “pole” of the spiral.

**Plate 2**

**Fig. 7**—Photomicrograph of the basal region of the wall of a cell of *V. ventricosa*.

**Fig. 8**—Photomicrograph of the point of attachment of a rhizoid, in cross-section.

On the right can be seen the “watch-glass” cell from which originated the rhizoid whose remains are attached to the wall on the left.

**Fig. 9**—X-ray photograph of an area of the wall of *V. ventricosa* in which one set of cellulose chains greatly predominates. (X-ray beam perpendicular to the surface.)

**Fig. 10**—X-ray photograph of the same specimen lying horizontally with the main set of cellulose chains parallel to the X-ray beam.