CELL-WALL STRUCTURE IN THE GROWTH-ZONE OF *PHYCOMYCES* SPORANGIOPHORES

II. DOUBLE REFRACTION AND ELECTRON MICROSCOPY

by

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§ 1. EXAMINATION OF THE DOUBLE REFRACTION (d.r.) OF THE WALL IN THE GROWTH-ZONE

a. *Determination of the spiral structure from the direction of extinction*

As we remarked in the Introduction to Part I (page 340 of this Vol.), OORT AND ROELOFSEN (1932) considered themselves justified in assuming a Z-spiral structure in the primary wall of *Phycomyces* sporangiophores on the basis of the direction of extinction, but since the data given were too scanty, this assumption has, with reason, not been accepted as a certainly.

It seemed therefore advisable to repeat these observations, employing the same methods. OORT AND ROELOFSEN have determined the angle of oblique extinction in the growth-zone of sporangiophores in stage 4, by cutting the cell obliquely in its growth-zone and by subsequently pushing a drawn-out glass thread into the cell by way of an incision made about 1 cm lower, until the wall fitted tightly around the glass cylinder. After this they heated it in alkali and stained in chlorzinc iodine to intensify the d.r.* The thread with the cell-wall around it, could easily be rotated until the small area of single wall, produced by the oblique cut through the growth zone, came into a good position for the extinction angle to be measured.

We tried again to apply this procedure which we previously followed, but we met with so many failures, that the method had to be abandoned as too time consuming. A simpler way was to prepare, in the manner described (in Part I § 2c), a flattened out cell, with a diamond-shaped area of single wall at its tip. It even turned out to be possible to make oblique cuts at several places in the growth-zone so that the single wall could be observed in various places; Fig. 17 gives a picture of this.

To enhance the d.r. the wall was coloured with alkaline congo red. Then it was washed in water and mounted in glycerine. With the aid of a strong light source (Leitz Monla lamp) the difference between the extinction angle of the single wall and of the adjacent double wall was subsequently measured between crossed nicols for a total of 60 preparations. The results were as follows:

* Double refraction.

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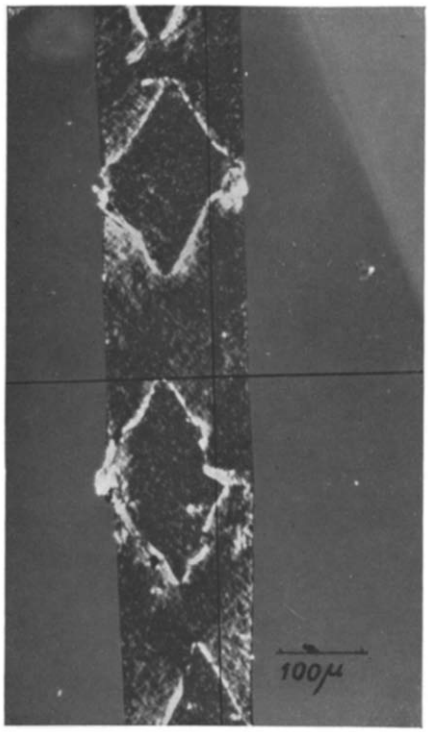

Fig. 17. Diamond shaped areas of single cell wall in growth zone of stage 4b. Stained congored; crossed nicols. Highest index of refraction exactly transverse

were just as disappointing as the previous ones. In the different preparations, both the transverse and the two spiral directions of extinction were found in all parts of the growth-zone.

Thereupon we thought that the fact that locally the cell wall was not exactly horizontal might have been playing a part. For that reason we have tried yet another method, viz. the one used by PRESTON (1938) for parenchyma cells. Sporangiophores that had been cleaned with alkali and stained with congo red, were allowed to dry out on a slide covered with a uniform layer of gelatin (we used developed, non-exposed photographical plate). Minute scratches were then made over the cell with a fine needle, and the whole then was mounted in methyl benzoate. One then observes local areas where only the single cell-wall is left, as shown in Fig. 19.

31 cases of transverse extinction;

16 cases of an S-spiral making an angle of 5–14° (average 6°) with the transverse;

13 cases of a Z-spiral making an angle of 5–7° (average 4°) with the transverse.

From this it might be concluded that S- and Z-spiral-structures are about equally frequent. In view of the constant right hand rotation of the cells in the stage investigated, this is not possible.

With the possibility in mind that the spiral structure might differ in different parts of the growth-zone, we have made a number of preparations, such as the one figured in Fig. 17, and also as indicated by OORT AND ROELOFSEN (1932) under method 2, *i.e.* by squeezing cells lying on a wet cover-slip until they burst in the growth-zone. The split thus formed runs in an axial direction, due to the fact that the wall-tension is greater in the transverse than in the axial direction. Fig. 18 shows such a ribbon.

The results of the extinction measurements

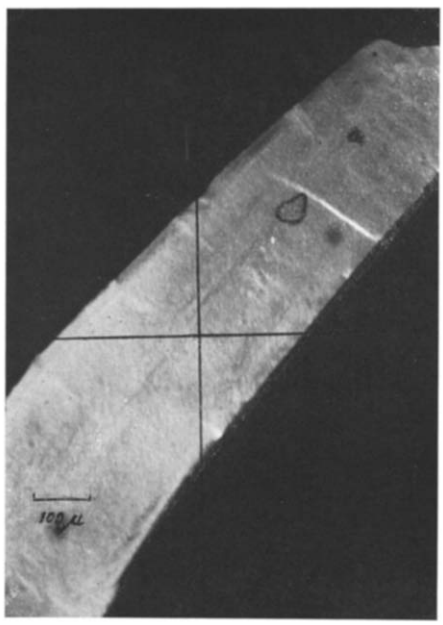

Fig. 18. Ribbon of single wall, obtained from the growth zone of stage 4b. Stained congo-red; crossed nicols

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However, the results of these measurements were not different from those already mentioned.

Moreover, the method was not free from danger, because the direction of scratching could sometimes be seen to influence the direction of extinction.

A remarkable observation was that in several cases an undeniable d.r., with the maximum refractive index running lengthwise, could be observed in spots where both cell walls had disappeared except for a very thin layer coloured weakly by congo red. It could not have been the gelatin itself and neither the cuticle as this is completely isotropic, a fact which we established with the thin cuticular membranes obtained from the bursting of the fluid droplets described in Part I. Electron micrographs show an outer layer consisting of an apparently isotropic network of fibrils, (§ 2). However, as this probably originated from a transversally orientated one by extension lengthwise (ROELOFSEN 1950), it might be possible, that in some cases this re-orientation had gone farther than the isotropic state. So, here was an indication of it.

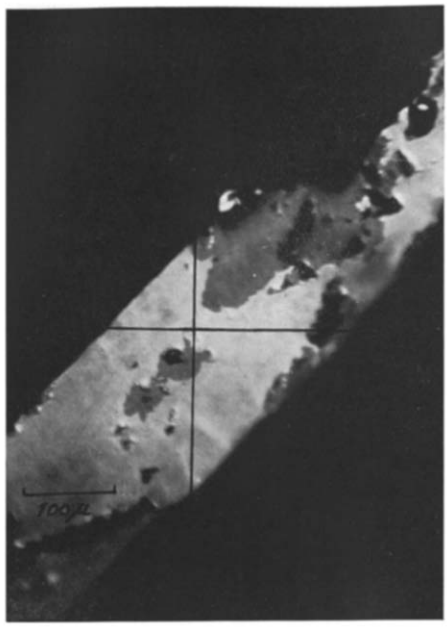

Fig. 19. Local areas of single wall in the growth-zone of stage 4b, obtained by scratching a cell glued on a gelatin-covered slide. Stained congored; crossed nicols

After having made these observations, it occurred to us that one source of error might be the fact reported by CASTLE (1938) that a faint positive d.r. is shown after staining with congo red in walls cleaned with alkali only, whereas the d.r. becomes strongly negative when the same preparations are cleaned by an additional heating for a short period in 5% HCl. In the growth-zone of vigorously growing cells that had not been stretched in the process of preparation, we have, however, never seen a positive d.r. (we did see it in cells where growth had ceased). But as a matter of fact the d.r. turned out to be strongly intensified, so that the determination of the extinction angles could in any case be performed more accurately. Furthermore, it became clear that before and after acid treatment the direction of extinction was liable to change. This can be seen from the data reported in the table on p. 360.

We cannot yet account for the phenomenon with certainty, but the following explanation is a possibility. We have in any case two and in the lower part of the growth-zone three layers of different orientation in the meristematic wall (see § 2). Only the middle layer possesses a transverse orientation. It is possible that after cleaning with NaOH only, this layer is not stained sufficiently and therefore shows a faint d.r. After cleaning with acid giving deeper penetration of congo red the central layer may take its full share of the d.r. and thus may alter the extinction angle of the wall as a whole. CASTLE (*l.c.*) thinks in terms of elimination of axially arranged non-chitinous material, but this does not seem probable to us.

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Direction of extinction after treatment with:	
NaOH	NaOH and acid
6° S	4° Z
transverse	4° Z
2° Z	transverse
10° S	transverse
9° S	transverse
13° S	transverse
transverse	transverse

We have treated a number of preparations in this way and we obtained the following result:

20 cases of transverse orientation
9 cases of a Z-spiral of 4–13°

Thus it becomes clear that a transverse, or almost transverse orientation can be found in walls cleaned with NaOH and acid, but that a Z-spiral orientation can also be regularly found. We got the impression that the latter phenomenon may be seen especially in cultures which show extremely strong rotation during growth due to abnormal growth-conditions such as infections. These cultures also show unwinding of spirals (Part I, § 2a).

Despite the fact, that a transverse extinction angle was found in many cells we consider ourselves justified in concluding that in all cells there had been a Z-spiral structure as long as they were in a turgid condition, but that, due to shrinkage during the preparation of the cells, the spiral structure was flattened so much, that the oblique extinction angle could only be observed in a few cases.

We have investigated to what extent the cell contracts in preparation. For that purpose a turgid cell was put on a large coverslip on which we had spread out a thin layer of liquid gelatin and this gelatin was made to solidify at once by putting the coverslip on a small ice-cube. By using a mm-scale lying under the coverslip the top was now cut off exactly 2 mm below the sporangium. After cleaning in NaOH and acid and staining in congo red, the length was again measured. On the average this turned out to be 69% of the original length*. It has to be admitted that the diameter of the cells also diminishes, but only to the extent of 5%. From this one can calculate that an original spiral of, *e.g.*, 5°, will become one of 3° 45′ in the preparations. Angles of 3° and less are recorded as transverse as a result of the faint d.r., so that it is understandable that in this way only the steeper spirals can be observed to be present in cells that happen to show strong rotation.

OORT AND ROELOFSEN (1932) employed a method in which a glass thread was pushed into the cell until it fitted tightly in the growth-zone. It will be clear that here the spirals cannot flatten (they may even be extended), and this may be the reason, why in this publication we came to the early conclusion that in most material a Z-spiral was present.

* It has already been shown (ROELOFSEN, 1950), that loss of turgor alone results in a contraction of about 15%; heating in NaOH gives a shortening of about 10%, and the acid treatment adds another 6% to this.

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b. *Quadrant-effect and dichroism*

In the polarization microscopes that we used, sporangiophores of *Phycomyces* stained with congo red usually showed a striking quadrant effect. By this we mean that the color between crossed nicols is slightly different in quadrants 1 and 3, from those in quadrants 2 and 4 $\left(\frac{1}{3}\frac{P}{P}\middle|\frac{2}{4}\right)$.

It is well-known that a quadrant effect may, generally speaking, be produced by various causes, viz.:

1. incompletely crossed nicols;
2. a slight strain d.r. in one or more of the lenses, or in the slides;
3. the presence, in the object, of d.r. layers possessing different extinction angles.

Thus, if factors 1 and 2 can be eliminated, the quadrant effect may serve as a means of demonstrating the presence of layers of different orientation. For our researches it was therefore worth while to investigate this point further.

In the literature the quadrant effect resulting from overlapping of thin uncoloured d.r. layers has often been described. As far as we know this was first done in a most elaborate manner by NAEGELI AND SCHWENDENER (1877, p. 332 seq.).

FREY-WYSSLING (1941) has carefully worked out that extinction is never complete and no longer occurs in the normal positions in objects consisting of two different layers. If these are very thin layers however, *e.g.* in crossed primary walls, one no longer sees a quadrant effect; such a set of layers behaves as one layer.

Several investigators have observed a conspicuous quadrant-effect in chloroplasts, especially in the large chloroplasts of algae. However, it is now thought that the phenomenon was due to the fact that the objectives employed were not strain-free. This was confirmed by FREY-WYSSLING AND WÜHRMANN (1947). However, FREY-WYSSLING (1949) has informed us that, according to more recent investigations, an explanation of the quadrant effect, different from the one recorded in the aforesaid publication, has been found. This, however, does not alter the fact that lenses which are not strain-free may cause a quadrant effect in some cases.

Dichroism for a particular wavelength causes a rotation of the plane of vibration for that wavelength, but FREY-WYSSLING AND WÜHRMANN (*l.c.*) showed that this cannot result in a quadrant effect, if only one layer is considered, because the rotation is the same in the various quadrants. With a pair of layers, however, dichroism has another effect. OORT AND ROELOFSEN (1932) have stated that the quadrant effect of two thin d.r. layers crossing at a sharp angle, is intensified to a great extent if dichroitic staining is applied, *e.g.* layers of cellulose or chitin coloured with chlorzinc iodine. This was demonstrated by the fact that in the bast fibers of rameh, flax and Vinca, in cotton and in the double, fullgrown wall of *Phycomyces*, the two positions of extinction shifted to the same pair of quadrants, whereas no shift was seen without the dichroitic staining. Whether the extinction position came into one or the other pair of quadrants depended on the nature of overlapping, whether Z or S.

The correctness of this can easily be verified with the aid of two small strips of cellophane stained with congo red (or chlorzinc iodine). An S-overlapping is redder (darker) in colour in quadrants 1 and 3 than in quadrants 2 and 4; with a Z-overlapping it is the other way round. The sharper the angle of overlapping and the smaller the d.r. of the cellophane and the sharper the angle to the left and the right of the position

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of extinction in which the colours are compared, the more conspicuous is the quadrant effect. Without staining, the quadrant effect is much less pronounced; it can then only be observed in a position parallel to the plane of vibration of the polarizer and by using a 1st order red plate. Then an S-overlapping shows a substraction colour and a Z-overlapping an addition colour*.

A qualitative explanation of the effect of dichroitic staining can easily be derived if it is assumed that the dichroism is absolute. In other words the green wavelengths in the case of congo red and all wavelengths in the case of CZI are supposed to be completely absorbed if vibrating parallel to the direction of the fibrils. See the following outline.

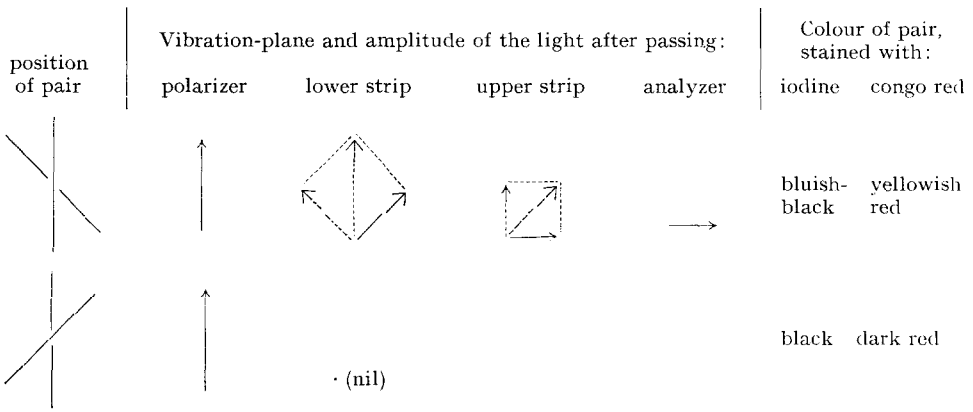

Illustration of quadrant effect in a pair of dichroitic cellophane strips, overlapping in Z-fashion

In our investigation of the d.r. of the meristematic cell wall of *Phycomyces* we have always stained with congo red or chlorzinc iodine in order to enhance the d.r.

It was remarkable that besides the expected quadrant effect in the *double* cell wall either meristematic or full-grown, an even stronger one was seen in the *single* meristematic (or full-grown) wall. At first we considered this to be due to the presence of two or more layers, orientated differently. This was confirmed later on by electron micrographs (§ 2). FREY-WYSSLING (1949) subscribed to this opinion (see also FREY-WYSSLING AND MÜHLETHALER 1950).

However, doubts soon arose, since FREY-WYSSLING (1941) had proved that no quadrant effect was to be expected with overlapping of very thin layers, at least non-dichroitic ones. In fact it appeared that with single dichroitic *Phycomyces* cell walls the quadrant effect could be ascribed to extremely slight strain d.r. of the optical system, despite the fact that this had been furnished by well-known manufacturers and was guaranteed to be free from strain. We found that the effect was due to the condenser lenses rather than to the objectives and that it could usually be eliminated by untightening the lens-holders a little or by turning them slightly.

These small optical errors produce a quadrant-effect only with thin cell walls, which in themselves are very weakly birefringent and then only after dichroitic staining. The phenomena described above for the overlapping of thicker cell-walls and cellophane

* This phenomenon is a well known criterion for distinguishing between fibers of flax or rameh on the one hand and hemp on the other.

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turned out not to be influenced by this small strain-birefringence, so that the explanation offered for these remains valid.

The meristematic wall of *Phycomyces* stained with congo red is an extremely delicate tool to test optical systems for freedom from strain and also nicols for exact crossing. Young cotton-hairs (10 to 15 days old) are also very suitable after a treatment with warm dilute NaOH and subsequent staining. A wrong position of the nicol prisms may also be demonstrated with the aid of a strip of cellophane stained with congo red, but this method is much less sensitive because the d.r. of the cellophane is too strong.

§ 2. ELECTRON MICROGRAPHS OF THE WALL IN THE GROWTH ZONE

OORT AND ROELOFSEN (1932) did not succeed in demonstrating with certainty a Z-spiral structure in the meristematic wall. CASTLE (1938) had expressed the belief that, apart from a transverse structure, a non chitinous material with axial orientation was present in this wall. CARNOY (1870) described the presence of many lamellae. To elucidate these points and also because electron micrographs were already known from cellulose but not yet from chitin walls, it was deemed desirable to investigate the meristematic wall of *Phycomyces* by means of this method. We found Dr A. L. HOUWINK willing to make these electron micrographs from preparations we made in collaboration with him in the Laboratory for Technical Physics in Delft.

These were finished in the beginning of 1949. Naturally they showed details of the submicroscopical structure of the wall, but instead of a spiral we found an almost transverse structure. Before publishing this, however, we preferred to await the result of our other investigations.

Meanwhile, there appeared a publication by FREY-WYSSLING AND MÜHLETHALER (1950) with electron micrographs of the same cell-wall. They employed sporangiophores in stage 1 and produced two kinds of preparations from these, one of the tips ("einigen Millimetern") and one of the basal parts. These they cleaned by boiling 4–5 times in 10% KOH and water and by oxidation with $KMnO_4$ followed by disintegration in a Waring blendor. In the extreme tips and on the outside of the wall in the remainder of the growth-zone, they found an isotropic net-structure of fibrils 150–200 Å thick. Except in the extreme tip, to the inside of this layer there had been deposited a layer consisting of a network of fibrils 250–300 Å thick with a preference for a transverse orientation. In some places they also found a layer against the inner side of the wall, in which the fibrils followed two main directions, making angles with the cell axis of 55 and 65° respectively. Sometimes one of the two directions was strongly dominant and they think that this is a transition towards the almost axial structure of the secondary wall. They mention the possibility that this double spiral structure may have resulted from an extension in an axial direction of the network with transverse structure, but they are nevertheless of the opinion that this "tennis racket" structure occurs naturally in the cell-wall. A similar structure has not yet been found in any other object. In Valonia one does indeed see two directions of structural units, but these are in separate layers (PRESTON, NICOLAI *c.s.*, 1948).

Our preparations have been obtained in a slightly different way and have a bearing on sporangiophores in stage 4b. As described in Part I, § 2c, we cut through the growth zone in an oblique direction, rolled the cell through 90° and cleaned it by heating for 30 minutes at 100° C in 5% NaOH and/or by keeping it at 37° C for 1–2 full days in

References p. 373. *(Text continued p. 367)*

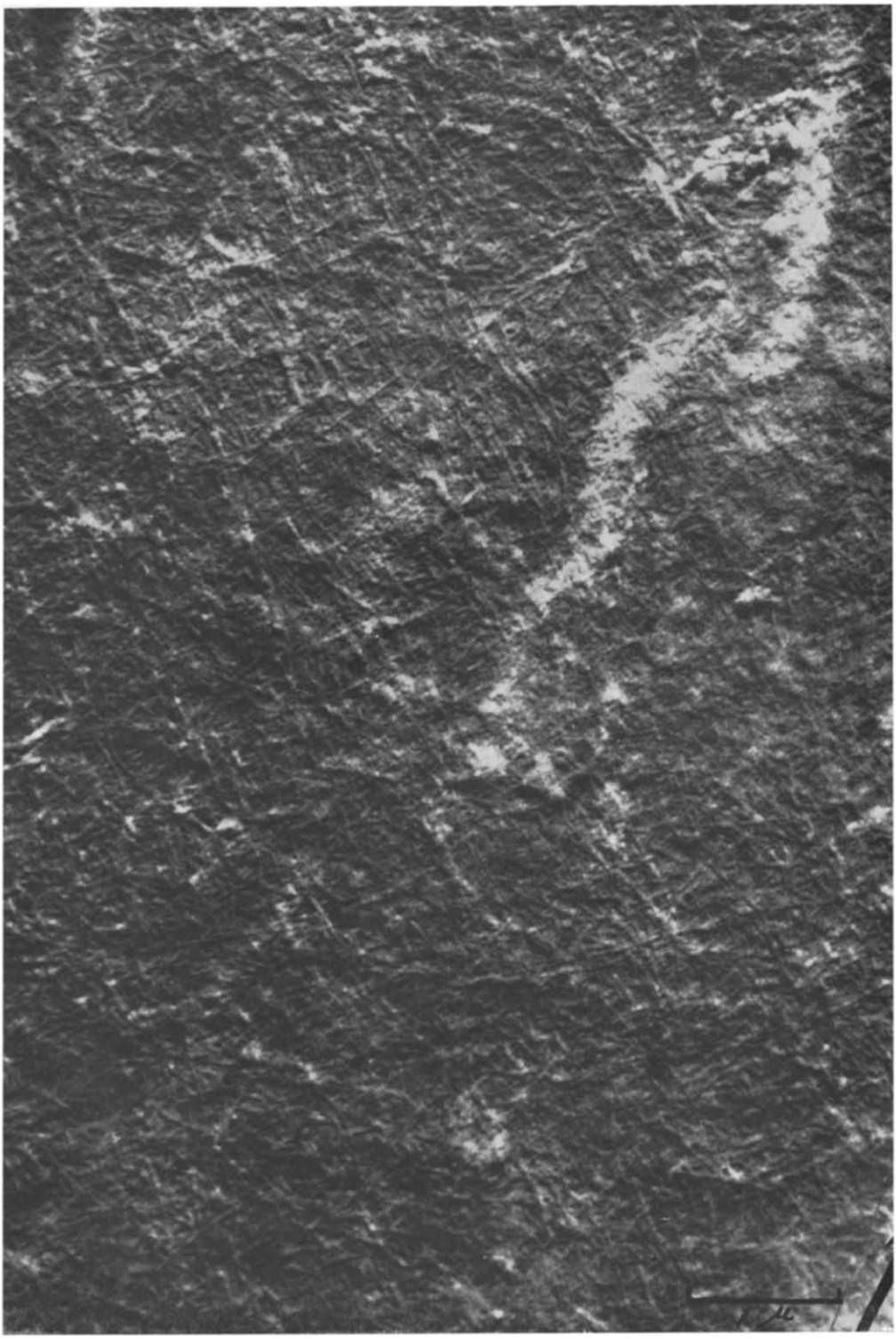

Fig. 20. Electron micrograph of the outside of the wall in the growth-zone 0.5 mm above compensation point. Cleaned in warm alkali and pancreatin. Thick line indicates cell-axis

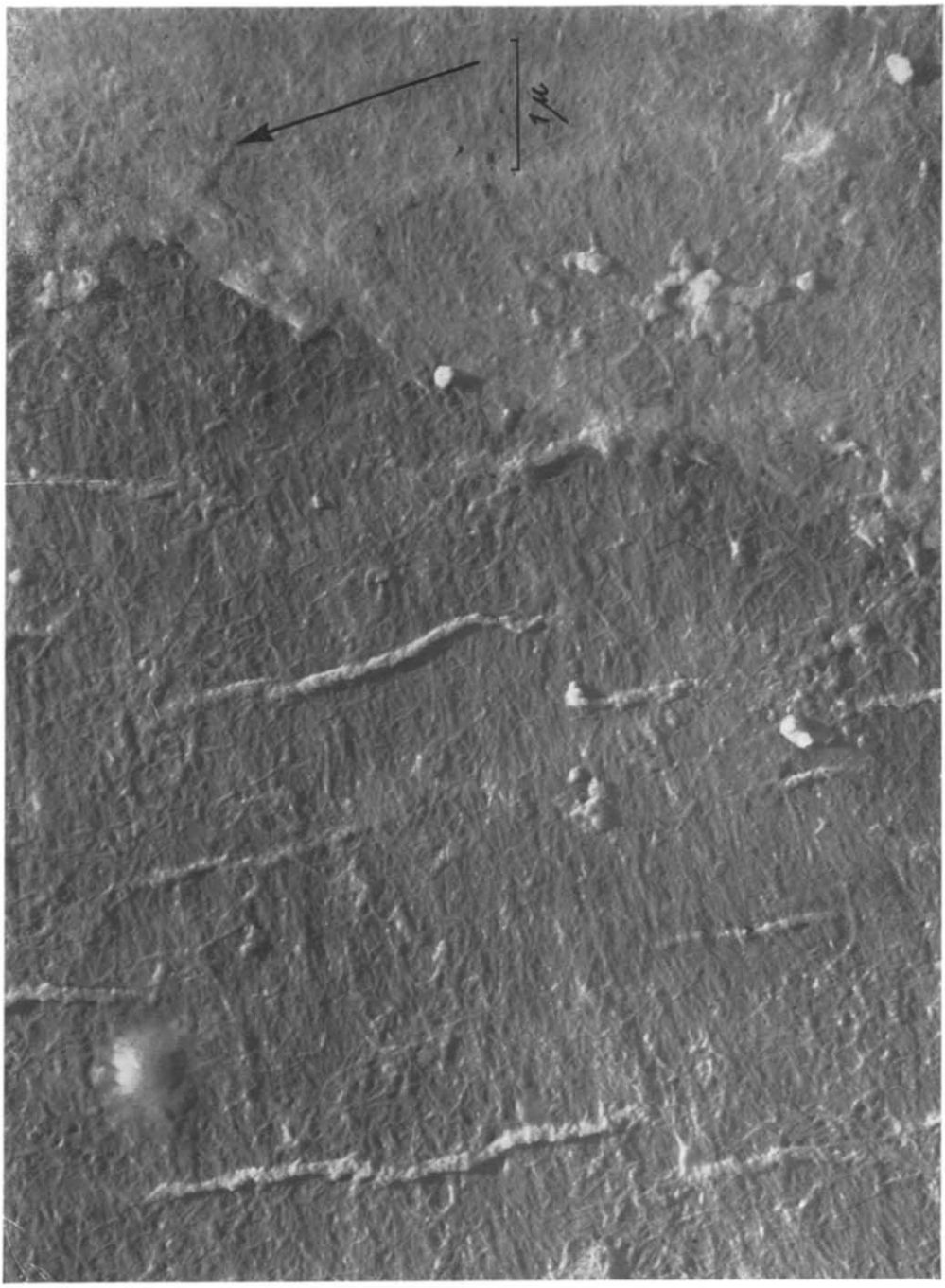

Fig. 21. Electron micrograph of inner and outer side (smaller area) of the wall in the growth-zone, 1 mm above the compensation point. Cleaned with pancreatin only

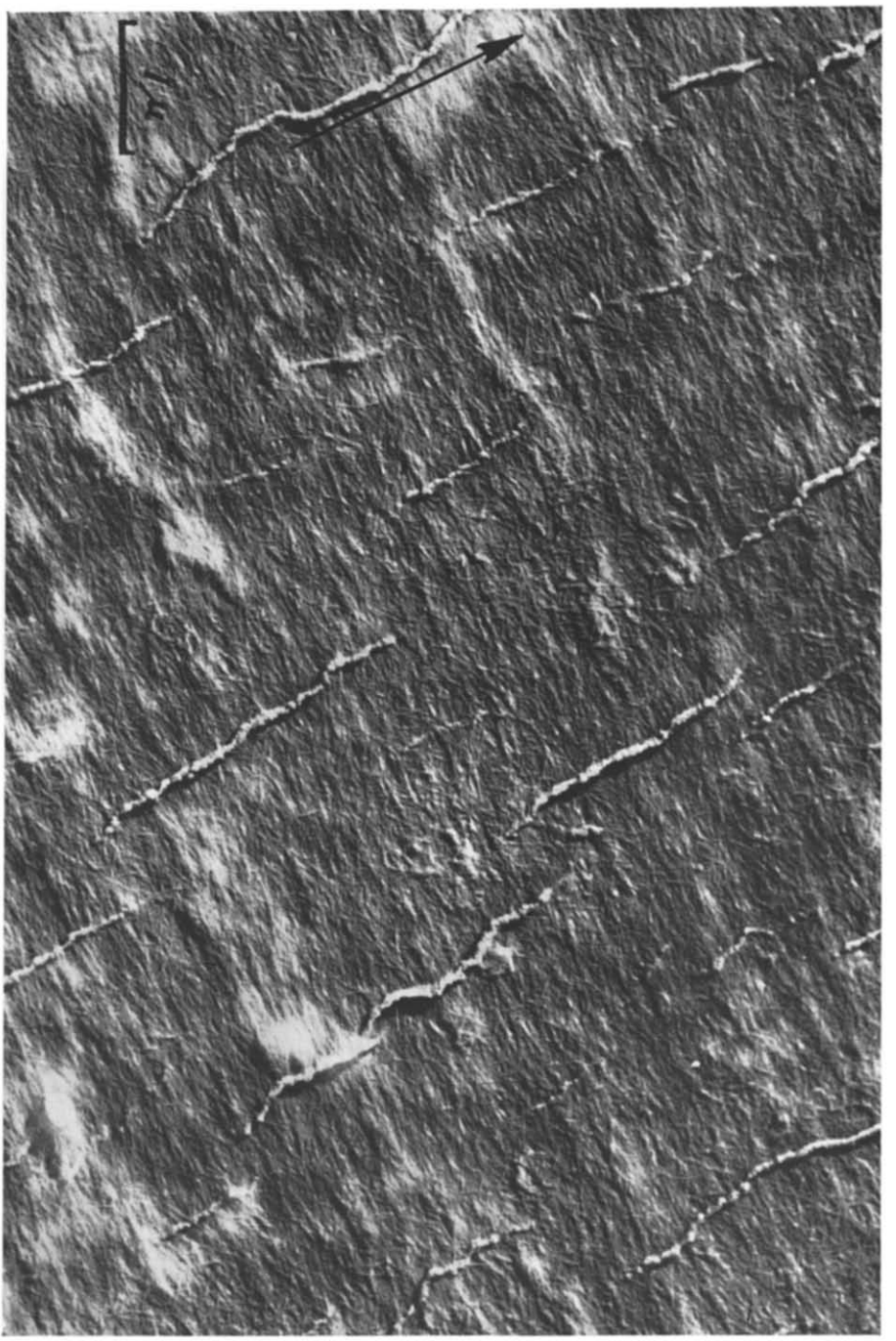

Fig. 22. Inner side of the wall 1 mm above compensation point of same cell as pictured in Fig. 21

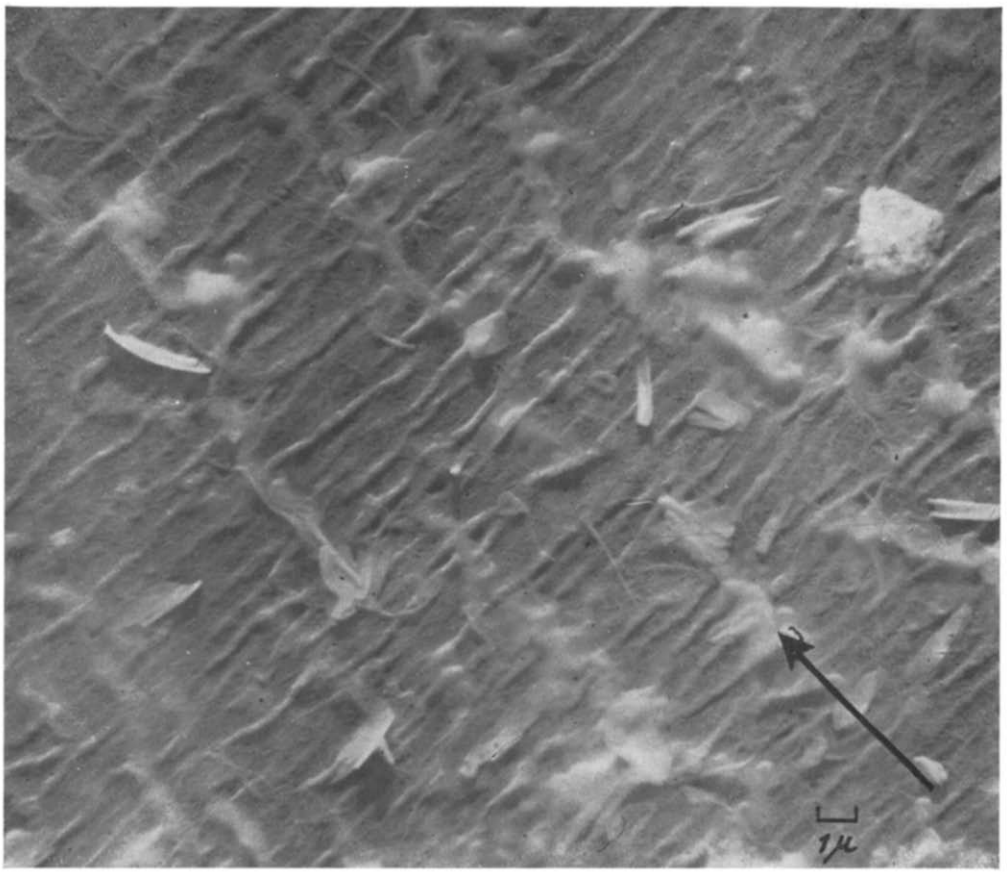

Fig. 23. Electron micrograph of inner side 0.5 mm above compensation point. Cleaned with alkali. Conspicuous transverse folds

a solution of pancreatin preserved with a drop of toluene. After that we stained the wall with congo red and noted the position of the compensation point of birefringence. Subsequently the cell, while lying in distilled water, was pulled onto the usual Parlodion covered specimen mounting grid. This was carried out in such a way that a small area of single cell-wall came over a hole in the grid. After the preparation had dried out, it was shadowed with chromium.

These preparations then, have been less subject to chemical attack and disintegration than were those of the above-mentioned investigators. Moreover, we could indicate exactly the direction of the long axis of the cell and the part of the growth-zone from which the electron micrograph was taken. We also knew whether the inner or the outer side of the wall was being photographed. We made certain of this point beforehand, but we could also tell this under the electron microscope from the "shadow" in those places where the single wall touched the double one.

The *outer side of the wall* in the growth zone of sporangiophores in stage 4 is to be seen in Figs. 20 and 21 (upper part). The preparation pictured in Fig. 20 had been previously cleaned with NaOH and pancreatin, but that shown in Fig. 21 only by

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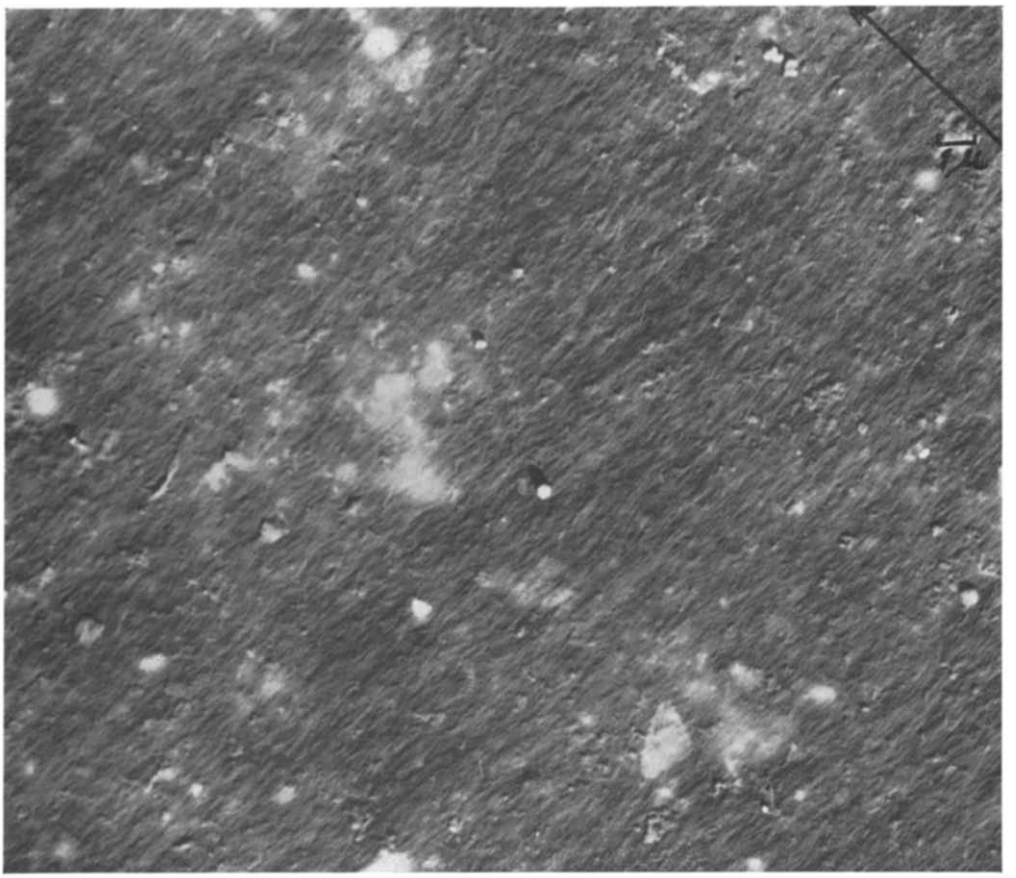

Fig. 24. Electron micrograph of inner side 1.5 mm above compensation point. Cleaned with alkali and pancreatin

heating for 10 minutes in water at 100° C followed by a 12 h treatment with pancreatin at 37° C. In both preparations a network of microfibrils is visible, which, as far as we can judge by the eye, is isotropic. FREY-WYSSLING AND MÜHLETHALER also thought that they had seen an isotropic structure on the outside. In the extreme tips of cells in stage 1 they recorded the same isotropic structure and they claim that the wall at this point consists of this one layer only. If, however, the latter observation is correct, the structure cannot be isotropic at that point, because in this extreme tip a negative d.r. is to be observed. So, there are indications that the outer layer originally had a transverse orientation, which later on was changed to an isotropic one.

This might be expected, because during the growth in surface area of the wall, microfibrils might be deposited with greater difficulty in the outer layer (which may already have been cutinized) than in the inner side, where (as we will see further on) there is a preponderantly transverse orientation and where the strong folding suggests that the wall structure is looser. The possible relation of this structure to spiral growth is discussed elsewhere (1950).

The *inner side of the wall* in the growth zone is to be seen in the Figs. 21, 22, 23

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and 24. It is clear that the fibrillar network has a preference for a transverse orientation, as was also found by FREY-WYSSLING AND MÜHLETHALER. A few fibrils only run in an axial direction and are more conspicuous for that reason. We did not find the "tennis racket" structure reported by the above mentioned authors. It is impossible to decide whether the structure is a purely transverse one or whether a very flat spiral is present. This is in agreement with the examination of the d.r. mentioned in § 1a. For the detection of spirals structure, the electron microscope is not as suitable an instrument as the polarising microscope, because:

1. it is difficult to determine the average direction of the fibrils;
2. the axis of the cell cannot be checked so easily;
3. the preparations have probably shrunk even more from severe loss of water, causing the spiral structure to become even flatter.

Towards the base of the cell the transversely orientated layer presumably gets progressively thicker, for CASTLE (1938) has found that the negative d.r. in the growth zone rises until a point about 1.8 mm below the sporangium has been reached, after which it declines sharply as a result of the deposition of secondary wall-layers.

Figs. 21 and 22 were made from one and the same cell and are taken from immediately adjacent parts. This cell had only been cleaned with warm water and pancreatin so that we can assume that all wall substances are present in it. Fig. 23 is from a preparation cleaned in alkali only, and Fig. 24 from one cleaned with alkali and pancreatin. The same picture is obtained by these different pretreatments, from which we may conclude that the cleaning with alkali does not noticeably change the wall structure.

The inner side of the wall just below the growth zone can be seen in Fig. 25, made from a preparation cleaned in alkali only. Here a strong preference for a roughly axial structure is to be noticed, though several fibrils run in a different direction, which is sometimes transverse. Nothing is to be seen of the layer with mainly transverse orientation which must be present underneath. The axial structure must be the beginning of the secondary wall.

Figs. 20–25 permit still further conclusions beside the ones regarding the microfibrillar structure. As a rule, one can observe *two kinds of fold* or undulation in the wall of the growth-zone; one orientated more or less transversely and one orientated axially. We consider them both to be the product of shrinkage of the wall caused by loss of turgor, by chemical purification and by drying out.

Sometimes the transverse wrinkles are only present in the shape of undulations, visible both on the outside (Fig. 20) and the inner side (Fig. 21, 22 and 24) of the wall. Sometimes they are pronounced little folds, as in Fig. 23. Fig. 13 (Part I) must have been made from such a wall. Since the wrinkles are always more or less transverse to the cell-axis but not always to the direction of shadowing, it is certain that their orientation is not only apparent but real. It is these wrinkles and undulations that give rise to the phenomena described in Part I, § 2c.

The axial folds that can be seen in Figs. 20–25 are smaller in numbers and are always larger and more pronounced. It is a striking fact that they are only to be found on the inner side of the wall, the outside showing just a local bulge as a result of the fold on the inside. This is most clearly demonstrated in the two parts making up Fig. 21. Presumably the inner side of the wall has a looser structure than the outer side. In Fig. 25 it can be seen that these large folds assume a transverse position as soon as the

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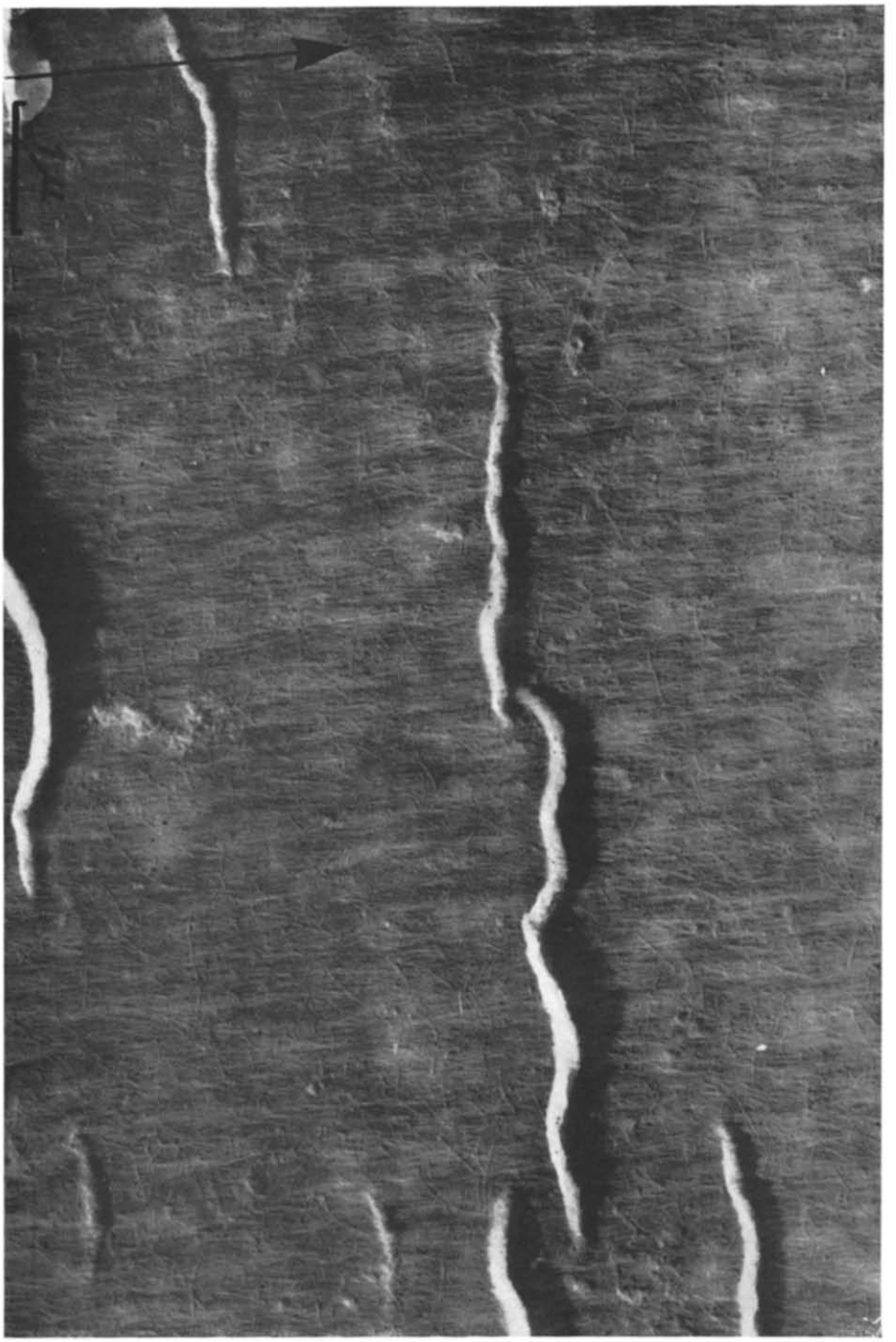

Fig. 25. Electron micrograph of inner side of the wall 0.2 mm below compensation point. Cleaned with alkali

cell wall fibrils take on a preponderantly axial orientation. It is not clear to us why there are two sizes of fold and why they have a different orientation.

Partly fixed microfibrils. In Fig. 21, and to a smaller extent in Fig. 22, 23 and 25, one notices microfibrils appearing only partly fixed within the wall, one end of each lying loose on the surface. It is probable that they had been, or were being, formed in the protoplasm and that the process of their deposition in the wall was interrupted. The fact that the microfibrils are interwoven, *i.e.* that they "creep over and underneath one another" has sometimes led to the idea that they might still grow while lying completely in the wall, (FREY-WYSSLING, MÜHLETHALER, WYCKOFF, 1948). To us, this does not seem to be necessary. The fibers in common paper for instance are also strongly interwoven, despite the fact that they are relatively much shorter and are deposited much more rapidly.

FARR (1949) believed she saw the gradual deposition of fibrils formed within plastids in Valonia. These observations were made with an ordinary microscope, by means of which, however, fibrils (200–300 Å in Valonia) cannot possibly have been seen. These may have been bundles of fibrils.

Thickness of the fibrils. In cells of stage 1 FREY-WYSSLING AND MÜHLETHALER (*l.c.*) find fibrils 150–200 Å thick on the outside, and 250–300 Å on the inner side. We have been unable to establish this difference in cells of stage 4. The thickness of the fibrils in our photographs is to be estimated at 150–250 Å, both for the outside and the inside. It remains a possibility that there is a difference in this respect between cells of stage 1 and those of stage 4, for it is a fact that between crossed nicols the tips of cells in stage 1 appear strikingly more delicate in their structure than the growth-zones in stage 4.

We were struck by the fact that in Fig. 21 so little is to be seen of the *cuticle* which must, however, be present on the outside, for this preparation had not been heated in alkali. The image of the double wall is admittedly fainter than that of the single one, but this may be caused by the fact that the object is thicker in the former case. One either has to accept the possibility that the cuticle, which indeed is very loose (Part I, § 2b), has disappeared in the process of preparation and cutting the wall, or one has to presume that the cuticle is so thin, that the sculpture of the wall underneath is shown just as well with as without it.

Fig. 26 is a picture of a cuticle membrane obtained from the liquid

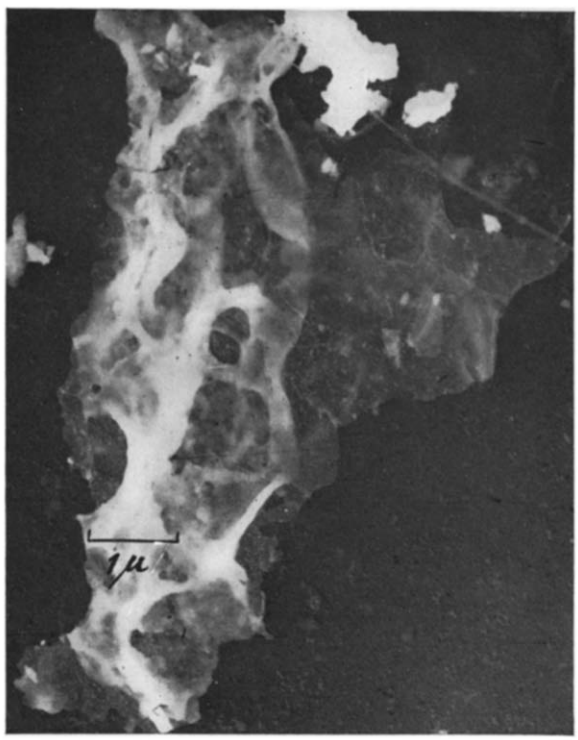

Fig. 26. Electron micrograph of piece of cuticle. Note lack of structure in area where the cuticle is not curled up

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droplets described in Part I, § 2b and pictured there in Fig. 12. Sporangiophores in growth-stage 2 were moved back and forth in a drop of water. As a result, the bubbles burst open and many tiny pieces of membrane floated on the surface. If the usual copper-grid with Parlodion-membrane is laid on the water surface and taken off again, the tiny pieces of membrane adhere to the Parlodion. One can also allow a drop to dry out on a grid. Sometimes pieces of the cuticle also adhere to the Parlodion if this is simply touched by a sporangiophore.

In such micrographs no structure at all is to be seen in the cuticle, it being clear at the same time that the latter is very thin, because hardly any shadow-area is present along the edges.

ACKNOWLEDGEMENTS

It is a pleasure to thank Dr A. L. HOUWINK and the Institute for Electron Microscopy at Delft for preparing the electron micrographs, Ir P. REINOUTS VAN HAGA for rendering assistance in making the photographs, and Drs B. J. D. MEEUSE and L. J. AUDUS for the translation and correction of this article.

SUMMARY

In addition to the experiments, described in Part I of this publication (*Biochim. Biophys. Acta*, 6 (1950) 340), on the spiral structure of the cell wall in the growth zone of *Phycomyces* sporangiophores, this wall was also studied with a polarising microscope. In several cells, cleaned with hot alkali and acid and stained with congo red, the single wall in the growth zone indeed showed an oblique direction of extinction, conforming to a Z-spiral structure. In most cells however, the extinction is apparently transverse. Probably, because as a result of the shrinking of the cell wall due to loss of turgor pressure and chemical cleaning, the spiral, originally very flat, becomes flatter still. With cells coloured with congo red after cleaning with alkali only, no reliable results could be obtained (§ 1a).

Dichroitic staining strongly intensifies the "quadrant effect" of two obliquely overlapping birefringent cell walls.

A weakly birefringent cell wall stained with congo red, is a sensitive test-object to discover strain birefringence in the optical system of the microscope (§ 1b).

Electron micrographs of the wall in the growth zone largely corroborate the observations of others. The outer layer consists of a network of micro-fibrils that seems to be isotropic (Figs. 20, 21). The inner layer shows a roughly transverse orientation; the estimation of the average fibril direction is too uncertain to detect eventual flat spiral structures (Figs. 21—24). In the lowest part of the growth zone and below it, almost axially orientated fibrils are deposited onto the layers mentioned; this is the secondary thickening (Fig. 25).

All chitin fibrils in the cell walls studied, are of about the same thickness: 150–250 Å units. One gets the impression that they are first fixed within the cell wall by one end (Fig. 21).

Isolated pieces of the cuticle are very thin and devoid of any structure (Fig. 26).

RÉSUMÉ

En continuation des expériences décrites dans la première partie de cette publication (*Biochim. Biophys. Acta*, 6 (1950) 340) concernant la structure en forme de spirale de la paroi cellulaire en état de croissance des sporangiophores de *Phycomyces*, nous avons étudié cette paroi au microscope polarisant. Dans plusieurs cellules, purifiées à l'alcali chaud et à l'acide, puis teinte au rouge Congo, la paroi de la zone de croissance montrait, en effet, une extinction oblique conformément à une structure en forme de spirale en Z. Dans la plupart des cellules, cependant, l'extinction semblait être transversale. Probablement le rétrécissement des parois cellulaires dû à la perte de pression interne et au traitement chimique avait aplati la spirale, déjà très plate à l'origine. Les cellules colorées au rouge Congo après traitement à l'alcali seul ne nous ont pas donné de résultats satisfaisants (§ 1a).

Une coloration dichroique intensifie considérablement "l'effet de cadran" produit par la superposition oblique de deux parois cellulaires biréfringentes.

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Une paroi cellulaire faiblement biréfringente teinte au rouge Congo est un objet sensible permettant de découvrir la biréfringence de tension du système optique du microscope (§ 1b).

Des photographies prises au microscope électronique de la paroi croissant confirment les observations faites par d'autres chercheurs. La couche externe consiste d'un réseau de micro-fibrilles qui semble être isotropique (Fig. 20, 21). La couche interne a une orientation à peu près transversale; l'on ne peut déterminer la direction moyenne des fibrilles de façon suffisamment précise, pour découvrir des structures éventuelles en forme de spirale plate (Fig. 21–24). Dans la partie inférieure de la paroi croissant et en dessous des fibrilles à orientation environ axiale sont déposées sur les couches mentionnées: c'est le renfort secondaire (Fig. 25).

Toutes les fibrilles de chitine de la paroi cellulaire que nous avons étudiées, ont environ la même épaisseur de 150–250 Å. Nous avons eu l'impression qu'elles sont d'abord fixées par un bout à l'intérieur de la paroi cellulaire (Fig. 21).

Des morceaux isolés de la cuticule sont très minces et exempts de toute structure (Fig. 26).

ZUSAMMENFASSUNG

Als Forsetzung der im ersten Teil dieser Arbeit (*Biochim. Biophys. Acta*, 6 (1950) 340) beschriebenen Versuche über die Spiralstruktur der Zellwand der Wachstumszone von *Phycomyces* Sporangiophoren, haben wir diese Wand mit dem Polarisationsmikroskop untersucht. In verschiedenen Zellen, die mit heissem Alkali und Säure gereinigt und mit Kongorot gefärbt waren, zeigte die einfache Wand der Wachstumszone wirklich eine schräge Auslöschungsrichtung, was mit einer Z-Spiral-Struktur übereinstimmt. In den meisten Zellen aber ist die Auslöschung offenbar transversal. Wahrscheinlich plattet die Schrumpfung der Zellwand, die durch den Verlust des Turgor-Druckes und durch die chemische Reinigung bewirkt wird, die ursprünglich schon sehr flache Spirale noch mehr ab. Mit Zellen die nur mit Alkali gereinigt und dann mit Kongorot gefärbt waren, konnten keine verlässlichen Ergebnisse erhalten werden (§ 1a).

Dichroitische Färbung verstärkte den "Quadrant-Effekt" zweier, sich schief überschneidender doppelbrechender Zellwände, bedeutend.

Eine schwach doppelbrechende, mit Kongorot gefärbte Zellwand ist ein empfindliches Prüfmittel zur Entdeckung von Spannungs-Doppelbrechung im optischen System des Mikroskopes (§ 1b).

Elektronenmikrogramme der wachsenden Wand bestätigen weitgehend die Beobachtungen anderer Forscher. Die äussere Schichte besteht aus einem Netz von Mikrofibrillen, das isotrop zu sein scheint (Fig. 20, 21). Die innere Schichte zeigt eine praktisch transversale Richtung; die Bestimmung der durchschnittlichen Fibrillenrichtung ist zu ungenau, um etwaige flache Spiralstrukturen aufzuzeigen (Fig. 21–24). Im untersten Teil der Wachstumszone und darunter befinden sich axial gerichtete Fibrillen auf den oben erwähnten Schichten; dies ist die sekundäre Verdickung (Fig. 25).

Alle Chitinfibrillen der untersuchten Zellwände haben ungefähr dieselbe Dicke von 150–250 Å. Es scheint, dass sie sich erst an einem Ende im inneren der Zellwand befestigen (Fig. 21).

Isolierte Teile der Kutikula sind sehr dünn und zeigen keine Struktur (Fig. 26).

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Received June 7th, 1950