THE STRUCTURE OF THE PRIMARY EPIDERMAL CELL WALL OF AVENA COLEOPTILES*

By S. T. BAYLEY, Ph.D., J. R. COLVIN, Ph.D., F. P. COOPER, and CECILY A. MARTIN-SMITH

(From the Division of Applied Biology, National Research Council, Ottawa, Canada)

Plates 32 to 35

(Received for publication, January 14, 1957)

INTRODUCTION

In the primary walls of most elongating plant cells, the cellulose microfibrils show a net orientation transverse to the major cell axis with wide angular dispersion about the common direction. This type of structure has been called "tubular texture" by Frey-Wyssling (1).

Not all the microfibrils in elongating cells conform to this pattern, however. In developing *Avena* coleoptiles, for example, longitudinal microfibrils have been detected in both epidermal and parenchyma cells. Heyn (2) concluded from an x-ray diffraction study, that the majority of cellulose microfibrils in dried epidermal cells run longitudinally. Mühlethaler (3) examined dispersed coleoptile cells directly in the electron microscope and found tubular texture in both epidermal and parenchyma cells. In addition, however, he observed that in epidermal cells there were large numbers of longitudinal microfibrils along the outer wall only, while between the primary pits in parenchyma cell walls there were ribs composed of longitudinal microfibrils showing little angular dispersion.

Since cell division in the epidermal cells of a developing coleoptile is completed at an early stage and subsequent growth results entirely from cell elongation, the walls of such cells are useful for testing models of cell wall growth. Their submicroscopic structure has therefore been reexamined by methods which minimize artifacts introduced by drastic chemical and mechanical treatments. Previous results have largely been confirmed; in addition, new structural information has been obtained, mainly from the examination of thin sections in the electron microscope.

Experimental Methods

Studies were made on coleoptiles of Lanark oats germinated on damp filter paper in the dark. Thin sections of epidermal cells and cell wall fragments were examined in the electron microscope, and, to investigate microfibril orientation, birefringence measurements were made

* Issued by National Research Council No. 4280.

on sections of coleoptiles and low-angle x-ray scattering patterns obtained from coleoptile specimens.

Electron Microscopy—Thin Sectioning.—Sections were cut from the central region of coleoptiles ranging in length from 2 to 40 mm.

To remove pectic and other non-cellulosic materials, coleoptiles were treated with pectinase (4) prior to fixation. Coleoptile segments, 1 to 2 mm. long, were immersed in a 10 per cent solution of commercial pectinase (Nutritional Biochemicals Corporation, Ohio) in 1 per cent peptone. The length of time necessary to give satisfactory removal of pectin without causing complete disintegration of the tissue varied with age of coleoptile from 1 to 6 hours.

Coleoptile segments, 1 to 2 mm. long, with and without prior pectinase treatment, were fixed in 1 per cent osmic acid at pH 7.3–7.5 in acetate-veronal buffer for 4 hours (5). They were then dehydrated and embedded in methacrylate (1 part methyl:4 parts *n*-butyl) with 0.1 per cent benzoyl peroxide as catalyst; polymerization was carried out either at 45° C. or 80° C. Sections were cut with glass knives either on a Servall Porter-Blum microtome or on a microtome designed by Hodge *et al.* (6) and built by the Scientific Equipment Corporation, Waltham, Mass.

Embedding medium was removed from sections with amyl alcohol followed by amyl acetate (7), after which the sections were shadowed at 15° with palladium-gold.

The thickness of a few sections was estimated with a Dyson interferometer microscope. For this purpose, a series of sections was cut uniformly thick as judged from the colour by reflected light; some of these sections were examined in the electron microscope while the remainder were mounted in air and their thickness measured at wave length 5460 A. The refractive index of the methacrylate-embedding medium was found to be 1.533 using immersion oils; variation in refractive index between samples was insignificant.

Other Electron Microscope Preparations.—Epidermal layers were stripped from 10 to 15 mm. long coleoptiles and treated in a pectinase solution as above for 6 hours. The cells were then dried onto a microscope slide and the walls stripped off with a formvar film, which was washed and mounted in the usual way.

Fragments of cell walls were prepared by the method of Scott *et al.* (8)[1]. Epidermal layers were frozen on solid CO_2, the cells fragmented, and the cytoplasm dissolved by immersion in ethanolamine for 6 to 24 hours. The wall fragments were then washed and mounted.

Birefringence Measurements.—Microfibril orientation was studied by the method which Wardrop and Preston (9) employed on conifer tracheids and wood fibres; in the present material, however, conditions are less favourable owing to the lower concentration of cellulose. The method consists essentially of cutting sections through the tissue at different angles to the cell axes and measuring the birefringence of the walls parallel to the direction of cut throughout the series of sections. The birefringence is greatest in the wall section whose plane is parallel to the microfibrils.

Segments, 3 to 4 mm. long, were cut from 20 mm. long coleoptiles, fixed, and embedded in methacrylate as described in the previous section. Sections about 8 μ thick were then cut at the desired orientation and mounted in water. Optical retardations were measured at wave length 5460 A on a polarizing microscope fitted with a de Senarmont compensator and the thickness of the sections measured in the clear portions of the methacrylate with a Dyson interferometer microscope as described above.

Low-angle X-ray Scattering.—Low-angle scattering of x-rays by cellulose fibres is ascribed to the crystalline regions. The scattering pattern is elongated perpendicular to the direction of the individual cellulose microfibrils indicating that the crystalline (ordered) regions are elongated parallel to the microfibrils. For samples in which the microfibrils are arranged heli-

[1] We are grateful to Professor Scott for providing details of the technique prior to publication.

cally, the scattering pattern forms a cross, the arms of which are at twice the angle of the fibrillar helix (10). Under similar circumstances, the wide-angle pattern of cellulose is drawn into wide arcs from which it is sometimes difficult to deduce the orientation of the cellulose chains (11). The low-angle pattern, however, gives a clear indication of microfibrillar direction and the reason appears to lie in the lamellar form of the crystalline regions in cellulose. (For a fuller explanation see reference 12).

The size of the crystalline regions and the extent of the x-ray scatter depend on the state of hydration of the cellulose. Studies on swollen cellulose (16), however, show that this dependence does not interfere with the interpretation of microfibrillar orientation from the scattering pattern. Furthermore, observation of this pattern, unlike that of the wide-angle pattern, is not seriously hindered by scatter from unbound water in the specimen. In studies of delicate material of the present type, therefore, the technique of low-angle scattering is particularly valuable since it enables microfibrillar orientation to be determined in native, undried tissue which has undergone little or no preparative treatment.

Low-angle patterns were obtained from whole coleoptiles (less foliage leaves), 20 to 30 mm. long, and from bundles of epidermal strips from coleoptiles of the same length. A modified Philips microcamera was used with Ni-filtered Cu Kα radiation. During exposures, the camera was evacuated and to keep the specimen moist, it was sealed in a thin-walled Lindemann glass capillary.

RESULTS

Electron Microscopy.—Coleoptiles which were fixed, sectioned, and examined without further treatment gave little information about wall structure owing to lack of contrast. After the removal of embedding medium and shadowing, their appearance was as shown in Figs. 1 and 2. The outer epidermal wall appears to consist mainly of a structureless mass of non-cellulosic material although towards the inside there is some indication of layering. On the outside, the cuticle can be distinguished as a structureless membrane about 0.2 μ thick. Individual variation in total wall thickness is quite large but over the range of coleoptile lengths examined it doubles roughly, reaching 2 to 3 μ in the older tissue.

Cellulose microfibrils were revealed in sections of coleoptiles which had been treated with pectinase before fixation. Without the removal of embedding medium, some indication of microfibrils was obtained (Fig. 3). After the removal of embedding medium and shadowing, individual microfibril segments are seen clearly in multiple layers within the outer epidermal wall (Figs. 4 to 10). Where two epidermal cells touch, these layers gradually fuse to form the normal type of primary wall between two cells (Figs. 5, 7, and 9).

Figs. 4 to 10 represent the appearance in transverse section of the outer epidermal wall of coleoptiles ranging in length from 2 to 3 up to 40 mm.; longitudinal sections were generally less informative because of the difficulty of getting exactly radial sections through individual cells. Owing to disruption of the microfibrillar arrangement during removal of embedding medium, it is difficult to be certain of the number of layers present in the wall. In general, however, there appear to be at least 10 to 15 even in the youngest coleoptiles examined (Fig. 4) while in the older, this increases to about 25 (Figs. 8 and 10). During

the same period, the density of deposition of microfibrils within a layer appears to increase also.

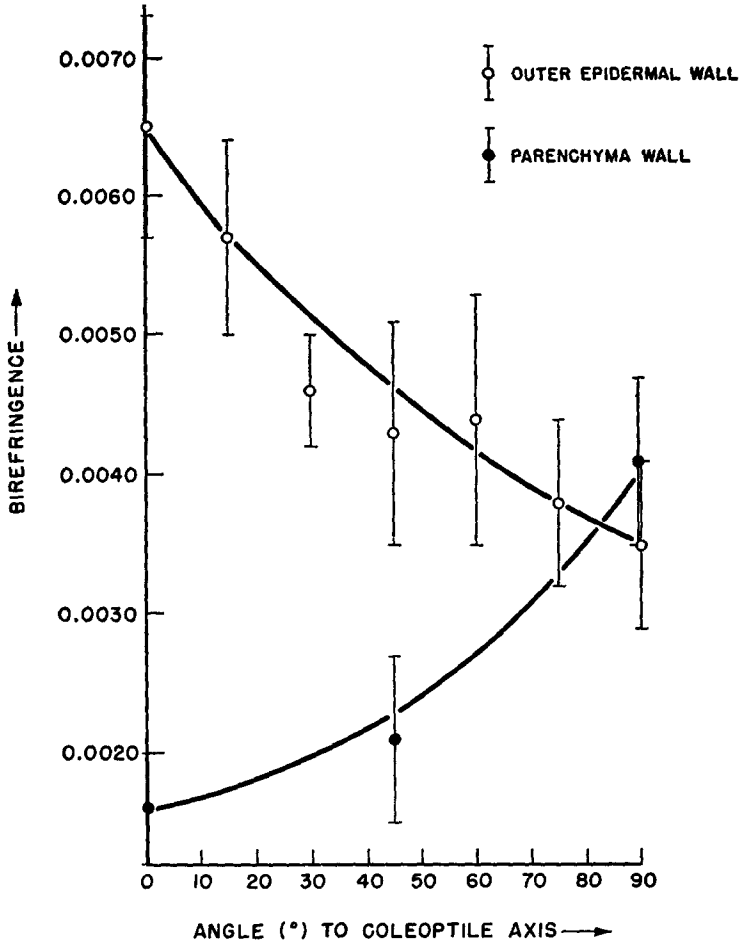
The appearance of isolated epidermal cell walls which had been treated with pectinase was similar to that observed by Mühlethaler (3). The interior walls consist of transversely oriented microfibrils while the outer wall is much thicker and superficially is composed of longitudinally oriented microfibrils. An interesting feature of the isolated cells examined here was that they often showed a "fringe" of parallel microfibrils issuing from the edge (Fig. 11). The microfibrils in such a fringe are predominantly parallel and extend for tens of microns. These fringes may be due either to tearing of the cell wall during preparation or to an extensive loosening of the microfibrillar structure through the removal of non-cellulosic material. In either case, the effect is much more extensive than is observed in other types of primary wall which have been torn, and probably reflects a real structural difference: the microfibrils in the outer layers of these epidermal walls cannot exhibit the wide angular dispersion or the interweaving which prevents extensive unravelling in normal primary walls (1).

The observations on isolated epidermal cells just described give a clear indication of microfibril orientation. The transverse orientation in the interior walls was confirmed by the appearance of wall fragments. In sections, the length and arrangement of microfibril segments were generally in agreement with these conclusions. Thus in longitudinal section, the microfibrils in the layers of the outer wall were of considerable length while in transverse section they appeared as segments whose lengths agreed closely with the measured thickness of the section (Fig. 7).[2] The converse was true of the interior walls of epidermal cells.

Confirmation of microfibril orientation was also obtained independently of electron microscopy by birefringence measurements and low-angle x-ray studies.

Birefringence Measurements.—Two series of sections were used. In one, all sections were from the same coleoptile, 20 mm. long, but of necessity represented different regions. In the second series, the same region, 5 to 10 mm. from the tip, from different coleoptiles of length 20 mm. was used. The results from both series were identical, within experimental error, and are plotted in Text-fig. 1. For comparison, similar results for parenchyma wall are included. The birefringence is definitely greater in longitudinal than in transverse section of the epidermal wall whereas the converse is true in parenchyma wall. This suggests predominantly longitudinal orientation of microfibrils in the epidermal wall and predominantly transverse orientation in parenchyma wall, results

[2] High accuracy was not aimed for in these measurements. Nevertheless, present experience suggests that transverse sections of measured thickness cut from a system of parallel microfibrils, for example the secondary wall of ramie, may provide useful specimens for magnification calibration of electron microscopes.



TEXT-FIG. 1. Birefringence of epidermal walls of 20 mm. coleoptiles as a function of the angle of the section to the coleoptile axis.

which are in accord with observations mentioned earlier. Admittedly, similar birefringence results for the epidermal wall would be given if the microfibrils in alternate layers made appreciable angles with the cell axis but of opposite sign. From the other observations, this does not seem likely, however.

In discussing birefringence, the contributions from other wall constituents, in particular the cuticle and waxes, must be considered. In this respect, methacrylate embedding is an advantage since, because of its refractive index, form birefringence is negligible. No optical study has been made of the cuticle. However, Roelofsen and Houwink (13) found that the cuticle of staminal hairs of *Tradescantia virginica* had a very small intrinsic birefringence. The cuticle of

the coleoptile is probably similar and since, in any case, it forms a separate outer layer to the epidermal wall, it seems safe to ignore its effect in the present discussion. More serious, however, is the effect of waxes within the walls. These have been studied in coleoptile walls by K. and M. Wuhrmann-Meyer (14) who concluded that the wax molecules formed cylindrical rods in the spaces between cellulose microfibrils. The wax molecules are arranged radially about the axes of the rods, which in turn are parallel to the microfibrils. The birefringence of these rods will therefore be negative with respect both to their own axes and to the cellulose microfibrils. Thus in the present study, the variation in the birefringence of the wax will be similar, but of opposite sign, to that of the cellulose. The effect of the wax therefore will be to reduce, but not alter the form of, the variation in birefringence with angle which would be observed from cellulose alone.

Low-Angle X-ray Scattering.—All the low-angle x-ray patterns obtained from specimens of coleoptiles 20 mm. long suggested some angular dispersion of the microfibrils. Nevertheless, the pattern obtained from a whole coleoptile examined wet was elongated parallel to the axis of the coleoptile; *i.e.*, transverse microfibrils predominate. A bundle of epidermal strips examined wet, on the other hand, gave a scattering pattern elongated perpendicular to the strips; *i.e.*, longitudinal microfibrils predominate (Fig. 12). The pattern from epidermal strips was not altered by treatment of the strips with pectinase for 6 hours. The process of stripping the epidermis is unlikely to alter the microfibril direction completely from transverse to longitudinal, so that these patterns are additional evidence for the longitudinal arrangement in the epidermal wall. For the whole coleoptile, the scatter from two thicknesses of epidermal wall is probably too weak to be recorded, so that the scatter observed will be due to interior walls composed chiefly of transversely oriented microfibrils.

Microphotometer traces were obtained from the low-angle patterns of wet epidermal strips and wet whole coleoptiles. If the crystallites in the cellulose microfibrils are assumed to compose a system of parallel cylinders scattering independently, then the exponential equation of Guinier (15) relating intensity of scattered radiation to scattering angle can be used to calculate the diameter of the cylindrical crystallites. The result obtained was about 40 A for both epidermal strips and whole coleoptiles. Although this is similar to the results quoted for other cellulose systems (16), it must be regarded only as an approximate value in view of the lack of monochromatic radiation and of knowledge of the scattering properties of the wet, undried cellulose microfibrils. Guinier and Fournet (12) have stressed the importance of these requirements before low-angle scattering patterns can be properly interpreted.

In addition to the central scatter, a ring corresponding to an interplanar spacing of about 44 A (using Bragg's equation) was obtained from both whole coleoptiles and epidermal strips and was more marked with dry specimens

(Fig. 13). This reflection, which remained after epidermal strips had been treated for 6 hours in pectinase, is probably due to waxes within the walls. Gundermann *et al.* (17) extracted and examined waxes from various plants. However these authors found that in the extracted form, the wax from *Avena* coleoptiles gave a reflection corresponding to a spacing of 60 A.

DISCUSSION

Structure.—The above results show clearly that the outer wall of the epidermal cell consists of multiple layers of cellulose microfibrils, the majority of which are oriented longitudinally. Between individual layers and in the broader region between the layers and the cuticle, there are encrusting substances the bulk of which can be removed by pectinase. The radial walls, on the other hand, contain a single layer of cellulose microfibrils which are oriented transversely.

The structural pattern of these walls is established before the coleoptile reaches 2 mm. in length. As the coleoptile elongates to 40 mm., the number of layers and the thickness of the outer wall roughly double. In addition, the longitudinal orientation of the microfibrils in the layers improves, as judged by the length of the microfibril segments in transverse section.

Mühlethaler (3) has suggested that the transverse microfibrils of the inner walls of the epidermal cell form a continuous layer on the inside of the outer wall; this layer then serves as a foundation on which the mass of longitudinal fibrils is deposited. The detail revealed in transverse sections does not support this suggestion, however, since the layers of microfibrils forming the radial wall between two neighbouring cells cannot be identified with any one layer of the outer wall. Instead, as previously mentioned, the single layer of the radial wall divides gradually until the full complement of layers in the outer wall is established.

A detailed picture of the transition in microfibril orientation from transverse in the radial wall to longitudinal in the outer has unfortunately not been obtained. From the lengths of microfibril segments in transverse sections, the transition appears to occur gradually with the change in number of layers, although in the inner layers it often takes place further from the junction than in the outer (see regions *B*, Figs. 7 and 8). The distribution of segment lengths in longitudinal sections also support these observations. In a few instances, the microfibrils in an inner, but not necessarily the innermost, layer have been observed to remain transversely oriented for almost the whole width of the cell (Fig. 6), but this does not appear to be common (*cf.* Fig. 5 from a coleoptile of the same age). A possible explanation of the transition is that, at the junction with the outer wall, the transverse microfibrils of the interior wall are divided numerically between the multiple layers and encompass the epidermal cell completely. With the exceptions just quoted, such transverse microfibrils have not been observed although they may be obscured by the greater number

of longitudinal microfibrils. However, their existence in the outermost layers seems doubtful in view of the formation of microfibrillar fringes described earlier. These fringes suggest that these outer layers of parallel microfibrils can be separated relatively easily from the remainder of the wall and are not woven in to any appreciable extent by microfibrils of markedly different orientation. Other explanations of the transition are that the transverse microfibrils end some distance within the outer layers or that they change their orientation.

Growth Mechanisms.—Various models have been proposed in recent years to explain wall growth. On the basis of observations on isolated cells, Mühlethaler (3) suggested that parenchyma and epidermal cells in *Avena* coleoptiles extend by bipolar tip growth. To elongate the cell, the protoplasm extends at the tips and deposits new wall material as it advances. However, later evidence from coleoptiles has not confirmed this suggestion. In epidermal cells, growth measurements by Castle (23) and autoradiographic studies by Wardrop (22) both showed that growth is not confined to the cell tips; autoradiographic studies on parenchyma cells gave similar results.

Wardrop (24) examined the distribution of pits in parenchyma cells of *Avena* coleoptiles and obtained strong evidence that growth occurred over the whole length of these cells. This author concluded that the points at which plasmodesmata penetrate the wall, in particular the primary pit fields, are the active regions of growth and are responsible for cellulose synthesis. The growth of cortical cell walls in onion root tip has also been explained in this way by Scott *et al.* (8). The "islands of synthesis" observed in conifer cambium by Preston and Ripley (25) were thought by Wardrop to be fragments of protoplasm displaced from plasmodesmata during preparation. Wardrop's proposal is a modification of the model of "mosaic growth" put forward by Frey-Wyssling and Stecher (26, 27). In this, the cell wall is penetrated transiently by the protoplasm at various points. At these points, cytoplasmic synthesis occurs forcing the microfibrils apart and extending the surface, so as to create "thin areas" into which new microfibrils are subsequently woven.

Both Roelofsen (18) and Tripp *et al.* (19) have observed that the walls of young cotton hairs are composed of microfibrils oriented longitudinally on the outside and transversely on the inside. Similar structures have since been found in other cells (20, 21) and to explain them, Roelofsen and Houwink (20) have proposed a theory of "multinet growth." These authors suggest that during cell elongation new microfibrils are deposited transversely on the inside of the wall. As growth proceeds, the extension of the wall causes a reorientation of the microfibrils already deposited, resulting ultimately in a longitudinal arrangement on the outside while the freshly deposited inner microfibrils exhibit transverse orientation. In his latest note, Wardrop (22) favours such a mechanism for parenchyma cells of *Avena* coleoptiles.

None of these models is fully satisfactory in explaining the present observa-

tions, however. For a multilayered wall, multinet growth would involve the deposition of distinct layers of transverse microfibrils on the interior of the wall. As elongation proceeded, the microfibrils should show a progressive change in orientation from transverse in the interior layers to longitudinal in the exterior layers of the wall. In addition, the outer layers should become more tenuous and gradually lose their identity. The micrographs do not, however, support such a model for the outer epidermal wall. Some variation in the microfibril orientation is apparent in the interior layers, particularly of younger walls. However, such a variation would be accounted for largely by the wider angular dispersion in younger walls and by the transition in orientation at the junction of the inner and outer walls of the cell already discussed. A more serious objection concerns the number and identity of the layers in the wall. For the range of coleoptile lengths examined, representing an elongation of epidermal cells of almost 20 times, the number of layers and the thickness of the wall double. New layers must therefore be deposited, but their number does not increase in proportion to elongation as would be expected if this were the means of reinforcing the wall. Furthermore, there is no indication that the outer layers in older coleoptiles become thinner and lose their identity. Indeed, the layering in the 40 mm. coleoptile shown in Fig. 10 is remarkable for its distinctness and regularity throughout the width of the wall. It is evident therefore that new wall material must be laid down in all layers as elongation proceeds.

Since the outer walls of epidermal cells contain no pits or plasmodesmata, mosaic growth could only be brought about through very deep transient penetration by the protoplasm. During penetration, or indeed as a prerequisite for penetration, the protoplasm would have to soften or dissolve the layers of noncellulosic material within the wall in some unknown way. The individual microfibrils would then be free to move relative to one another and allow either the extension of existing microfibrils or the intercalation of new. However no evidence has been obtained in the present study that the protoplasm does actually penetrate deeply into the outer wall.

A logical extension of the mosaic theory would be to assume that the wall components are reinforced continuously throughout the wall by the diffusion of low molecular weight precursors. These would then be attached to the ends of existing high polymer chains either enzymatically or spontaneously through suitable energy-rich bonds.

The epidermal cells of *Avena* coleoptiles are not the only instance where the walls of the cell during elongation are unevenly thickened and composed of cellulose microfibrils showing markedly different orientations; an example of uneven thickening is provided by the walls of collenchyma (28), while examples in which microfibrils of different orientations are present were given earlier. Furthermore it is feasible to assume that the mechanism for the growth of such walls is basically similar to that in walls of simpler construction. The

more complex walls therefore provide a severe test of any model for the growth of the primary wall and the orientation of the microfibrils. The epidermal walls discussed here are particularly interesting since not only is the elongation in them large but also, because of their thickness and accessibility, they can be readily studied by a variety of techniques. It is evident from the present results, however, that before an adequate model for the extension of these cell walls can be established, further information is required on the fibrogenesis of cellulose and on the mode of interaction of cytoplasm with cell wall constituents.

SUMMARY

The primary walls of epidermal cells in *Avena* coleoptiles ranging in length from 2 to 40 mm. have been studied in the electron and polarizing microscopes and by the low-angle scattering of x-rays. The outer walls of these cells are composed of multiple layers of cellulose microfibrils oriented longitudinally; initially the number of layers is between 10 and 15 but this increases to about 25 in older tissue. Where epidermal cells touch, these multiple layers fuse gradually into a primary wall of the normal type between cells. In these radial walls, the microfibrils are oriented transversely. Possible mechanisms for the growth of the multilayered outer wall during cell elongation are discussed.

The authors are grateful to Dr. G. A. Setterfield for assistance with thin sectioning and for discussion and criticism.

BIBLIOGRAPHY

1. Frey-Wyssling, A., Submicroscopic Morphology of Protoplasm, Amsterdam, The Elsevier Press, Inc., 1953.
2. Heyn, A. N. J., *Proc. Roy. Acad. Sc., Amsterdam*, 1933, **36,** 560.
3. Mühlethaler, K., *Ber. schweiz. bot. Ges.*, 1950, **60,** 614.
4. Chayen, J., *Nature*, 1952, **170,** 1070.
5. Palade, G. E., *J. Exp. Med.*, 1952, **95,** 285.
6. Hodge, A. J., Huxley, H. E., and Spiro, D., *J. Histochem. and Cytochem.*, 1954, **2,** 54.
7. Myers, A., Preston, R. D., and Ripley, G. W., *Proc. Roy. Soc. London, Series B*, 1956, **144,** 450.
8. Scott, F. M., Hamner, K. C., Baker, E., and Bowler, E., *Am. J. Bot.*, 1956, **43,** 313.
9. Wardrop, A. B., and Preston, R. D., *J. Exp. Bot.*, 1951, **2,** 20.
10. Heyn, A. N. J., *J. Am. Chem. Soc.*, 1948, **70,** 3138.
11. Preston, R. D., The Molecular Architecture of Plant Cell Walls, London, Chapman and Hall, Ltd., 1952.
12. Guinier, A., and Fournet, G., Small-Angle Scattering of X-Rays, (translated by C. B. Walker), New York, John Wiley and Sons, Inc., 1955, 179.
13. Roelofsen, P. A., and Houwink, A. L., *Protoplasma*, 1951, **40,** 1.

14. Wuhrmann-Meyer, K., and M., *Jahrb. wissensch. Bot.*, 1939, **87,** 642.
15. Guinier, A., X-Ray Crystallographic Technology, (K. Lonsdale, editor), London, Hilger, 1952, chapter 12.
16. Heyn, A. N. J., *J. Appl. Physics*, 1955, **26,** 519.
17. Gundermann, J., Wergin, W., and Hess K., *Ber. deutsch. chem. Ges.*, 1937, **70,** 517.
18. Roelofsen, P. A., *Biochim. et Biophysica Acta*, 1951, **7,** 43.
19. Tripp, V. W., Moore, A. T., and Rollins, M. L., *Textile Research J.*, 1954, **24,** 956.
20. Roelofsen, P. A., and Houwink, A. L., *Acta bot. néerl.*, 1953, **2,** 218.
21. Houwink, H. L., and Roelofsen, P. A., *Acta bot. néerl.*, 1954, **3,** 385.
22. Wardrop, A. B. *Biochim. et Biophysica Acta*, 1956, **21,** 200.
23. Castle, E. S., *Proc. Nat. Acad. Sc.*, 1955, **41,** 197.
24. Wardrop, A. B., *Australian J. Bot.*, 1955, **3,** 137.
25. Preston, R. D., and Ripley, G. W., *J. Exp. Bot.*, 1954, **5,** 410.
26. Frey-Wyssling, A., and Stecher, H., *Experientia*, 1951, **7,** 420.
27. Stecher, H., *Mikroskopie*, 1952, **7,** 30.
28. Esau, K., Plant Anatomy, New York, John Wiley and Sons, Inc., 1953.

EXPLANATION OF PLATES

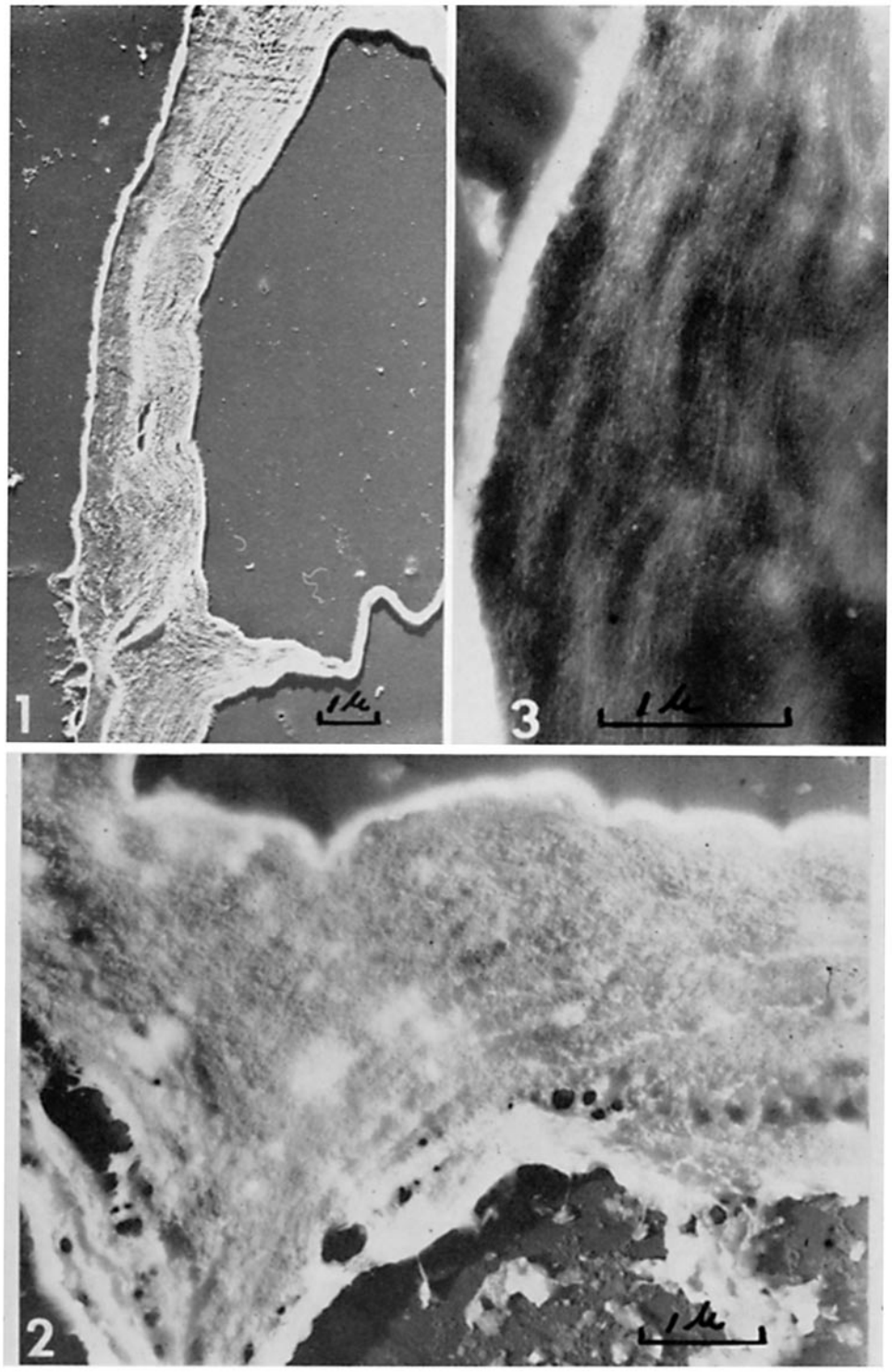
PLATE 32

FIGS. 1 and 2. Transverse sections of outer walls of epidermal cells of *Avena* coleoptiles; embedding medium removed and shadowed. Walls consist of amorphous mass although there is some suggestion of layering.

FIG. 1. 40 mm. coleoptile. × 8,000.

FIG. 2. 20 to 30 mm. coleoptile. × 18,000.

FIG. 3. Longitudinal section of the outer epidermal wall of 20 mm. coleoptile treated with pectinase for 1 hr. Microfibrils can be discerned. × 27,000.



(Bayley *et al.*: Epidermal cell wall of *Avena* coleoptiles)

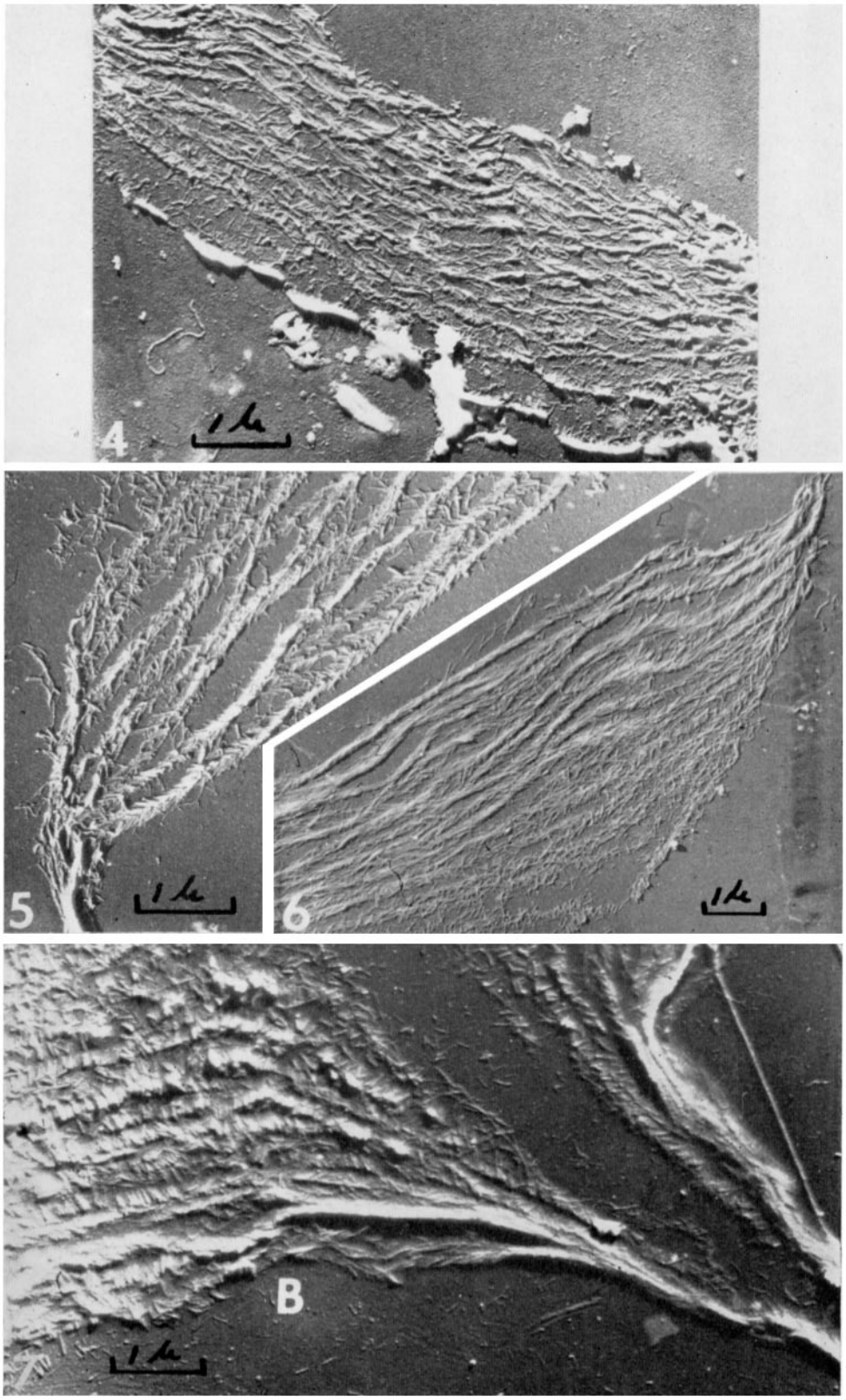
PLATE 33

FIGS. 4 to 10. Transverse sections of outer epidermal walls of coleoptiles of various lengths treated with pectinase; embedding medium removed and shadowed. In these sections, the cuticle has been loosened and except in Fig. 4 lies outside the field of view. Layers of microfibrils are clear, particularly where they have not fallen over (*A*, Figs. 8 and 9); elsewhere, the microfibril arrangement within layers can be distinguished.

FIG. 4. 2 to 3 mm. coleoptile about 1 mm. from the tip. The interior of the cell is uppermost. × 15,000.

FIGS. 5 and 6. 10 mm. coleoptiles, 3 to 4 mm. from the tip. The junction with the interior wall is shown at the bottom left and upper right respectively. Note the difference in microfibril orientation in the inner layers—longitudinal (short segments) in Fig. 5, approaching transverse (long segments) in Fig. 6. Fig. 5, × 15,000; Fig. 6, × 9,000.

FIG. 7. 20 mm. coleoptile, 5 mm. from the tip. The junction with the radial wall is at the bottom right. In the inner layers (*B*), there is an indication of the transition in microfibril orientation from transverse (long segments) to longitudinal (short segments). Measured thickness of section 1,300A. × 14,000.

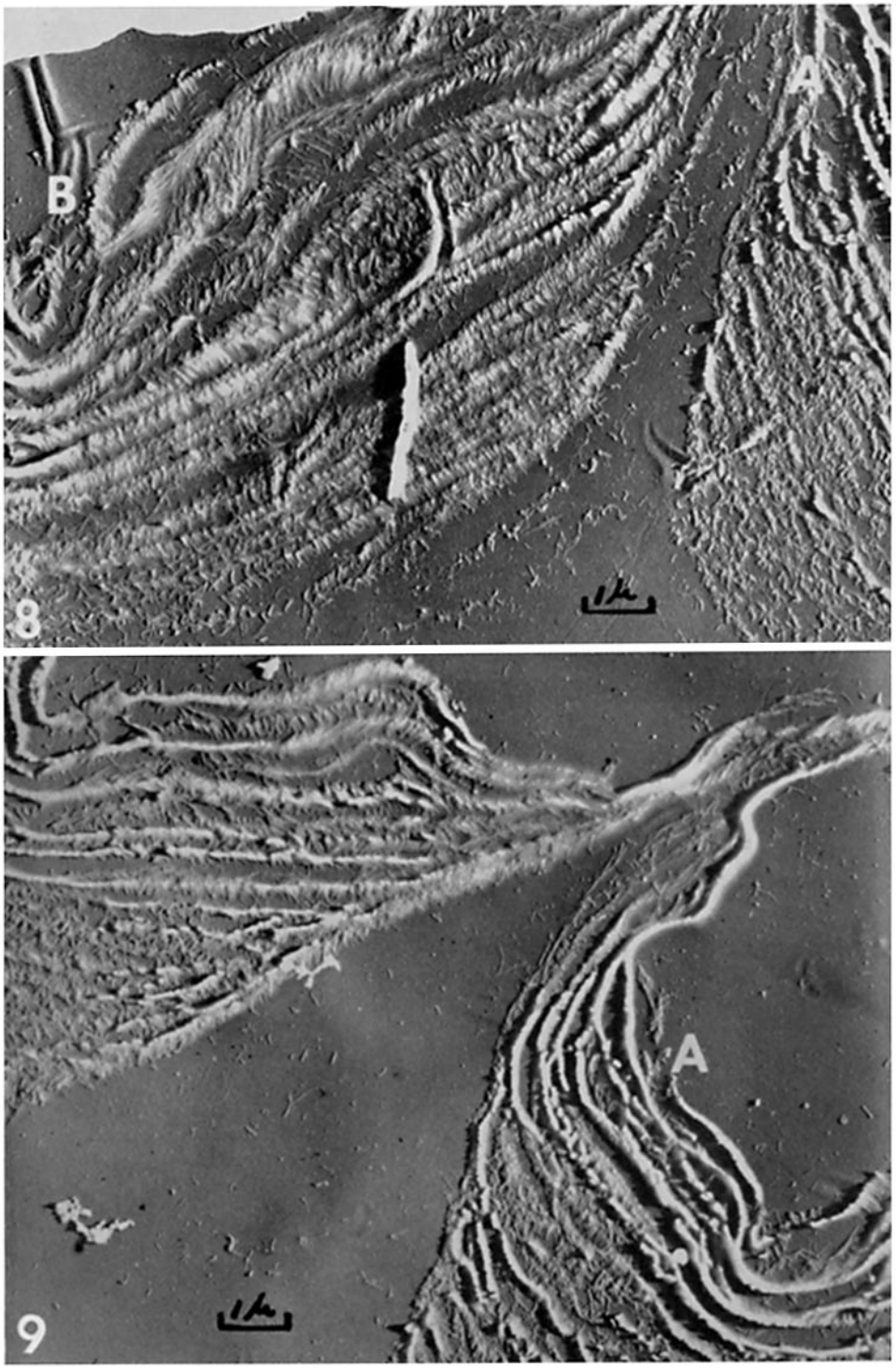


(Bayley *et al.*: Epidermal cell wall of *Avena* coleoptiles)

PLATE 34

FIGS. 4 to 10. Transverse sections of outer epidermal walls of coleoptiles of various lengths treated with pectinase; embedding medium removed and shadowed. In these sections, the cuticle has been loosened and except in Fig. 4 lies outside the field of view. Layers of microfibrils are clear, particularly where they have not fallen over (*A*, Figs. 8 and 9); elsewhere, the microfibril arrangement within layers can be distinguished.

FIGS. 8 and 9. 20 mm. coleoptile, 5 mm. from the tip. The junction with the radial wall is at the top right in each figure. There is an indication of the transition in microfibril orientation at *B* as in Fig. 7. × 10,000.



(Bayley *et al.*: Epidermal cell wall of *Avena* coleoptiles)

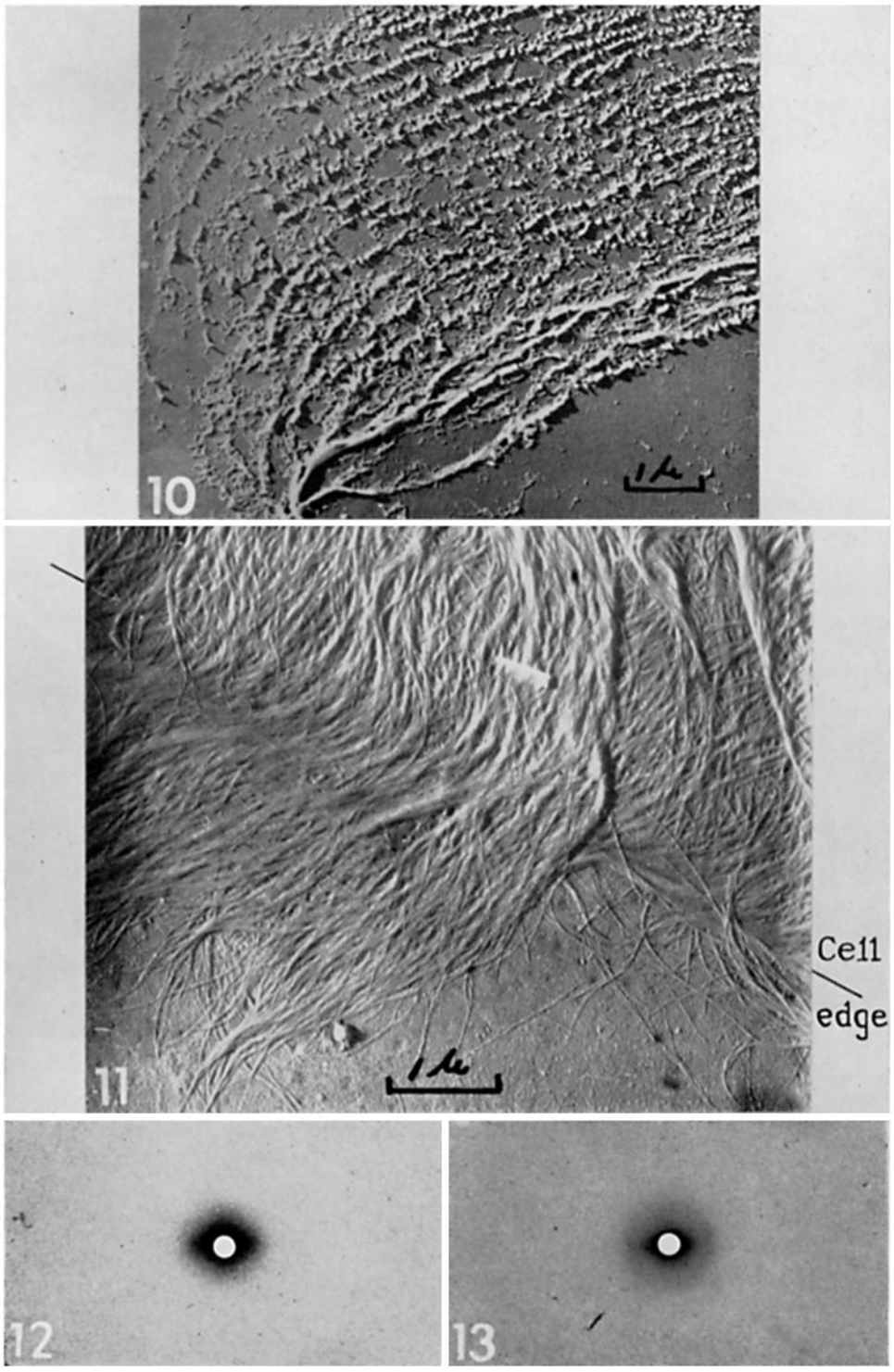
PLATE 35

FIGS. 4 to 10. Transverse sections of outer epidermal walls of coleoptiles of various lengths treated with pectinase; embedding medium removed and shadowed. In these sections, the cuticle has been loosened and except in Fig. 4 lies outside the field of view. Layers of microfibrils are clear, particularly where they have not fallen over (*A*, Figs. 8 and 9); elsewhere, the microfibril arrangement within layers can be distinguished.

FIG. 10. 40 mm. coleoptile, 5 to 10 mm. from the tip. The junction with the interior wall is towards the bottom left. × 11,000.

FIG. 11. Free microfibrils at the edge of an epidermal cell after prolonged pectinase treatment. × 16,000.

FIGS. 12 and 13. Low-angle x-ray patterns from epidermal strips of 20 to 30 mm. coleoptiles; cells vertical. Specimen wet in Fig. 12, dry in Fig. 13. Specimen-to-film distance 50 mm., optical enlargement. × 3.



(Bayley *et al.*: Epidermal cell wall of *Avena* coleoptiles)