Studies on Living Cells of Pea Seedlings. II. Intercellular Tubular Matter
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STUDIES ON LIVING CELLS OF PEA SEEDLINGS
II. INTERCELLULAR TUBULAR MATTER [1]

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THE INTERCELLULAR substance in meristematic tissues of higher plants is commonly considered to be composed of calcium pectate. When unmodified by lignification, suberization, etc., it gives a more or less characteristic reaction with ruthenium red (Mangin, 1893). It is truly plastic and morphologically distinct from the cell wall (Kerr and Bailey, 1934; Bailey, 1954). Accordingly, when ordinary schizogenous intercellular spaces are developing, this substance supposedly lines their inner surfaces. However, it has now been found that in the growing regions of the stem, as well as the root, certain intercellular spaces contain a different, tubular intercellular structure. This is characteristically soluble in ethanol, and is therefore not formed of calcium pectate. Such tube-like structures appear to be extremely labile, and are destroyed by pressure, as well as by dehydrating and embedding agents. Consequently, they are not present in alcohol-dehydrated and paraffin-sectioned material. In a limited number of studies on living material this intercellular matter has evidently been observed in the past, but interpreted as air found inside the film of water lining the capillaries of the intercellular space and forming a black rim at the interface. The problem of distribution and appearance of gas in intercellular spaces, as well as the old controversy concerning the lining of them are beginning to be re-examined (Sifton, 1945, 1957) in the light of the recent experimental studies with living material. The differences in solubility of oxygen and carbon dioxide in water are held responsible for increased internal pressure during photosynthesis and corresponding increase in air space development (Dale, 1957). A waxy substance lining the spaces is suggested to account for the differences in the capillary rise of various liquids in dried strips of *Dianthus* leaves (Häusermann, 1944), and the presence of a lipid pellicle in various parts of many plants is described by Scott, 1950; Scott and Lewis, 1953. The purpose of this investigation is to show: (1) that in addition to air the intercellular spaces of certain parts of growing stems and roots contain a solid substance which is soluble in ethanol and is not of pectic composition; (2) its occurrence in certain intercellular spaces and its absence from others; (3) its characteristic distribution pattern in the fast growing region of the stem, and its gradual depletion in the regions where growth has ceased; (4) its presence in air-dried sections; (5) its reactions towards dyes, acids, alkalies, and certain other chemicals.

The effects of enzymes, growth promoters and inhibitors upon these intercellular tubes will be discussed in a separate paper.

MATERIALS AND METHODS.—Parts of the internodes of 7-day-old etiolated pea seedlings (*Pisum sativum* var. 'Alaska'), grown in darkness with occasional weak red light at 25° C., were sectioned free-hand, or with the aid of a microtome, both longitudinally and transversely, and left in distilled water, 0.26 M sucrose, phosphate buffer pH 6.8, acetate buffer pH 4.8, or in other experimental media, until examined and photographed under the microscope. For sections by microtome, living stem segments were attached to a paraffin coated block by means of paraffin of melting point 53–55° C. For cross sections the stem was marked beforehand at 2 mm. intervals, and the sections from each fraction were kept separately. The internodes of the seedling were counted beginning from the bottom, the first being the oldest. Intensive growth of the plants at this age is occurring in the upper part of the third internode.

The concentrations of dyes employed were as follows: Janus green B, 0.005 per cent; neotetrazolium chloride, 0.001 per cent; ruthenium red, 0.01 per cent. In the tests for phospholipids Sudan black B was used according to Pearse (1954). Tests for other lipids were made with Sudan IV dissolved in propylene glycol (Pearse, 1954). In the tests with I_2 in KI, material was sectioned directly into Gram solution and left for several hours, and if sulfuric acid (67 per cent) was used, it was dropped on the side of the cover glass. The most convincing demonstration that the tubular matter discussed is not simply air was obtained after sec-

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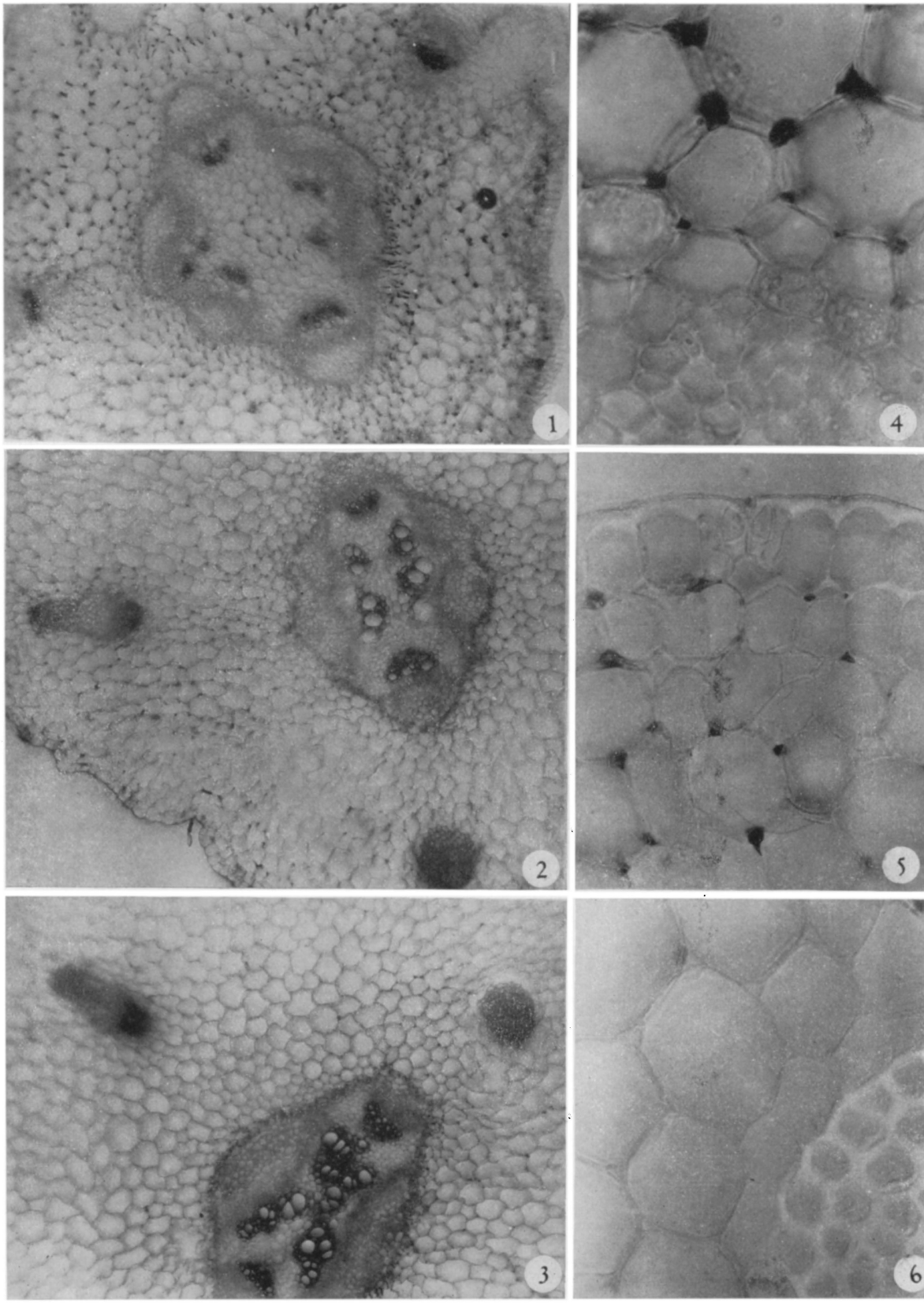
Fig. 1–6. Living material. Cross sections of the stem.—Fig. 1. Fast growing third internode. ITM found in cortex, about the stele, and about the cortical bundles. ×60.—Fig. 2. Second internode, growth has diminished, very little ITM. ×60.—Fig. 3. First internode, growth has ceased, ITM absent. ×60.—Fig. 4. Third internode, cortical parenchyma with large spaces filled with ITM (upper part). The cells of starch sheath, and those of the phloem strand have clear intercellular spaces (lower part). ×733.—Fig. 5. Air chamber under the stoma is clear of ITM, the latter is present in some spaces and absent from others. ×733.—Fig. 6. Cortical parenchyma and phloem strand of the first internode, ITM absent. ×733.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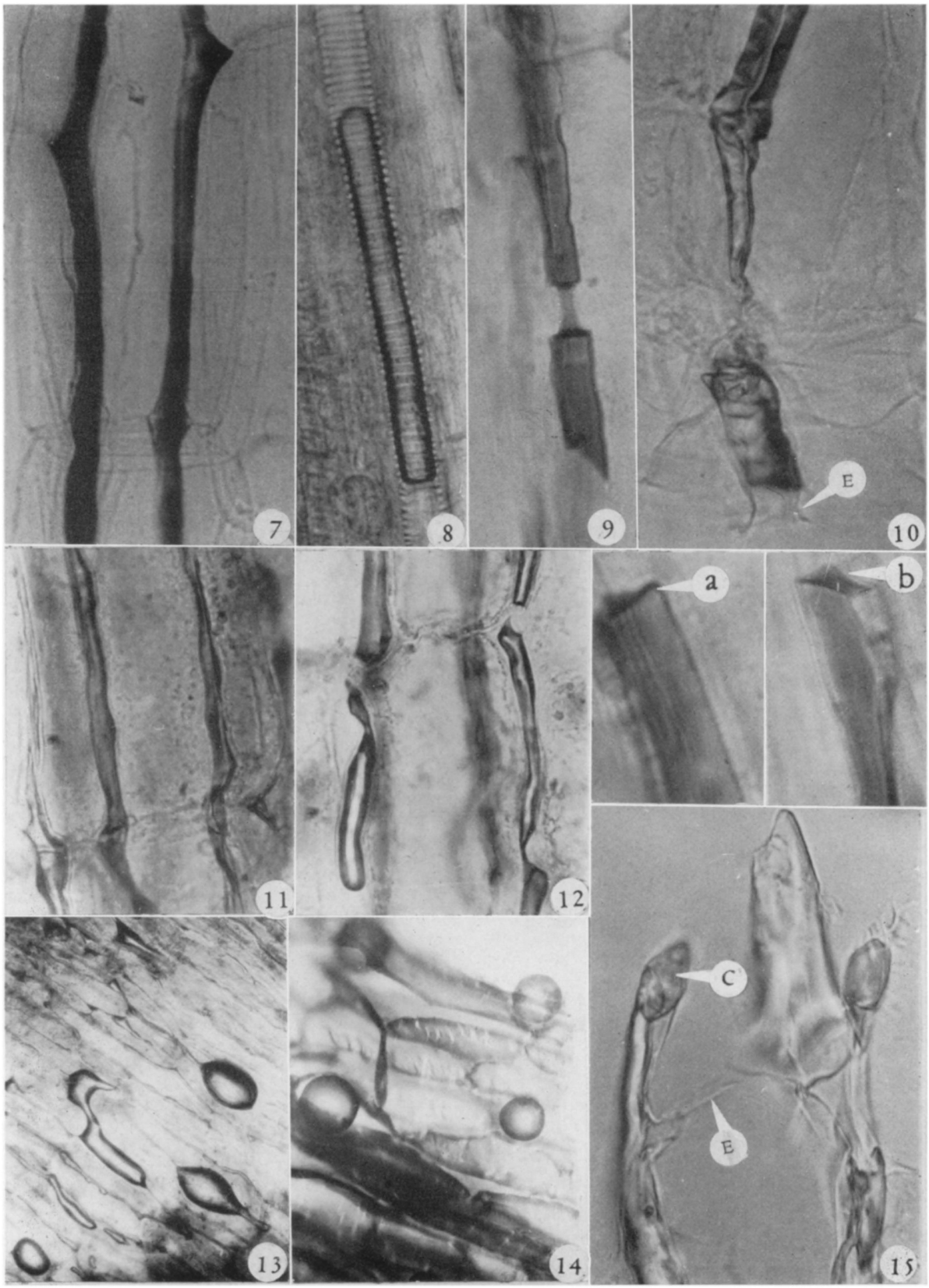
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tioning material, mounting it in water, and then drying it on a slide on a warming plate at 60° C. for several hours. Dried and contracted intercellular matter could be seen directly, or mounted in glycols, glycerol, or glycerine jelly, and examined under phase contrast.

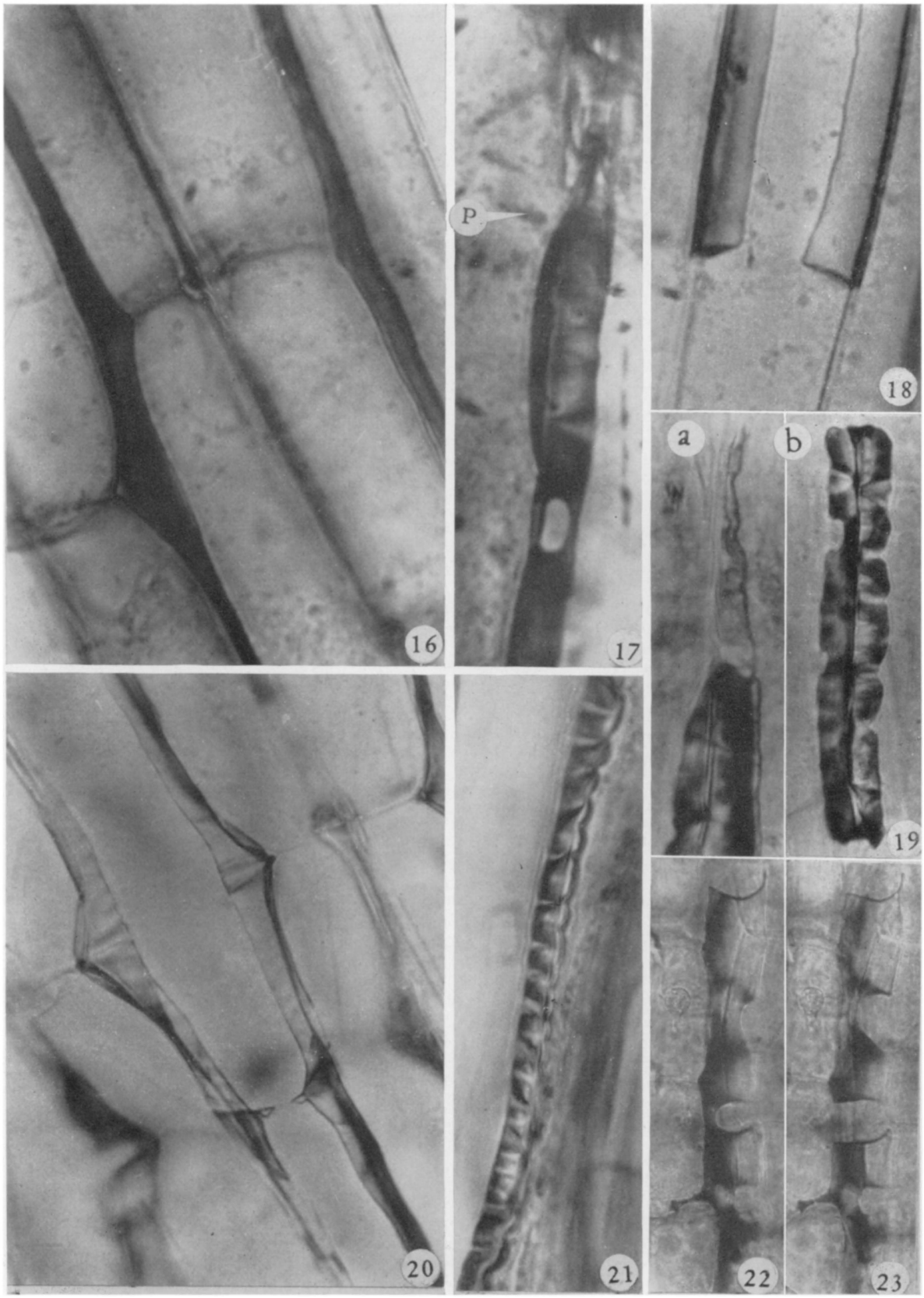
The effects of ethanol, ether, chloroform, acetone, strong acids and alkalies were tested by perfusing the mounted sections gradually with the liquid directly under the microscope and drawing it off by filter paper. The appearance of the preparations before, during and after experiments were continually recorded in photomicrographs. A 35 mm. camera attachment was used with a Zeiss microscope with bright field and phase contrast. Neofluar objectives 6.3/0.20, 16/0.32, 40/0.75, Ph. 40/0.75, 100/1.30 were used with K oculars 8 × with magnification changer for factors 1 ×, 1.6 ×, 2.5 ×. Negatives were enlarged as specified. The abbreviations ITM, intercellular tubular matter; JgB, Janus green B; NTC, neotetrazolium chloride, are used in the text.

RESULTS.—*Distribution of intercellular matter.*—In the fast growing part of the stem which is within 1–5 mm. from the top of the third internode (Christiansen and Thimann, 1950) the intercellular matter is particularly well developed. It could be seen in cross sections of fresh material mounted in one of the media mentioned above, or in preparations fixed in formalin and mounted in glycerine jelly. This part of the stem is somewhat quadrangular in cross section, contains a central stele with starch sheath, a well developed cortex, two cortical phloem strands lying in opposite corners of the stem which are also surrounded by a starch sheath, and two fibrovascular bundles which lie in the plane at right angles to the strands (Hayward, 1938). A portion of such a cross section is represented in fig. 1 showing the presence of dark intercellular matter distributed in part of the cortex, in the vicinity of the stele, of the fibrovascular bundles, of the phloem strands, and in small amount in the pith. The dark matter is conspicuously absent from the phloem, the xylem and the cambium. Under higher magnification a part of the cortex with parenchyma cells, starch sheath, and the cells of the strand are shown in fig. 4. The large parenchyma cells (upper part of fig. 4) show large intercellular spaces filled by dark matter, while the intercellular spaces of the smaller starch sheath cells, although plainly visible, do not contain the dark matter, neither is it visible between the cells of the bundle (lower part of fig. 4). The occurrence of intercellular spaces both with and without the dark intercellular matter in the same section of the stem is illustrated in fig. 5. Here the dark matter can be seen in some triangular and quadrangular intercellular spaces but not in others; it is also absent from the air chamber under the guard cells of the stoma (upper part of fig. 5).

In a series of sections at increasing distances from the tip, it could be seen that the intercellular matter began to disappear first from the middle part of the cortex, persisting longer in the regions near the epidermis and around both the cortical bundles, and the central cylinder. In the second internode, which is characterized by the abundance of primary xylem (fig. 2), the intercellular matter has conspicuously disappeared from the greater part of the cortex, persisting sometimes only in small amounts in the vicinity of the stele and of the cortical bundles. In sections from the first internode, which was the oldest and had ceased elongating (Christiansen and Thimann, 1950), almost all of the dark intercellular matter has disappeared (fig. 3). In longitudinal sections of the first internode most of the spaces are clear, and fragments of intercellular matter are visible in some of the others. In fig. 6 is shown a detailed view of the part of the stem from the first internode which corresponds to that from the third internode depicted in fig. 4. The large parenchyma cells show clearly the transparent intercellular spaces, and only occasionally can small remnants of the dark matter be observed. The walls of the phloem strand cells have become very thick and a pearly shine has developed. The parenchyma cells are alive, exhibiting perfect cyclosis, and showing positive staining of mitochondria when Janus green B is applied, thus indicating an abundance of air about these cells, since the mitochondrial staining is a strictly aerobic reaction.

Physical properties.—The physical nature of the intercellular matter is understood best from the studies of longitudinal sections. The intercellular substance between the elongating parenchyma cells appears as solid dark-colored strips showing pointed extensions at the places where two cells are joined (fig. 7), measuring sometimes over 1000 μ, and extending the length of several cells. This dark-colored matter may end abruptly showing a sharp pointed end, or it may exhibit a triangular (fig. 15*a*) or quadrangular (fig. 15*b*) opening, indicating the lining by this substance of the intercellular spaces of corresponding shape, and the presence of a hollow center probably filled by air or gas. At the edge of very thin sections made with the aid of a microtome, the intercellular matter can be seen protruding from the tissue and assuming the shape

Fig. 7–15. Physical properties of ITM. Longitudinal sections.—Fig. 7. Solid dark-colored ITM between living parenchyma cells. ×550.—Fig. 8. An air bubble inside a vessel showing dark rim and light interior. ×550.—Fig. 9. ITM becomes constricted after heating to 70–80° C. indicating changes in viscosity. ×550.—Fig. 10. ITM remains intact after repeated boiling on the slide; (*E*)—cut edge of the section. ×875.—Fig. 11–14. Liquefaction of ITM after 67 per cent sulfuric acid. For details see text. ×733.—Fig. 15. Triangular (*a*), quadrangular (*b*), and oval (*c*) ends of ITM. The tube in (*c*) was near section's edge (*E*) protruding into the mounting medium. ×1150.



of a tube with an oval opening (fig. 15*c*). Contrary to the uniform dark color of the intercellular matter, an air bubble, when it becomes trapped in a spiral vessel (fig. 8), appears light in the interior with a dark rim along the surface. Such an air bubble shows the physical properties of the gas and liquid interface in the capillaries, i.e. the water has a concave meniscus, the curvature of which is important in determining the surface tension (Adam, 1938). The intercellular matter, on the other hand, does not exhibit a concave-convex meniscus of the liquid-gas interface, but under ordinary conditions shows a surface typical for solids, which can be very complex in structure.

When a trapped air bubble (fig. 8) is heated on a slide, a great expansion of the bubble follows, due to the decrease in surface tension of the water and to increase in volume of the gas. The bubble expands about 10 times its original volume, and decreases correspondingly after cooling of the slide. In contrast, the intercellular substance does not visibly increase or decrease its volume after heating and cooling. This is shown in a portion of it located next to the cut edge at the end of a section (fig. 10, *E*) on a slide which was heated to the boiling point. Only after repeated boiling and cooling did the substance finally contract and dissolve. Heating of the slide to 70–80° C. causes the substance to become soft and pliable and to form a constriction seen in fig. 9. Other agents also cause the tubular matter to become semi-fluid and viscous, making it swell, contract, or break. For example, after a section is placed in propylene glycol the tubes become irregularly swollen, and break into smaller pieces. The openings of these pieces are sealed by the viscous material, thus trapping the gas located inside the fragments.

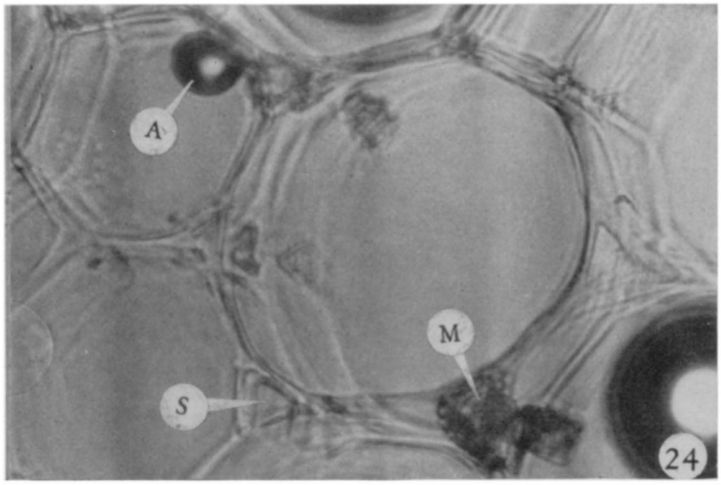
The most interesting observations of changes in viscosity in tubular matter were made after treatment with strong sulfuric acid (67 per cent), either in sections previously treated in I_2 in KI solution for the cellulose test, or mounted directly in the acid. In presence of iodine the tubular matter appears as a gray-colored substance with interrupted dark-brown lines and streaks (fig. 11). Application of the acid is followed by changes in viscosity of the tubular matter. It become semi-fluid, very shiny, assumes a concave-convex meniscus of the liquid-gas interface, and begins to fragment into smaller or larger parts (fig. 12). At the levels of breaks in the tubes, the sticky character of the substance is apparent, as heavy drops fall from the ends and seal off the fragments. Further swellings and contractions produce peculiar structures of which examples are shown in fig. 13. These irregular swollen balloons are connected with the attenuated tubes and are presumably filled with gas. Their surface is not smooth, protrusions, excrescences, folds and grooves being quite common. As more acid is added both large and small pieces begin to contract and diminish in size, and by the time the blue cellulose reaction has set in, and the pit fields become visible, only spherical bubbles are left in the intercellular spaces (fig. 14). These bubbles apparently have a hyaline coating at first, which is dissolved eventually, and the bubbles are free to float about the disintegrating tissues. The bubbles are not soluble in absolute ethanol, ether, chloroform or xylol.

The tests which follow indicate that the tubes are two-layered, consisting of: (a) a peripheral hyaline sheath which gives positive reactions with the lipid-soluble dyes, which concentrates iodine, and which exhibits iridescence in sucrose, cobalt salts, glycerine, propylene glycol, acids and alkalies, and (b) an inner layer adjacent to the central lumen with gas. This layer is more easily hydrolyzed and affected by pressure and will be referred to as the gray substance. The tubular matter exhibits ridges in the longitudinal (fig. 10, 17, 18, 30) and transverse (fig. 19, 21, 29–32) directions the exact nature and significance of which is not determined.

Staining with ruthenium red.—Ruthenium red produces an appearance of staining the hyaline sheath (fig. 18) but certainly does not stain the gray substance. Although a red color appears in the residue of the original intercellular substance, it is more intense in the tubular matter, as is indicated by darker lines in the photograph. Generally, the specificity of ruthenium red for pectic substances is very much in doubt (Kerr and Bailey, 1934; Bailey, 1954), as there are considerable differences in the affinity of the dye to pectic substances of different degrees of acidity (Kertesz, 1951), and under certain conditions coagulated protoplasm, nuclei, some lipids and polysaccharides may be stained (Bailey, 1954).

Staining with neotetrazolium chloride.—The reaction of the intercellular tubes towards the redox indicator dyes, neotetrazolium chloride and Janus green B are of particular interest because of the possibility that enzymatic action is involved. When

Fig. 16–21. Staining reactions of ITM.—Fig. 16. After infiltration by JgB in sucrose for 2 hr. the tubes become red-colored, the cell wall remains blue. ×1150.—Fig. 17. Dark brown ITM after Sudan black B, pit fields (*P*). ×1600.—Fig. 18. After ruthenium red. Inner portion of ITM remains gray, red color is visible along the edge of the tube. ×1150.—Fig. 19. After Sudan IV in propylene glycol. Red color is shown in the outer layer of the tubes. In (*a*) the gray substance is distinct from the attenuated hyaline end. In tube (*b*) the transverse lines and grooves are apparent. ×1150.—Fig. 20. Incubated with NTC in sucrose for 24 hr. Inner substance remains gray, red diformazan is visible in lines and streaks. ×1150.—Fig. 21. Incubated with NTC for 20 hr. and mounted in glycerine jelly. Diformazan is visible in vertical lines and streaks, gray substance shows colorless transverse segmentations. ×1150.—Fig. 22–23. Effects of ethanol on a tube mounted in weak acid. Successive stages of fragmentation taken 10 min. apart. For detail see text. ×1150.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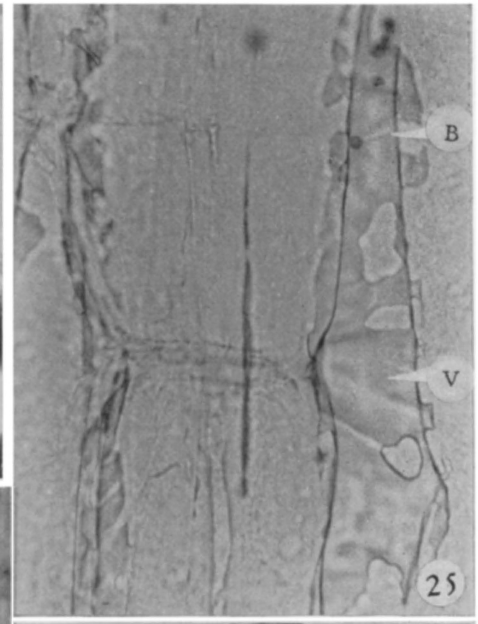
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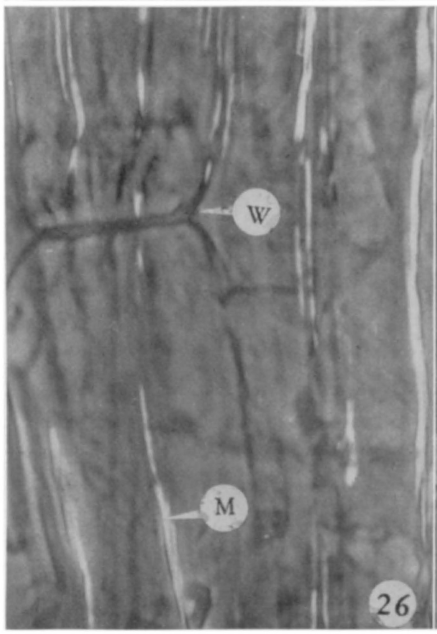
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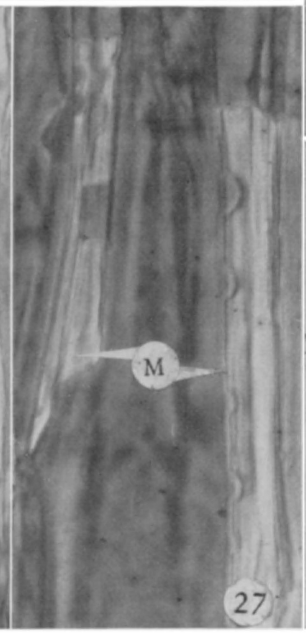
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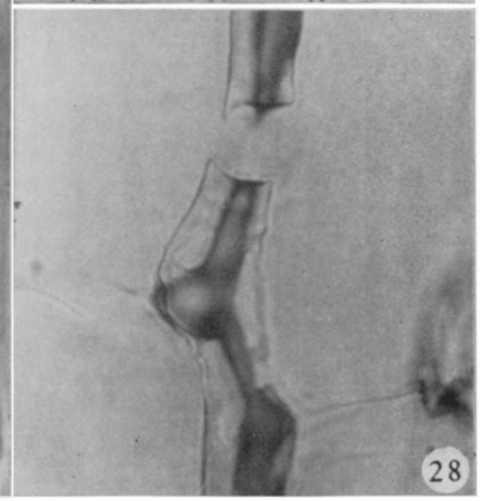
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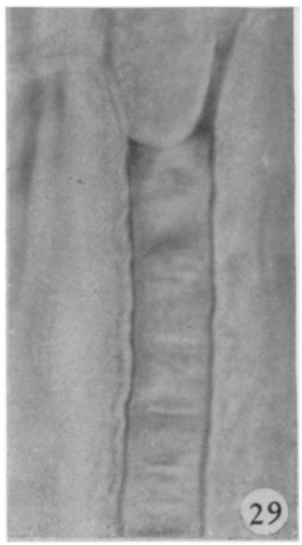
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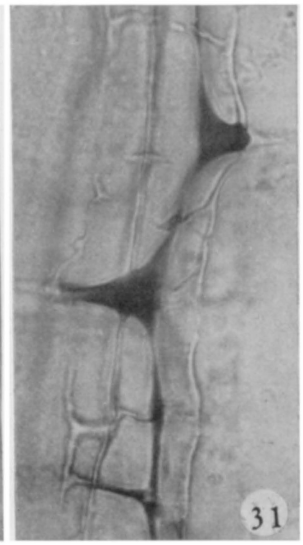
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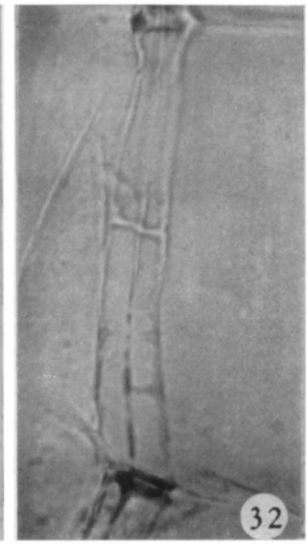
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living material is incubated with dilute solutions of NTC in sucrose for 30 min. to 24 hr., the streaks and lines of the tubular sheath become brilliant magenta (fig. 20), a color different from that of mitochondria which form dark-red diformazan (Sorokin, 1956). The color is either restricted to the lines (fig. 20), the gray substance remaining unaffected, or it diffuses throughout the tube. Both effects can be explained as due to the occurrence of the reaction in the hyaline layer. When this material is mounted in glycerine jelly, furrows and cross striations become apparent (fig. 21), which indicate the heterogeneous nature of the tubes. No color is visible in the cross lines. Fixed and dead material fail to show any reaction with NTC. That enzymatic action is involved in the staining with NTC is quite possible, however, the reaction can also be explained as a secondary effect due to solubility of diformazan in lipid. This was the explanation given by Currier and Zweep (1955) for the occurrence of formazan in the cell wall of *Anacharis canadensis*.

Staining with Janus green B.—The most effective demonstration of the intercellular tubes is by the reduction of JgB to diethylsafranin. The tubes become bright red and stand out from the blue-colored cell wall and cell contents (fig. 16). The effects of JgB upon the cells and tissues of the growing pea stem are varied: under aerobic conditions and in the presence of the cytochrome c-cytochrome oxidase system it produces a blue coloring in the mitochondria of all living cells; it also causes vacuolar precipitates in certain cells, and it is diffusely reduced by the cytoplasm to diethylsafranin in restricted regions of the topmost part of the growing stem (Sorokin, 1956). In dead cells there is general accumulation of the blue-green dye on cell walls, and eventually on proteins and lipids. In contrast, in the intercellular tubes JgB is reduced to red-colored diethylsafranin and the coloring is not limited to the streaks and lines, as was seen in the NTC reaction, but there is a gradient of the intensity of reaction, the tubes in some parts of the intercellular matter appearing entirely red, while in others the red color is more patchy. There is no reduction of the dye in material fixed in 10 per cent formalin, nor in that treated beforehand with propylene glycol. There is no further reduction of diethylsafranin to leucosafranin, and the red color is preserved in slides mounted in glycerine jelly. In the staining with JgB an enzymatic reduction in the tubes is even more probable, as compared with NTC, since preferential solubility of dye in the lipids without the enzymes would make the tubes appear blue rather than red, for the blue color is present in the medium in abundance from the very beginning.

Staining with Sudan black B and Sudan IV.—Because living tissues containing water form sticky, uniformly sized brown globules all over the preparations when perfused with Sudan black B dissolved in 70 per cent ethanol, all material for these tests has to be fixed beforehand and brought to 70 per cent ethanol to avoid this artifact. Fixation in 10 per cent formalin pH7 with addition of $Ca(NO_3)_2$ M/1000 and a rapid handling with 70 per cent ethanol prevent the tubular matter from dissolving and show it dark brown after the reaction (fig. 17). The pit fields of the cellulose walls are also brown-colored (fig. 17, *P*), but the typical Sudan black B reaction (blue-black coloring of phospholipids) occurs in mitochondria and possibly plasmodesmata which are located in the pit fields. Sudan IV dissolved in propylene glycol produces considerable and irregular swelling of the tubular matter which is due to propylene glycol. It shows also a clear transparent red coloring in the outer layer, but not in the inner substance of the tubes. Noticeable are cross striations of lighter colored substance in the gray matter (fig. 19*b*). Occasionally sharp limits are observed between the gray matter and the hyaline substance, the latter extending to an attenuated point (fig. 19*a*). However, pit fields with granular structures which stain blue-black with Sudan black B, are not stained by Sudan IV, perhaps because all sudans, with the exception of Sudan black B, stain triglycerides and fatty acids a much darker shade than phospholipids. The latter may actually remain unstained (Gomori, 1952). Thus the tests indicate that the hyaline sheath contains lipids, but not the phospholipids.

Solubility in ethanol and other chemical properties.—When sectioned material mounted in water, sucrose, or buffered solutions is perfused with 95 per cent ethanol, a rapid dissolution of intercellular matter results. The tubes begin to contract either from one end or simultaneously from both ends. The contraction is either continuous or inter-

Fig. 24–32. Fig. 24. Cross section dried at 60° C., mounted in glycol. Clear intercellular spaces (*S*), and filled by ITM (*M*) are found between large parenchyma cells. ×1150.—Fig. 25. A longisection treated by I_2 in KI, dried for several hr. at 60° C., and remounted in water. Inner substance of the tube is colored violet (*V*), dark brown lines are visible in the outer sheath (*B*). ×1150.—Fig. 26. Longisection dried at 60° C. for 2 hr., mounted in propylene glycol. Examined under phase contrast. ITM acquires brilliancy and polychromy (*M*), transverse and longitudinal cell walls remain dark (*W*). ×1150.—Fig. 27. Treatment similar to fig. 26. ITM resembles sculptured marble. ×1150.—Fig. 28. After 0.5 per cent ammonium oxalate. The hyaline substances is not affected, the inner matter becomes partially hydrolyzed. ×1150.—Fig. 29. After 85 per cent propylene glycol. Face view of the tube with transverse segments. ×1150.—Fig. 30. Treatment similar to fig. 29. Side view of the tube, transverse segments do not reach the middle ridge. ×1150.—Fig. 31–32. Gray inner matter is partially hydrolyzed after 5 days in 25 per cent glycerine, the hyaline substance remains intact (vertical ridges). ×1150.

rupted and leaves no residue. Because the solubility of air is considerably higher in ethanol than in water (Seidell, 1920), it is assumed that air, when present in a tube, also dissolves in the ethanol. Material treated in advance with weak acid is seen to fragment and a small piece, instead of dissolving after addition of ethanol is at first liquefied and expanded in size. Presumably this is due to decrease in surface tension since swelling and expansion in size also occurs after the use of a wetting agent (5 per cent Tween 80). Further addition of ethanol, however, produces contraction and dissolution. In other mounts of similarly treated material the perfusion with ethanol sometimes results in evolution of a large amount of gas, also probably because of the decrease in interfacial tension. This gas comes out from the triangular end of the tube and forces its way through the tissue towards the edge of the piece. At a rate of one per second, 35 bubbles emerged from the tube photographed in fig. 22. The picture was taken immediately after the last bubble had separated and the tube had begun to fragment. Further stages of fragmentation are visible in fig. 23, taken 10 min. later.

Although the tubes do dissolve in lower grades of ethanol, the process is distinctly slower and requires either raising of temperature, or prolonged retention in the medium. Fixation in formalin with addition of Ca ions preserves many of the tubes in 70 per cent ethanol.

The dissolution in ether is extremely fast, the tubes dissolving from the ends, without leaving any residue. Similarly, the tubes dissolve in chloroform, acetone, and xylol. Air bubbles do not dissolve in xylol.

Partial hydrolysis in acids, alkalies, and some other substances.—Acids, in general, cause dissolution of the tubular matter. However, the speed of the reaction and the difference in resistance to the solvent of the hyaline substance and the central matter varies in different acids. Effects of glacial acetic acid are similar to those of ethanol: complete and fast dissolution of both substances follows its application. In 1N HCl noticeable swelling of the tubes may be observed almost at once after the mount is made. Alternating dark and light patches become apparent and the tube gradually dissolves from the edge, forming a characteristic "Swiss cheese" effect. This process is slow and continues for several hours even when the cover glass is lifted and fresh acid added. Reaction towards 1N HNO_3 is varied. Some tubes form gas-filled balloons, similar to those described for sulfuric acid, while others are absolutely unaffected for 4 hr. and more. However, after 10 hr. all the tubular matter is dissolved. The weaker acids react differently. Acetate buffer pH 4.8 partially dissolves the gray component of the tube in 4 hr. leaving almost intact the hyaline substance. Similar results can be obtained by treating the sections with a weak acetic acid (3–10 per cent) for 20–30 hr.

Whether or not the tubular matter dissolves in alkalies depends upon its preliminary treatment. If the sectioned material is kept in phosphate buffer pH 6.8 and 1N KOH applied, purple iridescence becomes evident in the tubes at once, but there is no noticeable dissolution even after one hr. Pretreatment in acetate buffer pH 4.8 or in dilute acetic acid causes the application of KOH to result in evolution of gas which escapes from the sections, leaving the tubes intact, but with definitely indicated transverse lines, similar to those seen in fig. 21. The residue of the tubes which remains after the action of alkalies consists often of transverse segments. In cross sections, which usually appear black or dark-gray, 3.5 per cent NH_4OH causes the inner substance to dissolve gradually, clearing the lumen of the cross section of the tube, and leaving the hyaline matter in the form of iridescent triangles. The residue of hyaline substance in the form of vertical and horizontal lines may also be observed after treatment with 25 per cent glycerol for a period of 5 days which hydrolyzes the gray matter (fig. 31, 32).

Further evidence of the composite structure of the tubular matter can be obtained by treatment with 0.5 per cent ammonium oxalate (fig. 28). The hyaline sheath is not affected, but the inner matter becomes viscous, and is partially dissolved. Again the composite nature of the tubes may be seen in material treated with 85 per cent propylene glycol. A side view of a tube is shown in (fig. 29) where the transverse lines go all the way across the tubular structure. In face view, the transverse lines stop a short distance from the middle ridge (fig. 30).

Drying of the intercellular matter.—An obvious way to demonstrate the presence of solid substances in the intercellular spaces (in addition to air or gas) is by evaporating the water from the tissues, and in this way eliminating the possibility of occurrence of air-water interfaces in the capillaries. In a preparation mounted in water without a cover glass changes in the tubular matter during drying can be observed directly under the microscope. They consist in increased iridescence, cleavage, and disappearance of dark color, when the interfacial water becomes evaporated. The cleaved matter can be recognized from the shrivelled cell wall by a different refraction. However, to avoid this high refraction and to make photography possible, material has to be mounted after drying either in water, glycerine or propylene glycol. A cross section of cortical parenchyma treated as described above is shown in fig. 24. The protoplasts of these large cut cells disappear in all probability during the preparation, but the cell wall remains intact and shows both triangular and quadrangular intercellular spaces. Certain spaces are devoid of any visible material (fig. 24, *S*), others contain plastic matter (fig. 24, *M*). In longitudinal sections of similarly treated material examined under phase contrast, the tubular matter has acquired the appearance of

sculptured marble (fig. 27, *M*), great brilliancy, and at the same time pronounced linear polychromy. The intercellular spaces above and below the tubular matter, and the longitudinal and transverse cell walls remain dark (fig. 26, *W*). Significantly, when these preparations are examined under polarized light the intercellular tubular matter remains isotropic.

Sections treated with I_2 in KI, dried for several hours, and mounted in water, show swollen and partially hydrolyzed tubular matter. The inner part becomes distinctly violet in color (fig. 25, *V*) which was not apparent before drying; the outer part shows dark-brown lines (fig. 25, *B*), which possibly represent the result of concentration of iodine by the hyaline sheath. A lipid pellicle lining the intercellular spaces described by Scott (1950) also stained brown with I_2 in KI, and resembled in this respect the hyaline sheath.

SUMMARY

In the fast growing apical regions in the stem of pea seedlings certain intercellular spaces contain an ethanol soluble solid tubular lining in addition to air or gas. That the dark colored intercellular spaces contain a solid substance, and not merely a film of gas at the interface with water is indicated by physical criteria. (1) It does not show a concave-convex meniscus typical for water-gas interfaces. (2) It does not noticeably expand nor contract on heating and cooling. (3) It persists in dried sections and shows high refraction and polychromy when dried material is mounted in water, glycol, or glycerol, and examined under phase contrast. This substance is found in abundance in the regions of fast growth, decreasing gradually in the regions of slower growth, and disappearing almost entirely where growth has ceased. It is labile, easily destroyed by pressure and dehydrating and embedding agents, but is prominent in fresh material and can be fixed and mounted in glycerine jelly. The tubes contain two substances: a hyaline sheath, which gives positive reactions for lipids, and a gray inner part which is easier hydrolyzed in weak acids, alkalies, and 25 per cent glycerine. Both substances line concentrically the intercellular space leaving a lumen for air or gas. The tubes react with redox indicator dyes by forming red diethylsafranin after infiltration of the intercellular spaces with Janus green B, and red diformazan after neotetrazolium. They are rapidly dissolved in 95 per cent ethanol, ether, chloroform, xylol, glacial acetic acid, liquefied by strong sulfuric acid, partially hydrolyzed by other acids and alkalies, and show changes in viscosity by action of several agents.

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