Callose distribution and wall structure in the laticiferous cells of *Allium cepa*

By

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With 10 Figures

Received October 30, 1958

The presence of callus in the tube-like laticiferous cells of onion was first noted by Rendle 1889. Besides describing extensively the various types of callus-cell wall relationships, Rendle maintained that the end walls (i. e., so-called "septa" between successively superposed cells in the tube-like series) were pitted. Only one case of cell wall perforation was found. This concept seemingly was at variance with that of Hanstein 1860, who had described the superposed series of elements in onion as tubes. He had indicated that the cross walls of these tubes were perforated with sieve-like pores.

Rendle apparently was unfamiliar with a subsequent publication, in which Hanstein 1864 stated that the pores were not really open but were closed off. However, Molisch 1901 re-examined this problem and declared that there was an open continuity between the original cells which constitute the "slime tubes" of monocotyledons. (De Bary 1884 had been uncertain whether occasional perforations in the transverse wall of the laticiferous cell of onion were normal or caused by preparative techniques. Nevertheless, his figure 56 shows a pitted transverse wall.) Currier 1957 described the transverse wall as "pitted (sometimes perforated)".

In addition to Rendle, other workers have noted the callus deposits on the cross walls of these laticiferous cells. By the use of aniline blue and fluorescence microscopy, Currier & Strugger 1956 demonstrated the presence of callose in the cell walls of epidermis and mesophyll of the onion. Currier 1957 extended these observations to show that callose was indeed the substance present in the callus nodules on the transverse walls of the laticiferous cells. This observation corroborated the suggestion of Rendle 1889 that the reaction of this laticiferous callus was similar to that of sieve tube callus. Using resorcin blue, Eschrich 1956 also found a definite callose reaction in the callus deposits of these cells. Currier 1957 followed Rendle in assigning an irregular mode of distribution to the callus masses.

Eschrich 1956 has reviewed suggestions that callose material is laid down in response to foreign intrusions. Evidence, particularly in the case

of virus infections, is rather abundant. The present observations, which were carried out on bulbs of the onion variety, "Yellow Globe", serve to illuminate this thought. Following ESCHRICH, the writer has used resorcin blue as a specific callose stain.

The structure of the end walls of the laticiferous cells may be observed in figures 1 and 2. In these face views, it is apparent that the wall is reticulately thickened, the thickenings being limited to narrow bars about the more or less circular thin spots of the wall. Between crossed nicol prisms (fig. 2), the orientation of the cellulosic micelles in these bars is made obvious: the micelles are arranged in parallel order, and their longitudinal course is tangential to the circular thin spots. This structure is strongly parallel to that described for the sieve plates of *Cucurbita* by FREY-WYSSLING & MÜLLER 1957.

Using polarized light, the writer ascertained that the lateral wall of the laticiferous cell is characterized by a "tubular texture" (FREY-WYSSLING 1953): that is, the long axes of cellulose micelles are arranged principally in a direction perpendicular to the long axis of the cell.

A sectional view of the cross wall (or of a lateral wall between two adjoining laticiferous cells) shows that the thinner regions of the wall, i. e., the circular spots, are not microscopic pores. These thin regions are actually "closing membranes" in the large, pit-like depressions. Perhaps electron micrographs eventually may disclose extremely small plasmodesmatic perforations in these thin membranes, as are characteristic of the closing membranes of pits of parenchymatous cells (MÜHLETHALER 1950, SCOTT et al. 1956). Use of higher powers of the light microscope suggested that such might be the case.

Another line of evidence supports the above observation. Normal laticiferous cells are turgid. They are alive, with a single large nucleus (fig. 3), and the latex particles are in active Brownian movement. In a solution of 10% NaCl, plasmolysis occurs, involving a local withdrawal of the cytoplasmic membrane from the cell wall. Brownian movement ceases. When re-immersed soon in distilled water, the cell recovers. Hence, there, are osmotically active solutes within the vacuole of the laticiferous cell.

If a fresh cell be immersed in pure water, the vacuole enlarges, and the transverse walls become distended. Within a short time, plasmoptysis occurs, with the bursting of a small portion of the transverse wall. Immediately thereupon, latex begins to stream out through the newly-created opening. If a tubelike series of cells be included in the section placed in water, it is possible to see the successive rupturing of each transverse wall.

Bursting may also occur if mechanical pressure is exerted on the coverslip over the preparation. In this instance, the applied force is translated into hydrostatic pressure within the laticiferous cell.

In all cases, the rupture is explosive. It involves only a very small region of the transverse wall, presumably a pit membrane. No rupture was

ever found in a lateral wall. It is thus evident that the transverse wall of the laticiferous cell is normally unperforated and must be characterized as "pitted". The laticiferous cells are not in open continuity in the onion.

Deposits of callus occur frequently in these cells, particularly on the transverse walls. The callus is stained selectively with aqueous solution of either aniline blue or resorcin blue. In contrast to the observations of RENDLE 1889 and CURRIER 1957, it was possible to find characteristic modes of callus deposition. Figure 4 shows the most common form of callus. This type could be found singly or as several scattered "plugs" on well over 80% of the transverse walls. It consists of a conical projection in one cell, subtended at the base by an oppositely-placed lenticular disk in the adjoining cell. More rarely, a callus deposit would develop only on one side of the cell wall (usually a lateral wall), without opposed callus developing on the other side (fig. 5).

More extensive callus development is marked by fusions and decreasing regularity in the typical shape. Apiculate protrusions are rather frequent (fig. 7, 8), sometimes extending on both sides to form bizarre configurations (fig. 8). Occasionally, as noted by RENDLE 1889, the callus mass may extend to cover the whole transverse wall (fig. 9). Extensions along the lateral wall are sometimes noted (fig. 6), but here there is no topographical coincidence of callus deposition in adjoining cells.

The writer could confirm the observation by RENDLE that occasionally broad areas of the cell wall would assume a callose stain, even though no morphological callus deposit was evident. On the lateral wall, such areas may be quite extensive, surrounding already formed callus masses. Whether there is a transformation of some other polysaccharide material into callose or whether callose is being laid down de novo could not be determined. However, the fact that the projecting callus deposits consist principally of large masses of callose and the fact that, unlike other glucans of higher plants, callose is a polymer based on 1—3 linkages (ASPINALL & KESSLER 1957), suggest that the latter event is more probable.

When lipoidal crystals (STERLING 1959) are present at the transverse wall, some of these may be coated by a layer of callus (fig. 10). Resorcin blue will stain the mantle of callus without affecting the fatty crystal, and the crystal may be dissolved with alcohol with no effect upon the callus coating. (THALER & WEBER 1957 found callose mantles about crystals of calcium oxalate in *Abutilon*.) In one instance, a cystolithoid callus growth was found to enclose a cluster of fatty droplets (fig. 11). Figure 12 shows another cystolithoid callus. In this structure, the resorcin blue stain was very light. Some small foreign bodies seem to be embedded in the swollen portion of the callus.

These last-described callus growths are reminiscent of the cystolithoid formations of callose found in *Asperula* by WEBER & THALER 1956. The suggestion has been made that such abnormal cystolith growths are related

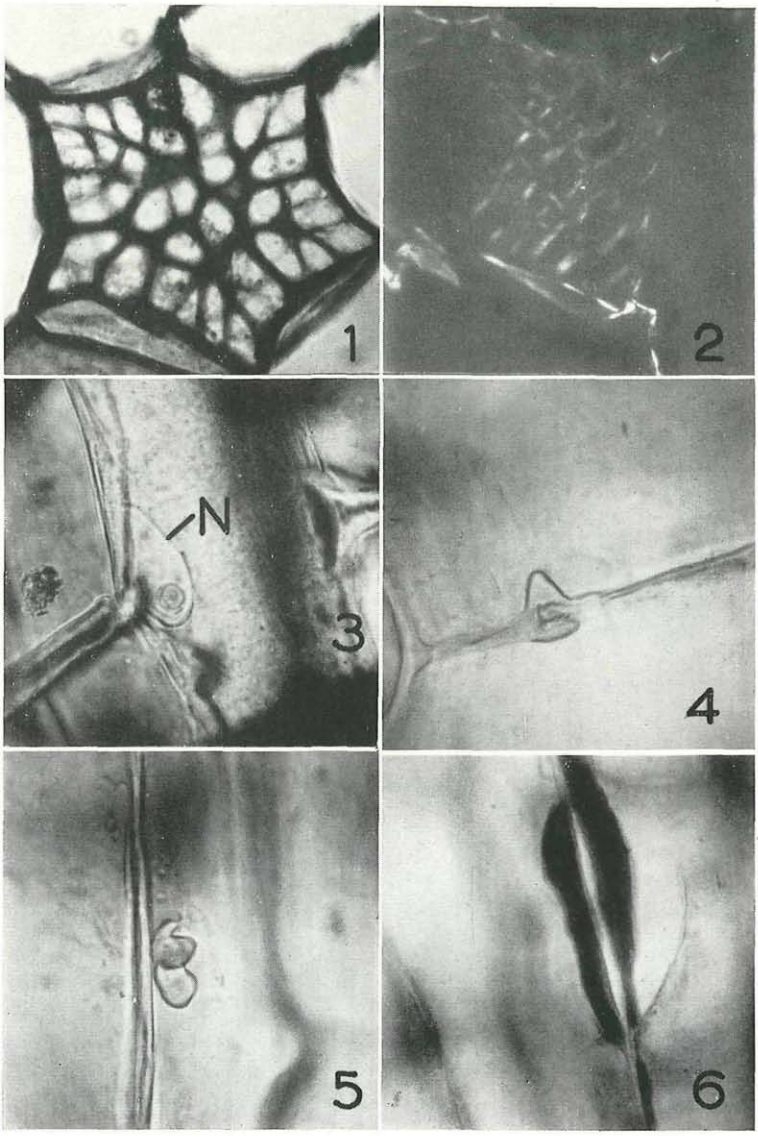

Fig. 1. Transverse wall of laticiferous cell, as seen in face view. Note thickened rims on borders of thin areas. × 710 — Fig. 2. Another transverse wall, as seen between crossed nicol prisms. Birefringence patterns indicate that cellulosic micelles in the thickened rims lie tangential to each encircled thin area. × 710. — Fig. 3. Portion of living laticiferous cell, showing nucleus (N) and nucleolus at left and finely particulate latex of central vacuole. × 440. — Fig. 4. Callus nodule, representing most typical shape found. Conical on one side of the transverse wall (above) and lenticular on the opposite side of that wall (below). Lightly stained with aniline blue. × 710. — Fig. 5. Callus nodules on lateral wall of laticiferous cell. Adjoining cell is laticiferous also. Light aniline blue stain. × 710. — Fig. 6. Callus deposits on lateral walls of two adjoining laticiferous cells. Note that opposition of deposits is not as precise as at transverse wall. Resorcin blue stain. × 440.

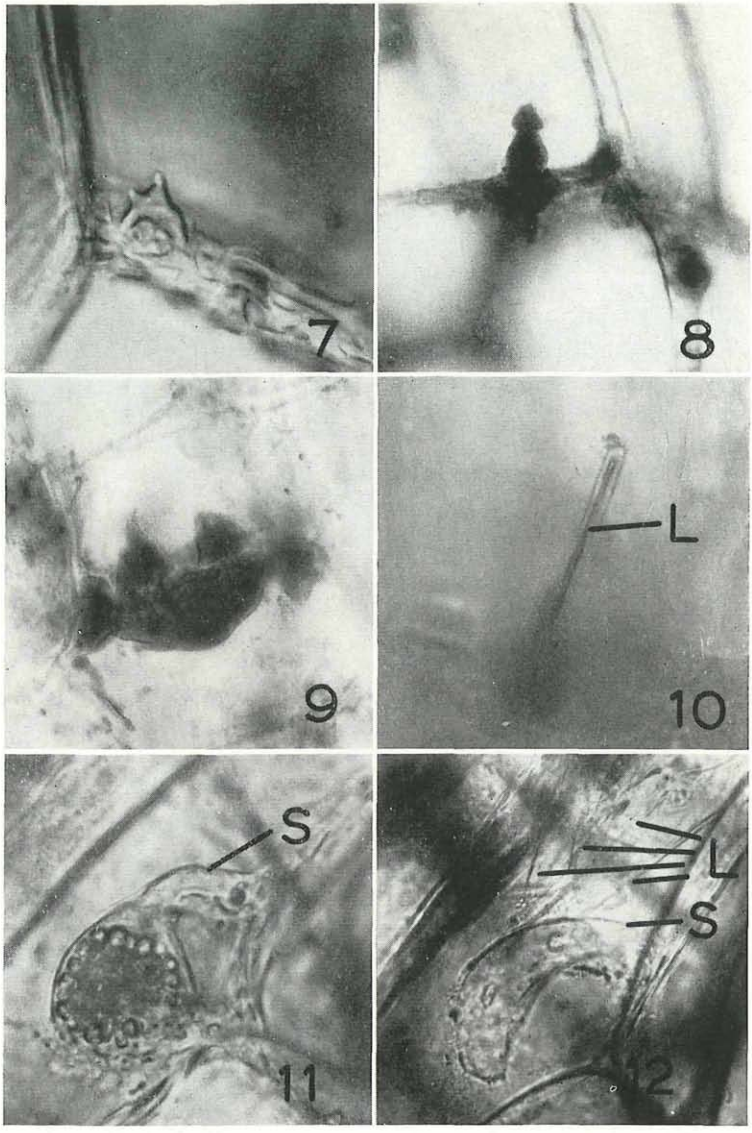

Fig. 7. Formation of apiculate protrusions on conical callus nodule at transverse wall (seen partly from above). Aniline blue stain. × 710. — Fig. 8. Double-cone development of callus nodule above and spike-like growth from lenticular nodule below. Tip of double cone (out of plane of focus) comes to a fine point. Resorcin blue stain. × 710. — Fig. 9. Extension of callus masses across transverse wall. Note that fused masses below tend to exhibit lenticular shape while cones above remain individual. Resorcin blue stain. × 710. — Fig. 10. Unstained cellus deposit about lipoidal crystal (L), at transverse wall. Crystal long axis is more or less perpendicular to plane of that wall. (Subsequently, the colorless deposit stained deeply when a drop of resorcin blue solution was added). × 710. — Fig. 11. Unstained cystolith-like callus growth enclosing a group of lipoidal droplets. Note narrow, elongated stalk (S). × 710. — Fig. 12. Unstained cystolith-like growth in cell with group of acicular lipoidal crystals (L). When resorcin blue was added, this growth was stained very lightly. Thick stalk of cystolith structure at (S). × 440.

to virus infection in that plant. It is of interest to note that the presence of lipoidal crystals in the laticiferous cells is attended by many circumstances which are similar to those surrounding virus infection (STERLING 1959). The cystolithoid relationship discussed here is another factor indicating possible virus activity.

Summary

The transverse walls of laticiferous cells of onion are microscopically entire, being pitted rather than perforated. These walls have characteristic callus deposits which are conical in shape. Occasional bizarre, apiculate protuberances may develop on the callus deposit. Reference is made to the occasional appearance of callus as a mantle about lipoidal crystals and to the cystolith-like growth of some callus masses.

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