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The Fine Structure of the Collenchyma Cell Wall*

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Summary. An investigation of the fine structure of the cell wall was carried out on representative species of four morphological forms of collenchyma, viz. annular, angular, plate and lacunate. In all forms lamellae were observed in which the orientation of cellulose microfibrils was transverse. These lamellae alternated throughout the thickness of the wall with lamellae in which the orientation of cellulose microfibrils was longitudinal. The distribution of pectic substances within the wall, when stained with ruthenium red and by the alkaline hydroxylamine-ferric chloride method of Reeve (1958), was generally not observed in lamellate form although instances of lamellae with high pectic content were observed.

Introduction

Investigators of plant cells have generally reported the lamellar nature of the collenchyma cell wall to be characterised by the occurrence of lamellae rich in cellulose and poor in non-cellulosic components alternating with lamellae poor in cellulose and rich in non-cellulosic components (Anderson, 1927; Majumdar and Preston, 1941; Beer and Setterfield, 1958; Roland, 1964, 1965, 1966). The non-cellulosic component has most often been considered to be composed primarily of pectic substances, or, more recently, hemi-celluloses (Roland, 1966). Preston and Duckworth (1946), although observing a lamellation of pectic substances, found a uniform distribution of cellulose throughout the wall in the collenchyma of *Petasites vulgaris*. The above studies have also shown the presence of an exclusively longitudinal orientation of cellulose microfibrils throughout the wall, except next to the lumen where the

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orientation appeared more nearly transverse. Majumdar and Preston (1941) considered the lamellation of the collenchyma wall to be continuous around the cell. Beer and Setterfield (1958), however, observed discontinuous lamellae which were deposited both outside and inside other continuous lamellae previously formed. Czaja (1961) suggested an entirely different concept of collenchyma cell-wall structure in which the cell wall was longitudinally heterogeneous in composition such that bands, predominantly of pectin, alternated with bands predominantly of cellulose.

The present study was undertaken in an endeavour to help clarify some of the differing interpretations on the structure of the collenchyma cell wall.

Materials and Methods

Representative species of the four most commonly considered morphological types of collenchyma were chosen for study, viz. angular, lacunate, plate and annular. The following species were examined:

Species	Morphological type of collenchyma	Location of collenchyma
Heracleum lanatum Michx.	Annular	Leaf petiole
Liquidamber stryraciflua L.	Annular	Leaf petiole
Abutilon striatum Dickson, var. *Thompsonii* Veitch.	Annular	Stem
Apium graveolens L., var. *dulce* Pers.	Annular to angular	Leaf petiole
Solanum tuberosum L.	Angular	Stem
Beta vulgaris L.	Angular	Leaf petiole
Rumex conglomeratus Murray	Angular to annular to plate	Leaf petiole
Viburnum opulus L., var. sterile, DC.	Angular to plate	Leaf petiole
Sambucus nigra L. var. *aureo-variegata*, West.	Plate to angular	Leaf petiole
Petasites fragrans Presl.	Lacunate	Leaf petiole

The material was restricted to the peripheral collenchyma of the stems and petioles. The collenchyma-like tissue of vascular-bundle caps was not examined.

Small segments of tissue were fixed in 70% ethyl alcohol or glutaraldehyde-osmium tetroxide and embedded in Epon. Thin sections were mounted on copper grids and shadowed with platinum-palladium following removal of the plastic (Mayor *et al.*, 1961) or stained with uranyl acetate and lead citrate. Replicas of macerated cells (10% pectinase at 37° for 24 hr) and of sections were also prepared before examination with an electron microscope. Additional material was treated with pectinase to remove pectic and other substances prior to embedding and sectioning. The distribution of pectic substances was studied by

the general staining reaction with ruthenium red or by the alkaline hydroxylamine-ferric chloride reaction of Reeve (1958). Cellulose was detected using chlor-zinc-iodine or by the I-KI-sulphuric acid reaction (Jensen, 1962). The Reeve reaction was also used for electron microscopic examination of the material (Albersheim *et al.*, 1960).

Results

When seen with the electron and light microscopes, lamellation of the collenchyma cell wall was a consistent feature of the four morphological forms of collenchyma examined. The nature of this lamellation, however, differed substantially from reports by previous workers.

The most striking feature of the electron micrographs of the cell wall was the presence of lamellae in which the orientation of cellulose microfibrils was transverse. These lamellae were not restricted to a layer bordering the cell lumen, but occurred throughout the wall thickness alternating with lamellae in which the orientation of cellulose microfibrils was longitudinal (Fig. 1) [1]. Between successive lamellae, an abrupt reorientation of cellulose microfibrils was observed (Fig. 2). That the microfibrils observed were predominantly composed of cellulose and not other wall substances was indicated by their presence in pectinase-treated material (Fig. 1)[2]. In all four morphological types of collenchyma examined this wall pattern was evident.

In the heavily thickened areas of the cell wall, lamellae in which the orientation of cellulose was longitudinal were generally considerably thicker than those in which the orientation was transverse. In the thinner areas of the wall there was a marked reduction in thickness of

Fig. 1. Electron micrograph of transverse section of collenchyma cell wall in *Apium gracolens dulce* treated with pectinase. Alternate lamellae are composed of microfibrils oriented in transverse or longitudinal directions. An apparent uniformity in distribution of wall substance is evident. Shadowed with platinum-palladium following removal of the embedding medium. Position of lumen beyond upper left corner. × 48,000. *C* Cytoplasm; *I* Intercellular space; *L* Lamella in which the orientation of cellulose microfibrils is longitudinal; *T* Lamella in which the orientation of cellulose microfibrils is transverse

1 In this paper the term lamella is used to designate a stratum of cell wall characterized mainly by the microfibrillar orientation of its cellulose component. It is used to indicate structure at the level of both light and electron microscopes. It is usually several to many cellulose microfibrils in thickness, although in the thin areas of the collenchyma wall it may be as thin as one layer of microfibrils.

2 It is not meant to be implied that the commercial pectinase employed here specifically removes pectin, as in all probability other wall substances are removed as well. Since cellulose would be the most difficult wall component to degrade, the objective here is merely to indicate the presence of microfibrils which must certainly be primarily composed of cellulose.

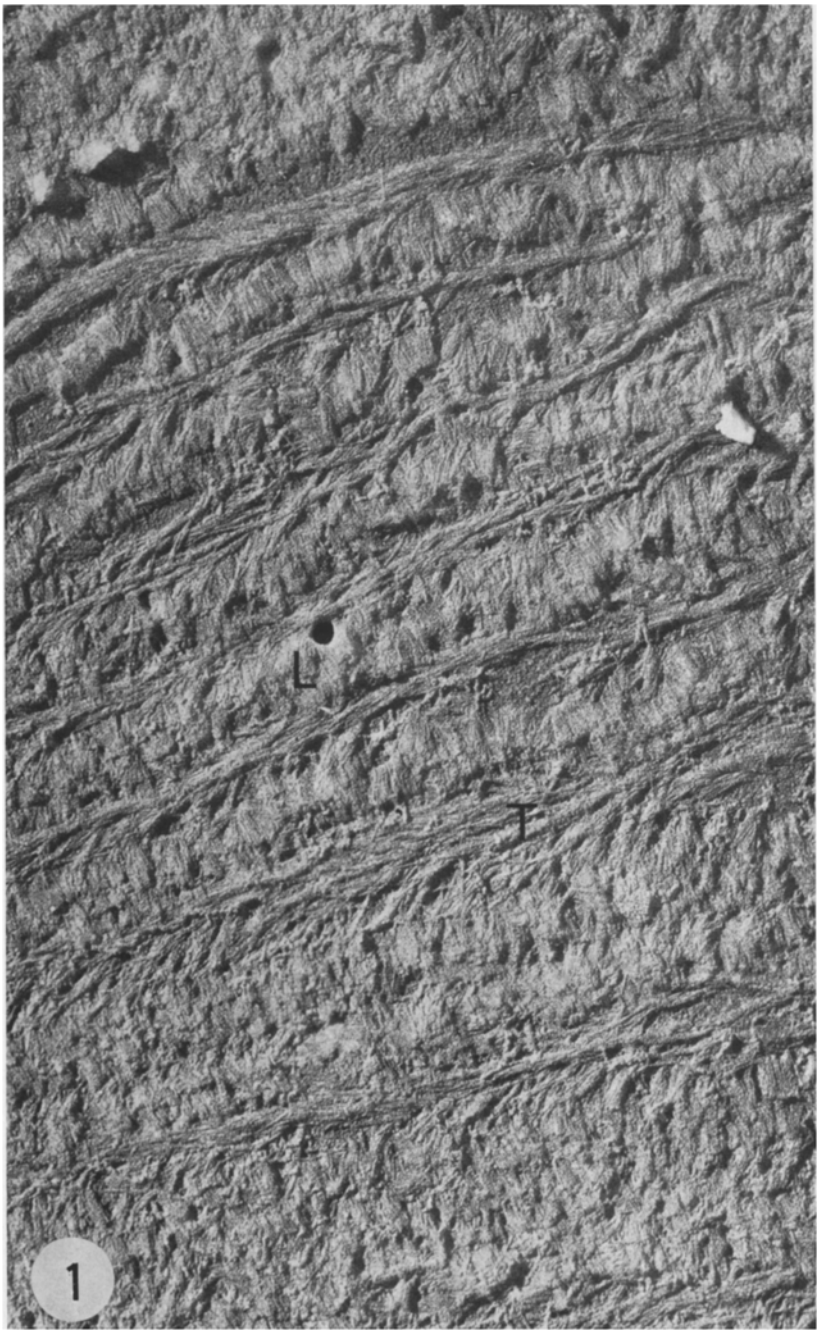

Fig. 1

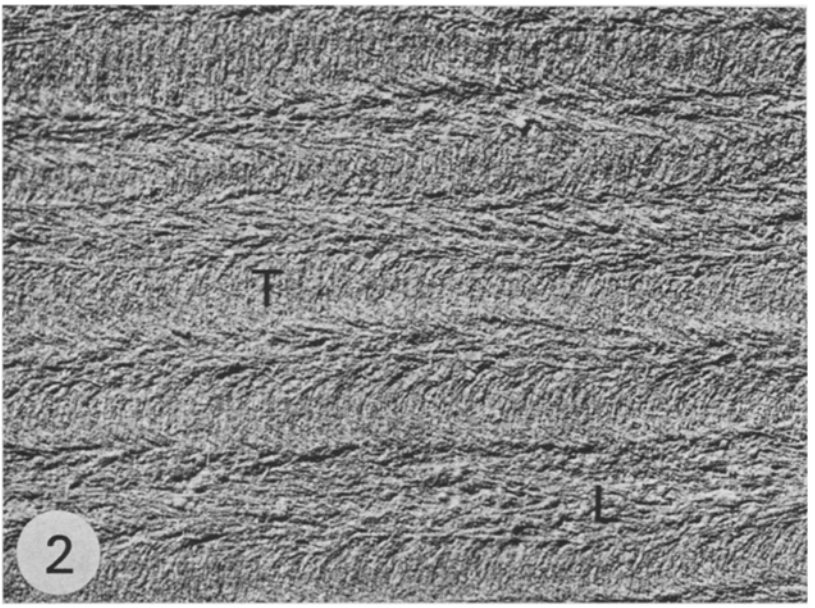

Fig. 2. Electron micrograph of approximately radial section of collenchyma cell wall in *Rumex conglomeratus* showing transition of microfibrillar orientation between successive lamellae. Fixed in 70% alcohol. Shadowed with platinum-palladium following removal of the embedding medium. Cell axis parallel to long edge of micrograph. × 66,000

all lamellae, but most notably this reduction appeared to occur in those lamellae in which the orientation of cellulose microfibrils was longitudinal (Fig. 3). Thus, in the thin areas of the wall, the predominant lamellae were those which exhibited a transverse orientation of cellulose. This explains the relatively high degree of birefringence observed in the thinner portion of the walls when viewed in transverse section between crossed nicols (Fig. 4). That all lamellae appeared to be continuous around the cell was indicated in electron micrographs (Fig. 3) and in photomicrographs of sections stained for cellulose with iodine. The discontinuous lamellae reported by Beer and Setterfield (1958) and

Fig. 3. Electron micrograph of transverse section of thin area of collenchyma cell wall in *Rumex conglomeratus*. Reduction in thickness is most marked in lamellae showing a longitudinal orientation of cellulose microfibrils. All lamellae appear to be continuous through the thinner portion of the wall. Glutaraldehyde-osmium tetroxide. Uranyl acetate lead citrate stained. × 30,000

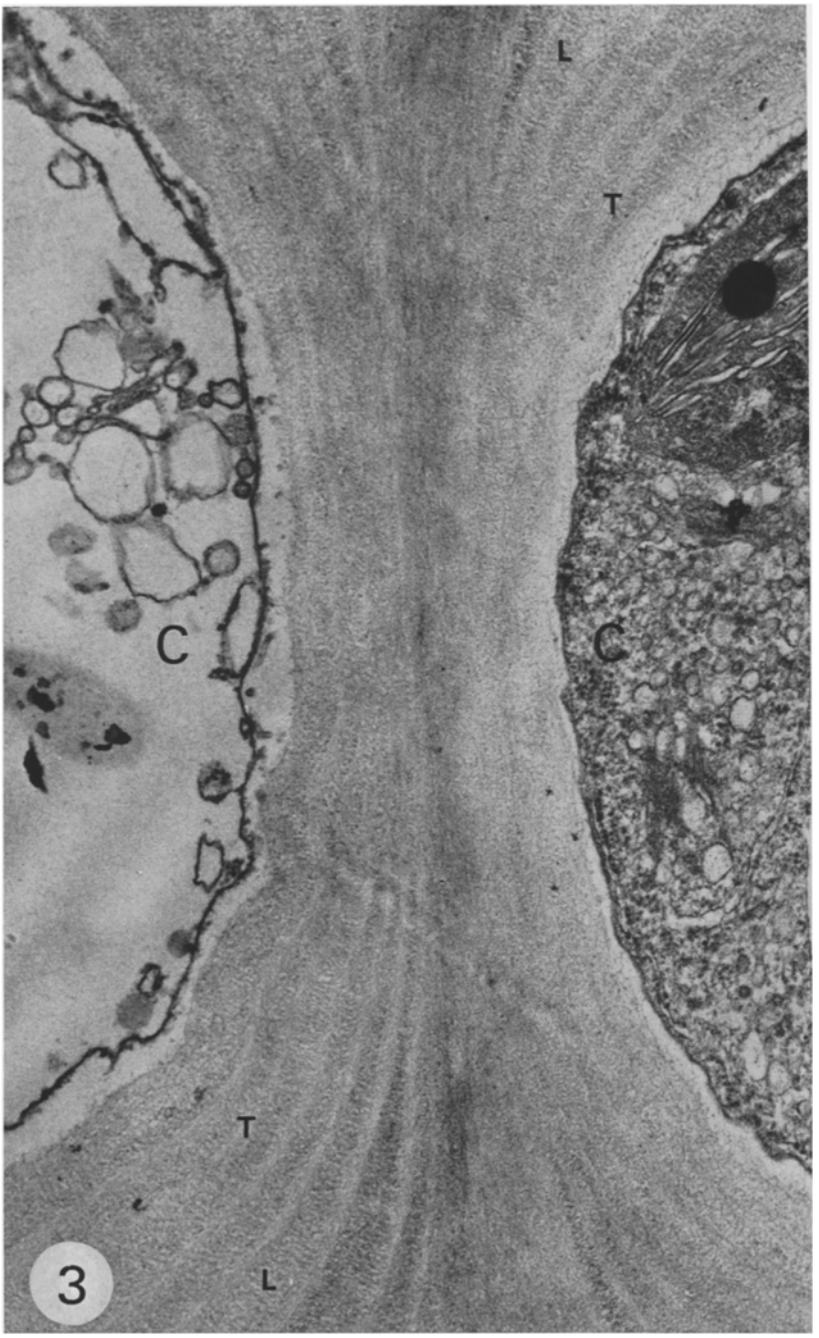

Fig. 3

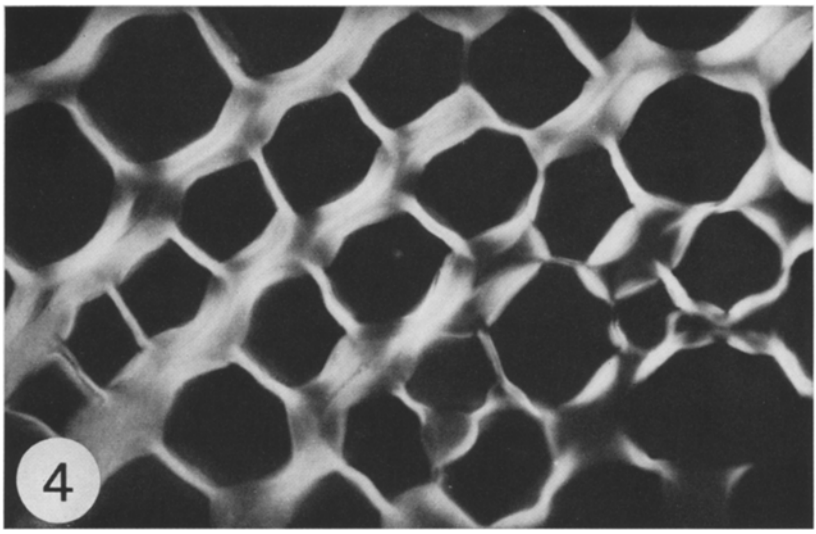

Fig. 4. Photomicrograph of collenchyma tissue mainly of the plate form in *Sambucus nigra aureo-variegata*. Unstained transverse section viewed between crossed nicols showing relatively high birefringence in thin walls. × 500

indicated by Roelofson (1966) could not be detected. Neither was there any evidence to support the concept of wall structure as proposed by Czaja (1961).

Staining of fresh sections with ruthenium red and hydroxylamine-ferric chloride produced varying results. Under the optical microscope both methods showed a lamellar distribution of pectic substances in only three species, viz. *Apium graveolens*, *Solanum tuberosum* and *Beta vulgaris*[3]. Examination with the electron microscope of the collenchyma of *Apium* and *Petasites* stained by the Reeve method produced conflicting results as well, with both continuous and lamellar distributions being observed (Fig. 5).

Fig. 5. Electron micrograph of transverse section of collenchyma cell wall in *Petasites fragrans* stained by the Reeve method showing a lamellation of pectic substances. Those lamellae having the higher pectin content appear to be those in which the orientation of the cellulose microfibrils is longitudinal. × 68,000

3 Although ruthenium red is acknowledged to stain certain hemicellulosic components as well as pectic substances, it is interesting to note that under the optical microscope the species showing lamellation with this stain are the same as those that exhibit lamellation when stained by the more specific hydroxylamine-ferric chloride method.

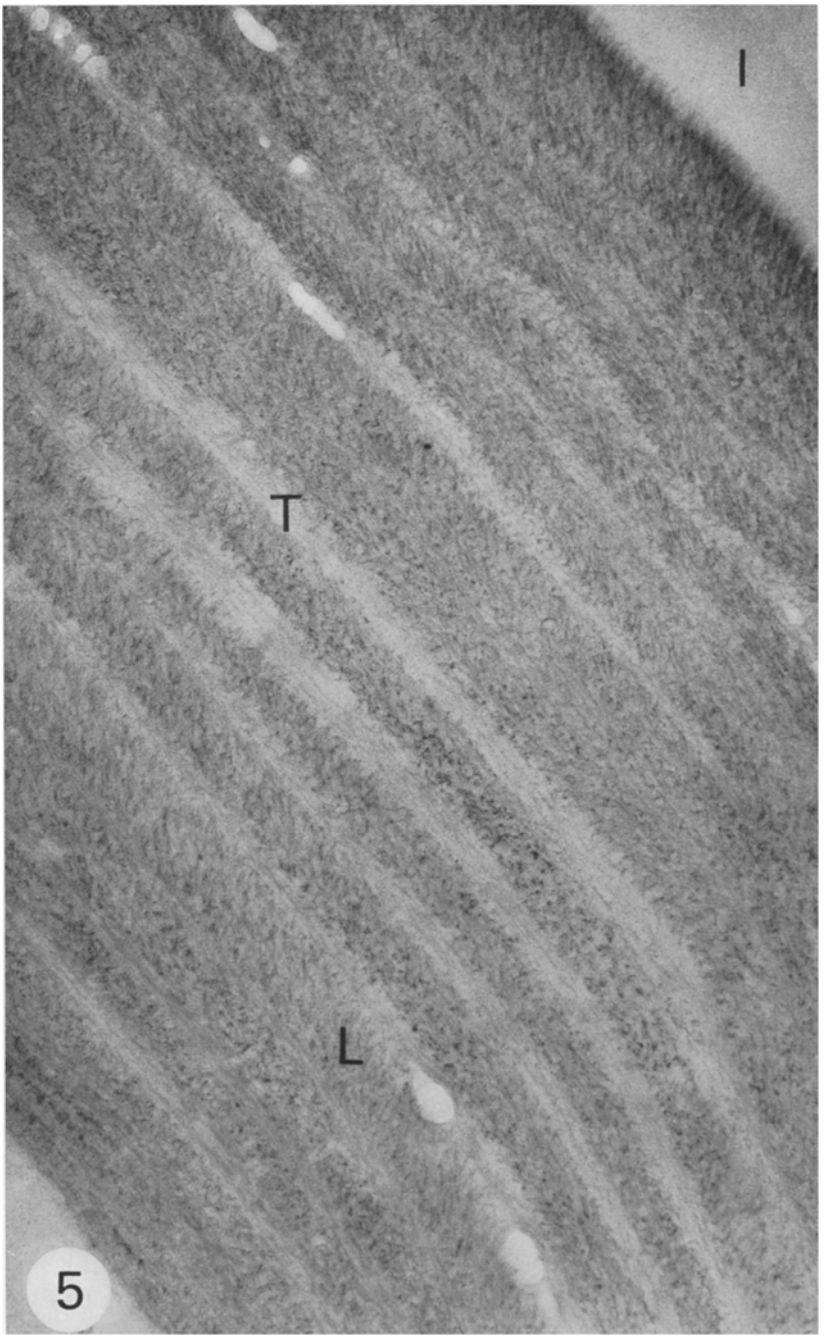

Fig. 5

Discussion

The presence throughout the wall of regularly occurring lamellae in which the orientation of cellulose microfibrils was transverse, alternating with lamellae showing a longitudinal orientation of cellulose, appears to be a hitherto unrecorded fact, with the exception of a similar observation by Wardrop (personal communication) on the lignified collenchyma of *Eryngium*. This regular alternation of lamellae of mutually perpendicular cellulose orientation differs from the findings of previous workers (Majumdar and Preston, 1941; Preston and Duckworth, 1946; Beer and Setterfield, 1958; Roland, 1966). These authors concurred on an exclusively axial orientation with the exception of the innermost layer which was considered to have an orientation of cellulose microfibrils different from the longitudinal or transverse. Although the X-ray diagrams of Preston and his co-workers show an absence of transversely oriented cellulose, the evidence obtained here appears conclusive. The electron micrographs of Beer and Setterfield (1958) and of Roland (1964, 1965, 1966) also fail to show the presence of regularly occurring lamellae having a transverse orientation of cellulose. This appears to be partially due to the embedding medium used, viz. methacrylate, which tends to produce disruption of the wall, especially with extracted material, by expansion of the plastic during polymerization.

The distribution of pectic substances within the cell wall remains unclear. Observations with optical and electron microscopes provided evidence of relatively continuous distribution of these components in many instances but a lamellar distribution in others. When a lamellar distribution was apparent in the electron microscope, however, the lamellae most intensely stained appeared to be those in which the orientation of cellulose microfibrils was longitudinal (Fig. 5).

In view of the complex organization of the wall, the process of growth in collenchyma cells would appear difficult to explain in terms of the multinet concept. Although a certain amount of passive movement and reorientation of microfibrils probably occurs in the outer lamellae as the cell elongates, the presence of successive lamellae with mutually perpendicular cellulose orientation throughout the wall appears inconsistent with the operation of the multinet mechanism.

Regarding passive elongation of the outer wall lamellae, replicas of macerated elongated collenchyma cells of *Apium* sometimes show bands of transversely oriented microfibrils, spaced longitudinally, in the outermost lamella. On the other hand, the outermost lamella sometimes displayed a random organization. It seems unlikely that such a random organization could result from diameter growth of the cell for, while

mature cells are larger in diameter than their meristematic precursors, the increase in diameter is in no way commensurate with the considerable extension in the axial direction. Other growth theories, e.g. mosaic growth or protoplasmic tip growth, do not appear to be operative in the collenchyma cell wall since incorporation of radioactive glucose has been shown in preliminary work here to be approximately uniform along the length of the cell.

It is suggested that, in view of the apparent uniformity of distribution of microfibrils before and after treatment with pectinase (Figs. 1 and 2), the distribution of cellulose in successive lamellae of the wall is relatively uniform. The alternation in orientation of cellulose microfibrils in successive lamellae here established indicates that lamellation apparent in the wall at the optical level would reflect not primarily a difference in composition between successive lamellae, but a differing structural arrangement of the cellulose microfibrils.

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