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REVIEW

The cholesteric type cell wall: nucleation of defects in the structural order and its relation to spherical cell shape

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INTRODUCTION

Liquid crystals can be synthesized but also occur naturally. They represent a state of ordered matter that is intermediate between mobile fluid and stable solid. They can be seen as structures that are a first step away from crystalline regularity (Mackay 1990). Liquid crystals have found a wide range of applications, in fundamental fields as well as in technology. Their potential importance has been acknowledged by the recent Nobel Prize in Physics awarded to P. G. de Gennes for his work in this area (see for example his basic book on the physics of liquid crystals, 1974).

Two classes of liquid crystals have been shown to play an important role:

(1) the 'smectics', which form sheets of mobile molecules perpendicularly oriented to the surface; among these are the amphiphilic molecules (fatty acids) of cytoplasmic microvesicles and cytomembranes;

(2) the 'nematics', among which are the main variety of 'cholesterics', in which the molecules are perpendicular to an axis and in which their orientation rotates continuously and smoothly through a small angle from plane to plane, forming a characteristic chiral arrangement or 'helicoid' (the so-called 'helicoidal pattern'). In the biological field, they are frequently found in the assemblies of long molecules such as DNA (Livolant 1989), and more especially in fibrous composites built of collagen or chitin in animal extracellular matrices (Bouligand 1972; Woodhead-Galloway *et al.* 1978; Mazur *et al.* 1989; Giraud-Guille 1992).

In the plant cell wall, the helicoidal pattern has been demonstrated in a great variety of examples (see review Neville & Levy 1985) with specific features. Helicoids can be found both in primary walls, where they are usually short-lived, and in secondary walls where they are stable. We have recently focused on the growing primary wall and shown how the wall behaves as a dynamic structure during surface expansion. This case exemplifies an unstable balance between the capacity to organize a cholesteric order and the tendency to destroy this order, either by shearing or by alteration of the genesis of the system at the plasma membrane/wall interface (Roland *et al.* 1992). The present paper deals with the secondary wall and especially with the capacity of the cholesteric complex to curve and adapt to the spherical shape of the cell. This is achieved by nucleation followed by new assembly leading to structural defects which relieve the internal strains. The phenomenon was studied previously in the case of endocarp (Reis *et al.* 1992). Here, special attention is given to the assembly of the cellulose microfibrils in the noteworthy thick-walled cells in which the layers remain unclosed; they have lateral free ends and thus limit the internal strains.

We have compared the strategy of the construction of the helicoidal layering in primary and in secondary walls with model systems.

FLAT AND CURVED HELICOIDAL SURFACES

General: distortion in liquid crystals

The helicoidal systems are generally described as flat and symmetrical arrangements of molecules called 'planar twist' or 'twisted plywood'. Their assembly can be seen as a conflict between a local order and a long-range order (Mackay 1990). As biological systems are often curved and bent, the modified order combines internal translations and rotations of molecules. Two types of defects can be defined: (1) the 'dislocations' resulting from local translocations and linear displacements; (2) the 'disclinations' (from the Greek *kline*: slope) due to displacements by rotation. These defects, which are rare in ordinary crystals, abound in liquid crystals (see Harris 1977).

In liquid crystals, the order is characterized by a vector, the director, which gives the direction of the local anisotropy. A bend introduces highly compressed or dilated domains. To avoid, or limit, the bend of the director field, the system usually nucleates dislocations and disclinations (Manneville 1991). The change of orientation of the molecules breaks the continuous helicoidal symmetry; the distribution of defects allows the system to adapt the three-dimensional arrays and relieve the stressed and strained body (Fig. 1).

Numerous authors have stated that the liquid crystalline phase favours the nucleation of disclinations. Each sheet of molecules absorbs the strain by buckling. Figure 2 illustrates diagrammatically two possible cases of deformations either by removal of a sector in the plane (formation of a cone) or by insertion of a new portion of material. In the latter case, a typical saddle-like surface is created with a complex curvature (four lines of change in orientation start from a singular point).

The situation of the helicoidal wall thickenings

Various possibilities exist for the location of wall thickenings around a given cell whose form is constrained by planar faces and curved domains. The case of 'primary walls' has to be considered separately; many primary walls remain thin and monolayered against the

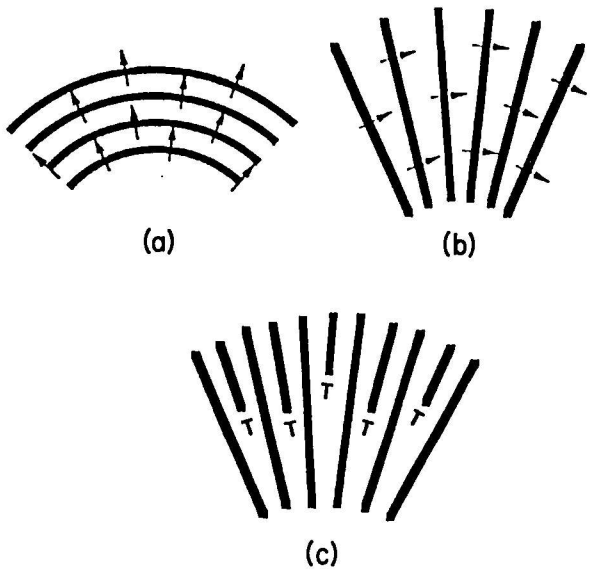

Fig. 1. Deformations in liquid crystals. (a) Splay deformation of the director field (arrows). (b) Bend deformation. (c) Distribution of dislocations, resolving a local variation in surface. (Adapted from Manneville 1991.)

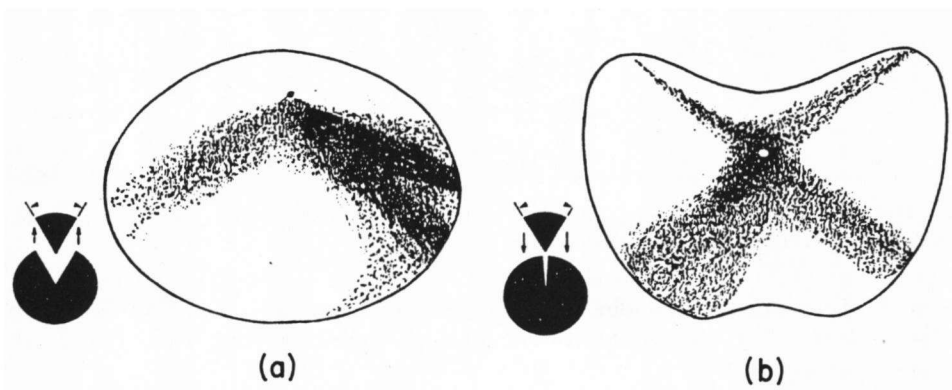

Fig. 2. Surfaces absorbing a strain by buckling. (a) Removal of a sector from a flat surface; the sheet buckles to form a cone. (b) Insertion of a sector; the sheet collapses and ripples; it creates a saddle-like body with four radial lines of change in orientation. (Adapted from Harris 1977 and from Charvolin & Sadoc 1990.)

middle lamella; when they thicken (for example in the cortical parenchyma of many stems, in the young epidermis and in growing supporting tissues such as collenchyma) the helicoidal deposits typically develop *independently* from one another at the 'cell edges' (the so-called 'ribs', see Roelofsen 1959 and Fig. 3a) or on certain cell facets. These deposits remain actually flat: they form planar twists in which the causes of topological defects are minimized (Roland *et al.* 1992).

For the 'secondary walls' considered here, the bend of the layers generally is obvious and one can *a priori* suppose that the curvature implies a progressive readjustment with

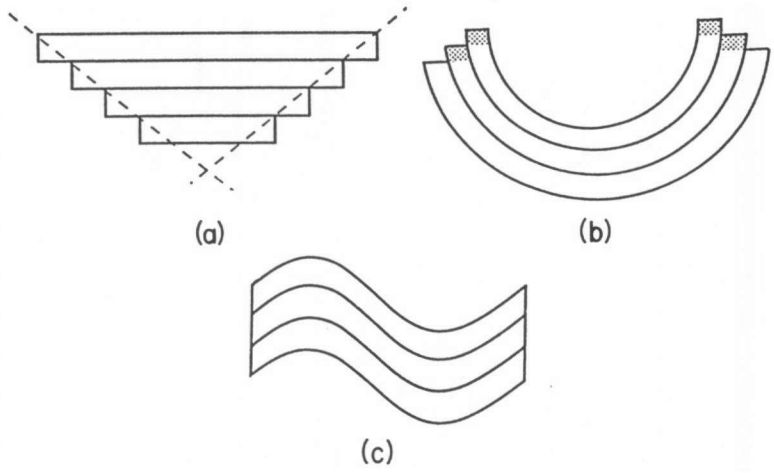

Fig. 3. Flat and bent layerings. (a) A rib (edge thickening); the dotted lines represent a cell corner. (b) Effect of curvature: overlay of the boundaries (hatchings) if the surface of the successive layers remains constant. (c) Folds and undulation.

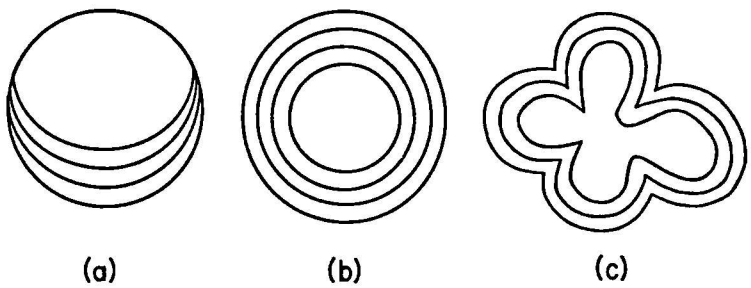

Fig. 4. Different surface extensions of the secondary wall layering. (a) Incomplete (unclosed layers): crescent-shaped or hemispherical. (b) Spherical. (c) Polylobed.

either an overlap of the boundaries or a progressive reduction, of the surface of the successive layers (Fig. 3b). Alternatively, folds might develop to absorb and balance the surface changes (Fig. 3c).

The situation for the location of the secondary wall layerings is depicted in Fig. 4. The 'open surfaces' (Fig. 4a) correspond to incomplete layers (unclosed) with lateral free ends, which reduce the lateral constraints caused by 'emboxing' and spherical shape. Instances of this are rare in nature but do exist, for example in certain sclerified tissues of bark (see below). The 'closed surfaces' (Fig. 4b) and their derivatives, the 'polylobed surfaces' (Fig. 4c), are frequent and usual in many sclerocytes. The endocarps whose mature secondary walls reduce the volume of the cell lumen to a large extent are likely to represent the more advanced cases of wall thickening of this type (Reis *et al.* 1992).

THE CASE OF THE 'UNCLOSED' SECONDARY WALL LAYERS

The occurrence of sclereids in the bark of stems is well-known from numerous anatomical observations and especially in the bark of Lauraceae where they have a

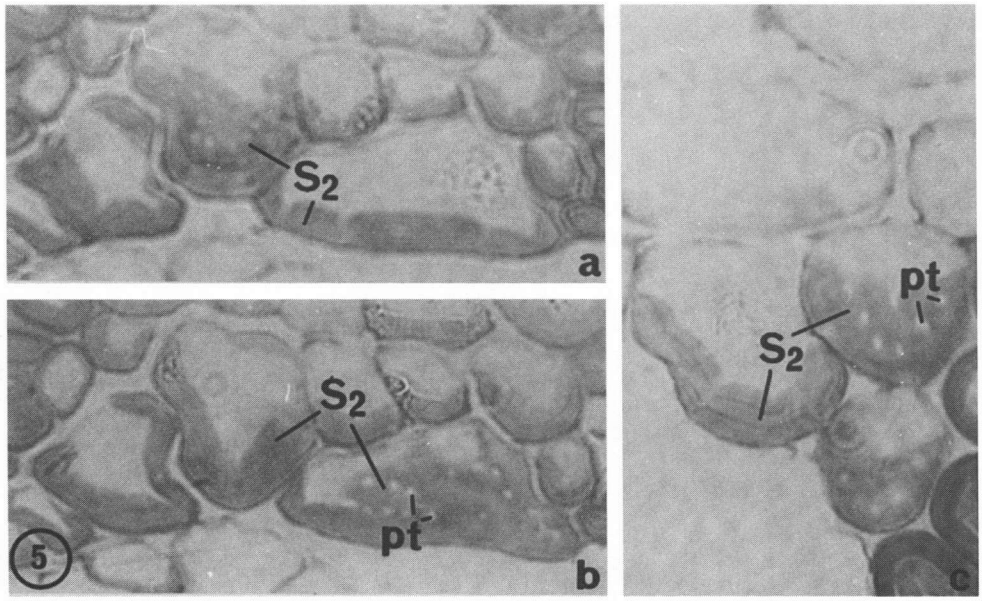

Fig. 5. Sclereids with crescent-shaped secondary wall. Stem of laurel. Fixed extracted (sodium hydrochloride for 2 × 1 h followed by methylamine for 6 days) and plastic-embedded specimens. Semithin sections were stained by toluidine blue, and examined in the light microscope. (a), (b) Serial sections: transverse and tangential views of the same secondary walls (S_2). (c) Pits (pt) are more clearly seen in glancing sections (× 800).

taxonomic significance (Esau 1964; Bamber & Summerville 1979). In Ceylon cinnamon (*Cinnamonum zeylanicum*), the sclereids are arranged at the periphery of the parenchyma and in the non-functioning secondary phloem. In laurel (*Laurus nobilis*), they form clusters disposed between bundles of the fibres commonly referred to as pericyclic.

In the light microscope, they appear as large ovoid cells, with crescent-shaped wall thickenings (Fig. 5). Such roughly hemispheric walls are usually open toward the periphery of the stem. They are highly birefringent between crossed nicols. They are progressively incrusted with lignins; the intense HCl–phloroglucinol staining and the autofluorescence (UV light, wavelength 270 nm) show that they are far more lignified than the pericyclic fibres and the xylem cells. They are storage elements, filled with numerous plastids containing large starch granules, and they stay alive for several years. The layering of the secondary wall is obvious, even at a low magnification, in cross sections in the electron microscope. In glancing sections it gives a 'moiré effect'. Both aspects are seen in Fig. 6a. The thickenings are crossed by pit canals connected to a neighbouring sclereid, to a parenchyma cell, or even to a fibre.

In the flattest portions of the wall, the arcing effect is particularly clear and regular (Figs 6b and 7). The walls maintain a steady and monotonous periodicity over 10–20 successive layers. The arcs are all oriented in the same direction. They are, as expected, cancelled or inverted when the sections are tilted by the goniometric stage. The whole is typically cholesteric-like. The point of this example of unclosed secondary thickenings is to illustrate a rare natural helicoidal pattern which has developed according to a high degree of lateral freedom. In this way, the development of secondary thickenings is comparable to a 'cholesteric wedge' (de Gennes 1974). Initially, the first microfibrils are anchored in the primary wall; the helicoid can then build up its natural pitch towards the

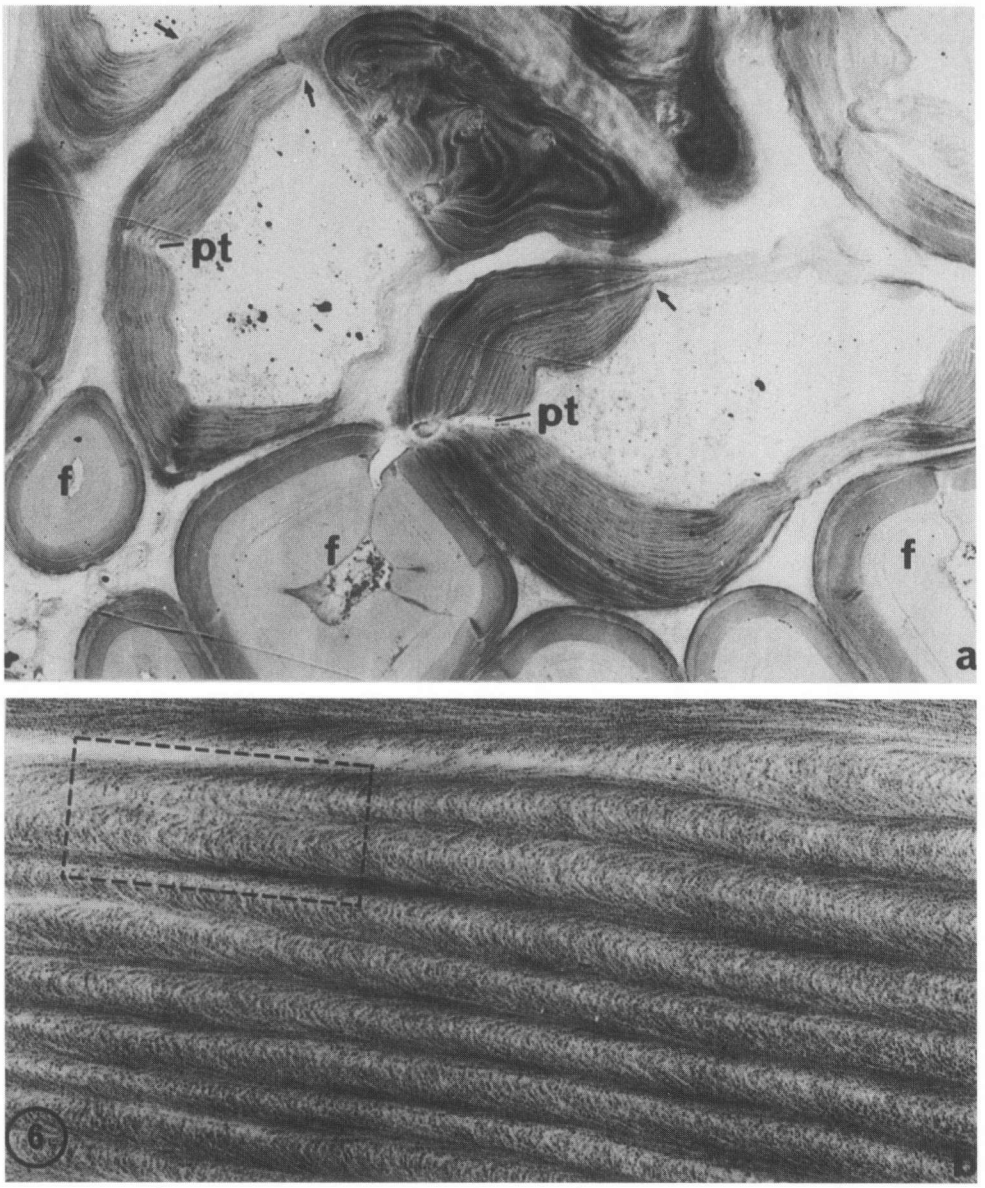

Fig. 6. Unclosed layering of secondary walls. Stem of laurel. Same specimen preparation as in Fig. 5. Ultrathin sections stained with PATAg (periodic acid–thiocarbohydrazide–silver proteinate) staining of polysaccharides. (a) Histology: sclereids with secondary walls seen in profile and in tangential views (moiré aspects) close to a bundle of fibres, f: arrows indicate lateral free ends of the layers: pt = pit canal (× 5000). (b) Detail of the arced pattern; notice the presence of an *edge dislocation* (limited by dotted lines) (× 20 000).

internal and lateral free surfaces. Its helix axis is normal to the cell surface with a pitch in the range of 200–400 nm. The lateral free boundaries of the secondary wall are tapered (Fig. 6a, arrows) and at that level the successive lamellae more or less overlap one another (Fig. 8). Often, the very ends appear as unravelled.

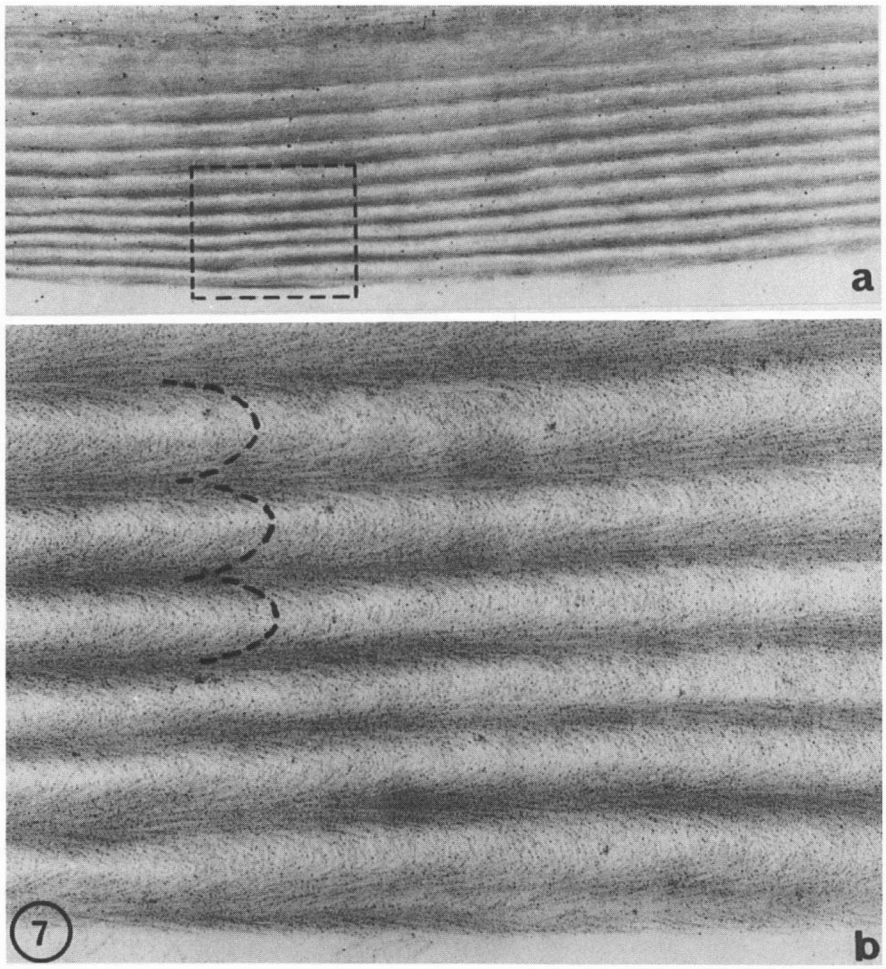

Fig. 7. Flat and regular arcing pattern. Sclereid of cinnamon. Fixed, extracted (sodium hydrochloride for 4 × 1 h followed by methylamine extraction for 4 days) and plastic-embedded specimens. Ultrathin sections were stained with PATAg for polysaccharides. (a) General view of an undistorted area with flawless multilayering (× 11 000). (b) Detail corresponding to the limited area (× 45 000).

It can be seen that such a wall is not completely flawless even in its straight portions. The typical defects that are found are 'edge dislocations'. Figure 6b shows that the central helicoidal line is interrupted and replaced by an empty core. This deviation is oriented towards the free boundary of the cell wall; it produces a thinning of the construction. The edge dislocations are mainly encountered near the scalloped lateral boundaries of the layers. The overlay of the free extremities of the successive layers corresponds to the situation depicted in Fig. 3b: from a morphogenetic viewpoint, the bend is not compensated by a progressive reduction of the surface of assembly of the successive layers.

Among the other symmetry-breaking examples some saddle-like disclinations were noticed (especially between pit canals). At the place where the layers were strongly bent and sectioned tangentially to the surface of the wall, 'spiralized series' were seen (Fig. 9). The genesis of this aspect was explained by Bouligand (1972, 1986) as a conical distortion

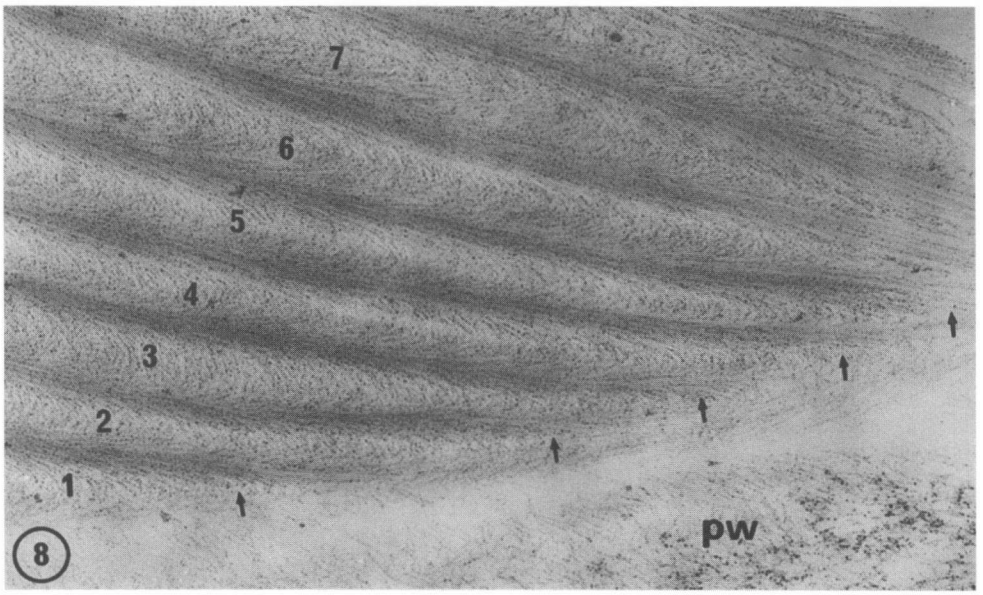

Fig. 8. Overlapping of the arced layers at the boundaries of the secondary wall. Sclereid of cinnamon, preparation: as for Fig. 7. The lateral free end is towards the right. Arrows indicate tapered ends of successive layers numbered from 1 to 7. Pw = primary wall (× 57 500).

associated with a sectioning effect. In some domains, enlarged layers suggested a change of periodicity but it remained usually unclear whether this was due to a more glancing sectioning and/or to an actual modification of the pitch.

Generally speaking, structural defects remained rather rare in the unclosed situation when compared to the following spherical (entirely closed) walls.

COMPLETE SPHERICAL FORMS: (POLY)LOBED AND CLOSED WALL LAYERS

Cells with a spherical shape and thickened secondary wall are frequent. They are specially abundant in the hardest cores of the plant. Numerous 'stone cells' and 'stone tissues' present in fruits have this type of construction. The wall is multilayered (up to 50–100 layers) and the arced pattern is typical. The progressive reduction of the cell lumen is often dramatic. Obviously, the *constraints imposed by the spherical form of the cell are not an obstacle to the construction of a real helicoidal system of cellulose.* The strains are absorbed by buckling and breaking of local symmetry, due to defects and folds. This point has been detailed in a recent paper (Reis *et al.* 1992), yet, for comparison, it seems important to emphasize the role of the folds in relieving the constraints.

The polylobed shaped cells are especially remarkable from this point of view. They are found, for example, in young endocarp of walnut. There the cells become tightly associated like the pieces of a three-dimensional jigsaw puzzle. This is achieved by an early undulating expansion of the primary wall surface (Fig. 10) followed by a sinuous deposit which forms the secondary wall (Fig. 11). The more obvious defects encountered in that case are the numerous saddle-like disclinations (Fig. 11a). They ensure the connection

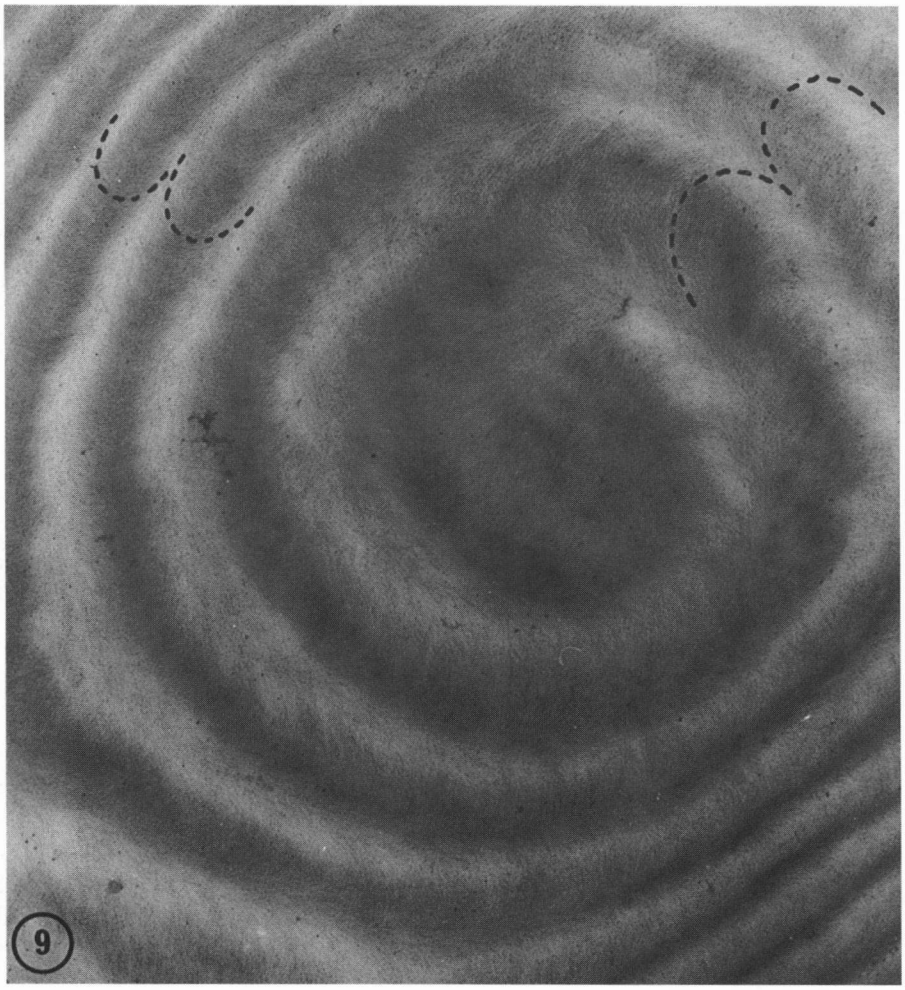

Fig. 9. Spiralized series of bow-shaped microfibrils. Sclereid of cinnamon, preparation: as for Figs 7 and 8. Tangential section through a strongly bent part (× 18 000).

between the different regularly layered basins (Fig. 10) and are abundant between lobes as well as within pit-fields.

A noticeable point is the maintenance of a rather constant width of the layers in the highly folded domains which appear flawless over long distances (Fig. 11b). Data suggest that the helicoidal pitch is not strongly affected by the folded repeats. Sinuosity could be a process to average out the internal constaints. Thus the maintenance of a stable and large surface of assembly may preserve both variations of potential and nucleation of defects.

This situation is rather different from the case of elongated sclereids and long fibres in which the helicoidal arrangement tends to be blocked in preferred orientations (Roland *et al.* 1987). This occurs, in many instances, as a series of more or less regular bursts almost exclusively between the S_1–S_2 and S_2–S_3 layers (Vian *et al.* 1986, 1992). For unknown reasons the cylindrical shape appears less compatible with cholesteric assembly than the much less constraining spherical shape. The case of secondary walls of root hairs that can

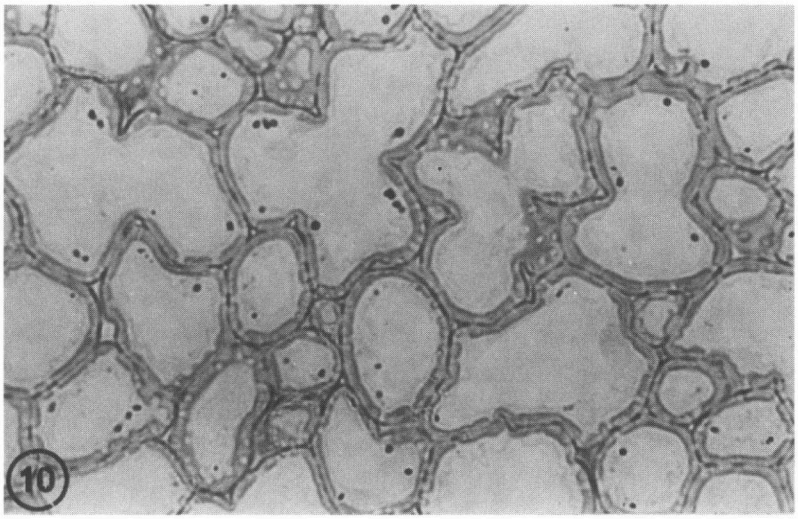

Fig. 10. Polylobed sclereids. Young stage of the endocarp of walnut prepared as described by Reis *et al.* 1992. Non-extracted specimen. Semithin section stained with PAS (periodic acid–Schiff reagent) and examined in the light microscope. The sinuous deposit of the secondary wall has just begun following the undulating surface growth of the primary wall. Note the basins in the secondary wall (× 600).

have helicoidal or parallel textures according to their aquatic or terrestrial development also remains an open and interesting question (Sassen 1993).

CONCLUSION: COMPARED STRATEGY FOR THE TWISTED ASSEMBLY IN PRIMARY AND SECONDARY WALLS

The functional importance of cholesteric-like arrangements of cellulose microfibrils in plant cell walls is mainly mechanical. Helicoids form a multiaxial structure that is capable of working in a complex deformation mode. It is effective against multioriented tensile stresses, pulls or shearing forces.

The cholesteric-like construction appears to be a basic structural feature adaptable to different situations. It has plastic as well as rigid properties and can be found in both primary and differentiated secondary walls. However the topology and the regime of the thickenings are different in the following two classes of walls:

(1) In *thickened helicoidal primary walls*, the helicoids are unevenly distributed and short-lived during growth. They typically form independent ribs at the cell edges. They remain flat and the planar helicoidal system presents a regular layering in which the flaws are minimized (Roland *et al.* 1992). It is a real straight twisted plywood in which no microfibrillar entanglement exists. In terms of surface extension and growth, the planarity, the reduction of defects and the relative independency of the edge reinforcements probably facilitate internal creep and sliding of the molecules. Each thickening unit may extend with a relative autonomy and more easily than if it was in a complete distorted 'emboxed' system.

(2) In *secondary helicoidal walls*, on the contrary, curvature of the layers is the rule. The global spatial requirement set by the bend and by the 'emboxed' situation is accommodated by numerous defects which break the continuous helicoidal symmetry

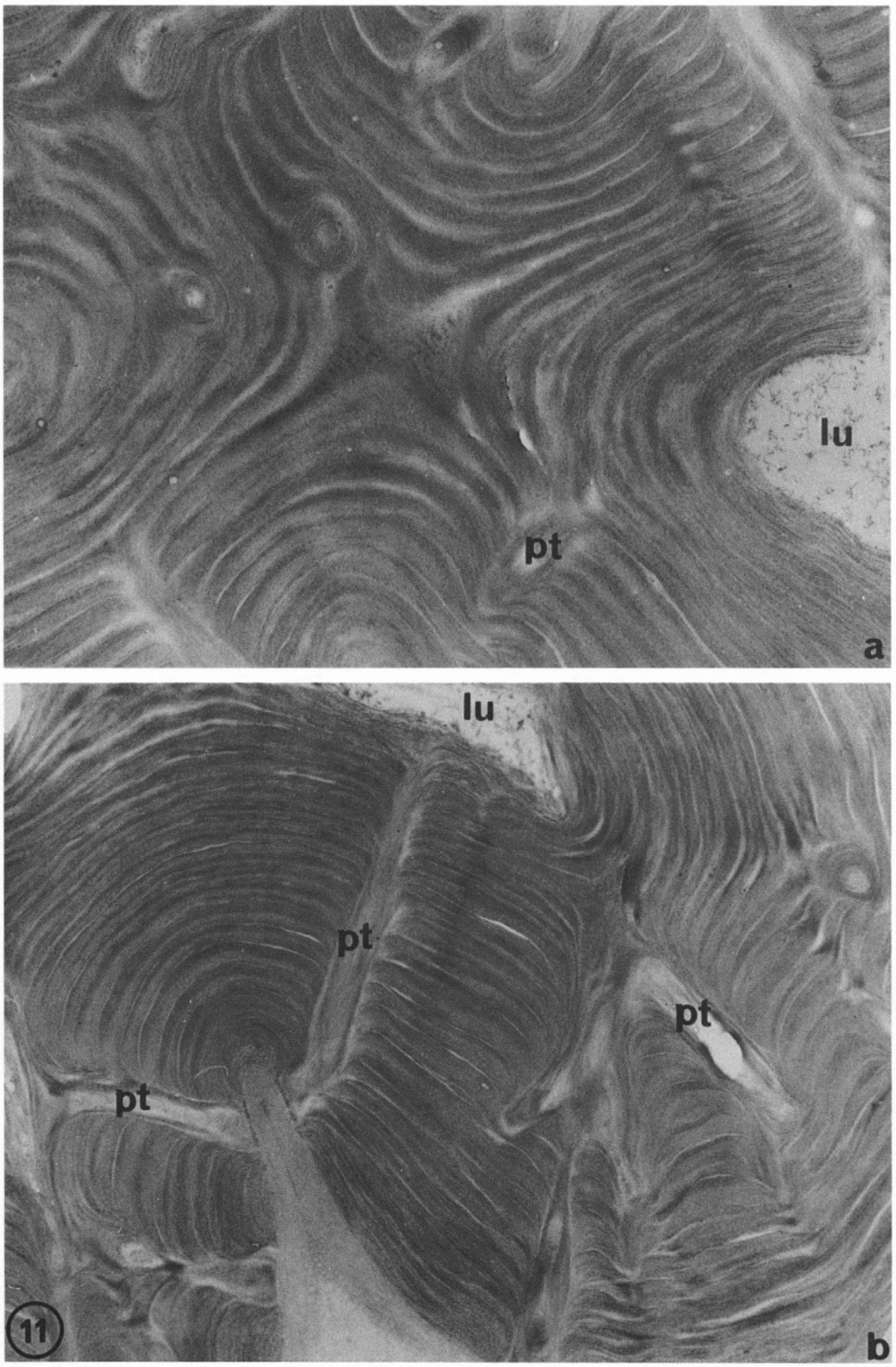

Fig. 11. Polylobed and multilayered cell wall. Endocarp of walnut prepared as described by Reis *et al.* 1992. Ultrathin sections were stained with PATAg for polysaccharides. Parts of the mature secondary wall of walnut with: (a) saddle-like disclination connecting concentric domains (× 11 000); (b) regular layering in folded portions (× 8000), pt = pit, lu = cell lumen.

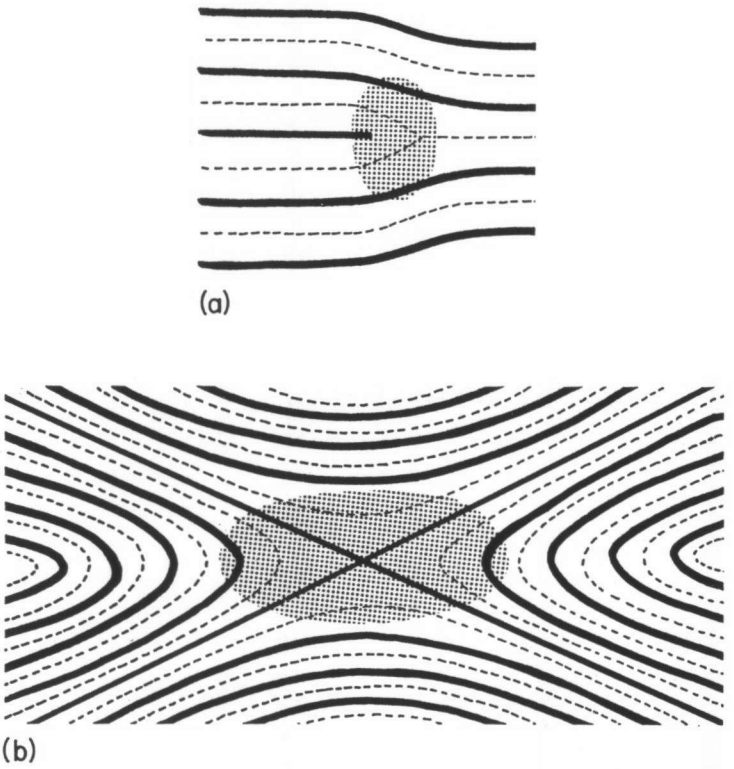

Fig. 12. Diagram of two typical defects found in the helicoidal pattern. (a) Edge dislocation. (b) Saddle-like disclination.

(Fig. 12). Disclinations are nucleated in places where domains of differing microfibril orientation meet. Symmetry is most frequently broken by the saddle-like structures, thus allowing the surrounding fields to reconnect smoothly. The response of the wall is remarkably identical to that of liquid crystals that experimentally are under constraint. Contrary to what occurs in the primary walls, the microfibrils do not keep their degree of freedom.

From the morphogenetic point of view, the data suggest a mechanism for a three-dimensional positioning of the wall components that is similar to the self-assembly process of cholesteric mesophases. At the moment when the common opinion that cortical microtubules orient the newly deposited microfibrils is challenged by an increasing number of studies (Emons 1989; Emons *et al.* 1992; Levy 1991), self-assembly could be an alternative process. The liquid crystal-type reassembly of native cellulose-glucuronoxylans obtained *in vitro* under biological conditions (Reis *et al.* 1991) strengthens the hypothesis that a transient cholesteric mesophase must occur at the very moment when cellulose is ordered in the periplasmic space. Interestingly enough, similar chiral ordering was recently obtained from cellulose crystallites derived from kraft wood pulp from black spruce (Revol *et al.* 1992). Above a critical concentration the colloid dispersion was shown to separate spontaneously into a chiral nematic liquid crystalline phase. On drying, this phase solidifies into regularly twisted fibrillar layers that mimic the structural organization of helicoids in nature.

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