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# A New Interpretation of Plasmodesmatal Ultrastructure

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*Summary.* It is shown that simple, unbranched, plasmodesmata between young xylem ray cells of willow have no direct intercellular continuity apart from the plasmalemma which limits the cytoplasm and lines the plasmodesmatal canal. Each plasmodesma is traversed by a 200 Å diameter tubule (the desmotubule) which has a wall with probably 11 subunits arranged around a central cavity through which runs a 40 Å diameter rod. This rod is connected to the inside of the tubule wall by fine filaments. At the ends of each plasmodesma the plasmalemma and cell wall are closely appressed to the tubule, thus precluding direct continuity between the cytoplasm of adjacent cells. Through the central part of the plasmodesmata the tubule is separated from the plasmalemma by a 90—100 Å wide gap. Cytoplasmic microtubules in the same tissue have a diameter of approximately 250 Å and a wall probably composed of 13 subunits: both desmotubules and cytoplasmic microtubules therefore have a centre-to-centre subunit spacing of about 47 Å. It is suggested that the desmotubules are not microtubules but may be nuclear spindle fibres which become trapped in the wall during cell plate formation. The endoplasmic reticulum, while closely approaching the plasmodesmata, is not continuous across them. It is thought most unlikely that the endoplasmic reticulum traverses plasmodesmata, as the dimensions of the central tubule — found here as well as by other workers — are smaller than those which would be expected to allow a stable molecular configuration in a unit membrane. The plasmalemma, where it lines the plasmodesmatal canal, appears to have particulate subunits in the outer opaque layers and the presence of these subunits may be attributable to the need for stability in membranes arranged about so small a radius.

## Introduction

Electron microscopic studies of plasmodesmata have resulted in a number of suggestions concerning the structure of these complexes but have, as yet, failed to resolve the precise nature of any intercellular connexion.

Most commonly it has been suggested that the plasmodesma comprises an intercellular tube, lined with plasmalemma, and containing a strand of endoplasmic reticulum which may have become trapped in this position during cell plate formation (Buvat, 1960; Porter and Machado, 1960; Whaley, Mollenhauer and Leech, 1960; Frey-Wyssling and Mühlethaler, 1965; López-Sáez, Giménez-Martín and Risueño, 1965; Wardrop, 1965). While it seems to be generally accepted that the plasmalemma does line the canal through the cell wall, there is not the same unanimity concerning the "core" of the plasmodesma. Cronshaw (1965) describes the simple plasmodesmata

found between ray cells in *Acer rubrum*, and refers to a central, electron-opaque, core. Other authors have described different types of structure ranging from the simplest form to anastomosing complexes with considerable internal elaboration (KOLLMANN and SCHUMACHER, 1962, 1963; WOODING and NORTHCOTE, 1965; O'BRIEN and THIMANN, 1967). However, the fundamental problem concerning the ultrastructure of the plasmodesmatal core remains unanswered. For this reason, an investigation was made of plasmodesmata between adjacent ray cells in differentiating secondary xylem, as it is known that such plasmodesmata are simple and normally unbranched.

## Material and Methods

Material was obtained and processed exactly as described by ROBARDS (1968). In brief, cambial tissue of *Salix fragilis* was fixed in 3% glutaraldehyde, post-treated with 1% osmium tetroxide, dehydrated, and embedded in a mixture of Araldite and Epikote. Silver sections were mounted on uncoated 400 mesh grids, stained in lead citrate and viewed in an *AEI EM6B* electron microscope at 60 kV.

Microtubules were examined in transverse sections cut from differentiating gelatinous fibres where it is known that the microtubules are arranged parallel to the fibre length and, therefore, parallel to the stem (ROBARDS, 1968).

Image reinforcement micrographs to enhance radial symmetry (MARKHAM, FREY and HILLS, 1963) were prepared from plates showing clear, transverse, sections of plasmodesmata or microtubules at 100,000× magnification. In some cases reinforcement diagrams were prepared direct from the original plate using 10× photographic enlargement. For convenience, greater magnification was sometimes obtained by enlarging the original plate 10× onto an Agfa-Gevaert Scientia 23D50 plate which was then contact printed onto another, similar, plate. This plate, at 1,000,000× magnification, could then be used with greater ease than the original. The diagrams were normally made using Agfa-Gevaert Brovira hard or extra-hard photographic paper. The paper was rotated about a central pin which was precisely positioned in the geometric centre of the circular structure being examined. By constructing a suitable protractor base-board it was possible to ensure accurate rotations through 9 to 16 arcs in each circle — sufficient to encompass the range of structures encountered in the present work. Experience showed that control of the photographic processes (paper grade, developing times, etc.), as well as the accurate centration of the structure onto the baseboard, were critical in enabling comparable results to be obtained: for this reason conditions as similar as possible were used for each single set of reinforcements. The initial selection of structures suitable for the reinforcement experiment was made using the criteria of concentricity, sharpness, and an even photographic image: random very light or very dark areas on the original plate either destroyed any reinforcement or produced misleading results.

## Observations and Results

The continuity of the plasmalemma between adjacent cells via the plasmodesmata was confirmed. As found in other situations in higher plants (ROBARDS, 1968), the plasmalemma was asymmetric, with the

inner layer more opaque than the outer (Figs. 2 and 3). The structure of the plasmodesmatal core was less easily resolved, but in suitable sections (Figs. 2, 3 and 4) it was established that, within the plasmalemma-lined canal, there was a tubule of approximately 200 Å diameter containing a central rod of 40—50 Å diameter. These two structures

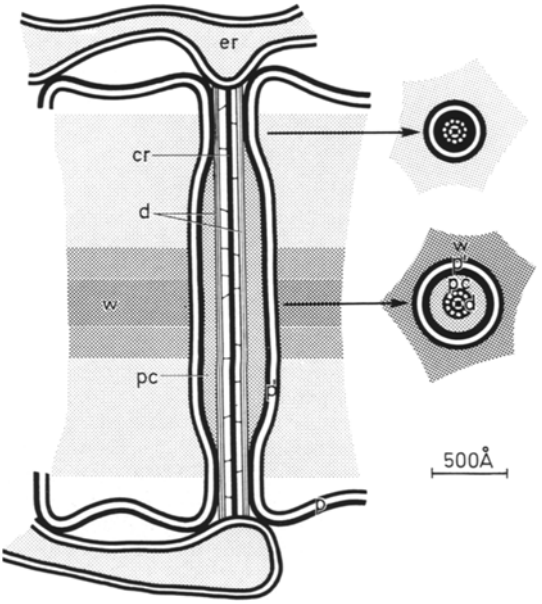


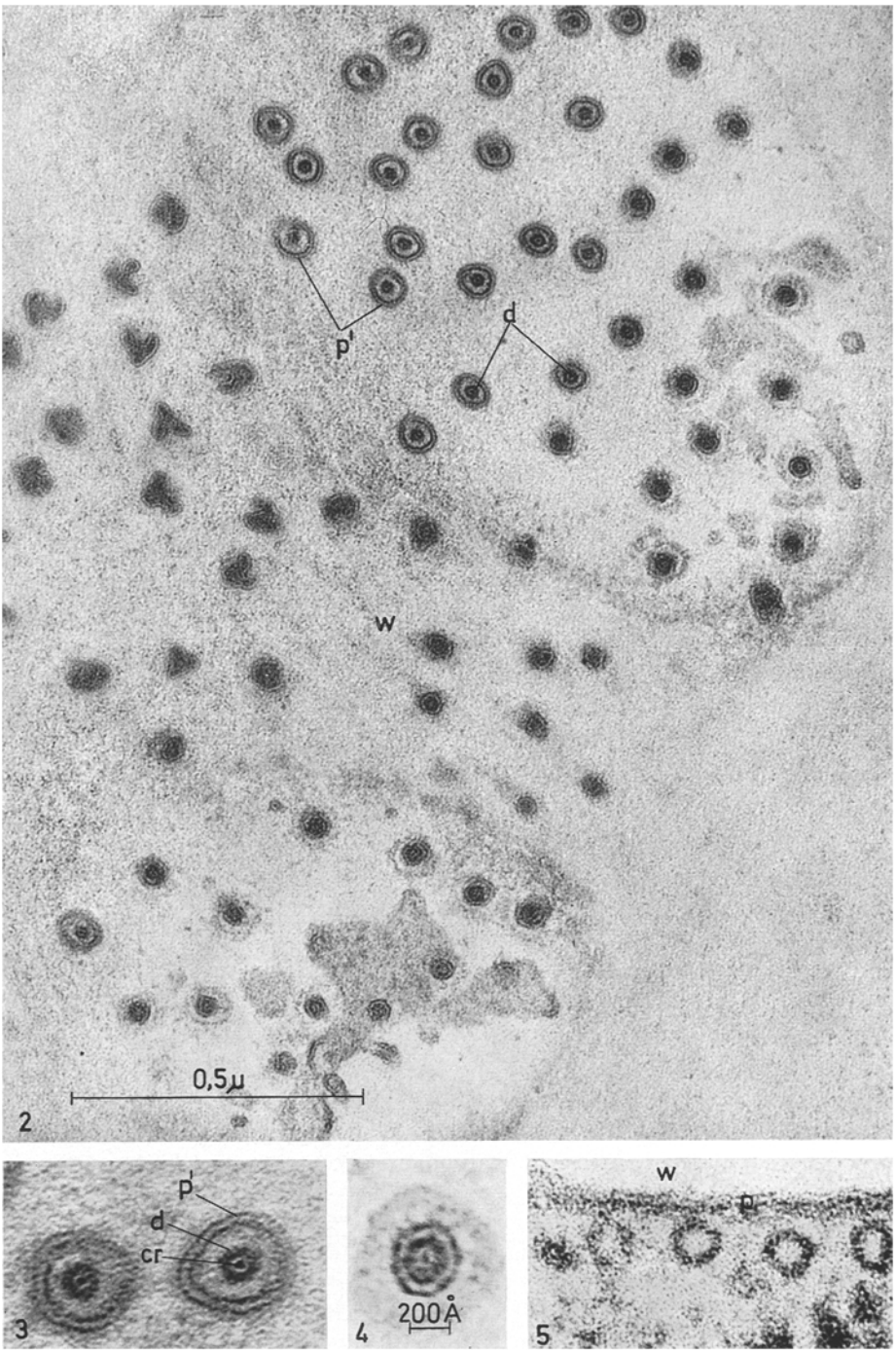

Fig. 1. Diagrammatic representation of longitudinal and transverse sections of a simple plasmodesma, drawn to approximate scale. No attempt has been made to indicate the granular nature of the plasmalemma, but the inner layer is shown thicker than the outer to represent the polarity seen in sectioned material. *cr* central rod; *d* desmotubule; *er* endoplasmic reticulum; *p* plasmalemma; *p'* plasmalemma through plasmodesmatal canal; *pc* plasmodesmatal cavity; *w* cell wall

Fig. 2. Grazing section through a wall between ray cells. Plasmodesmata have been sectioned transversely at all levels. × 80,000

Fig. 3. Transverse section approximately through the mid-line of plasmodesmata. × 260,000

Fig. 4. Transverse section through the narrow "neck" at one end of a plasmodesma. × 260,000

Fig. 5. Transverse section of microtubules in a differentiating gelatinous fibre. The microtubules are larger than the desmotubules seen in Figs. 3 and 4 but the fundamental similarity is evident. Such microtubules gave maximum reinforcement at $n = 13$. × 260,000




Figs. 2—5

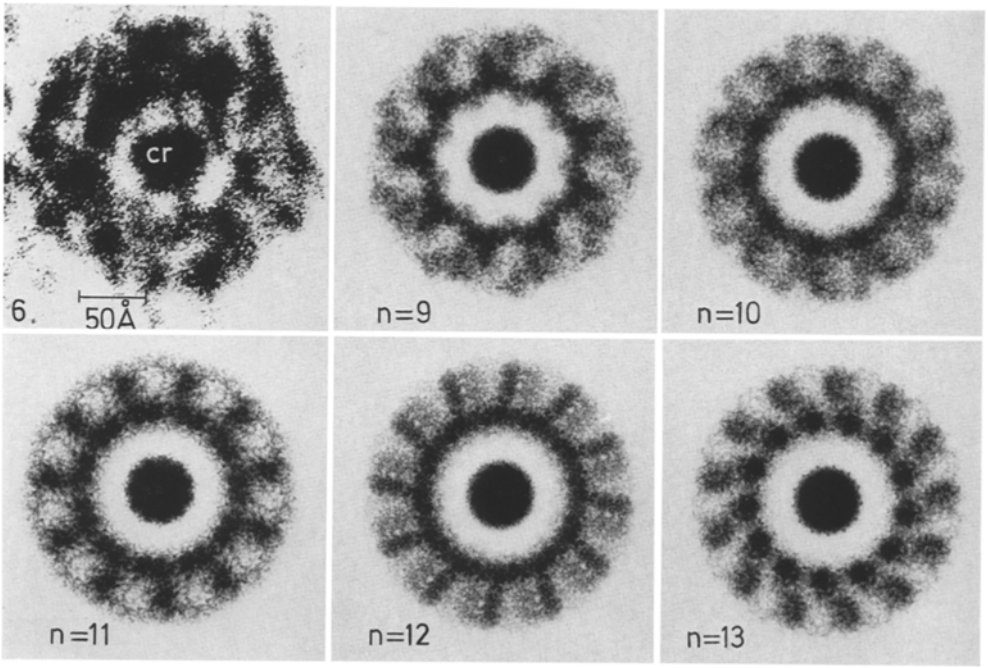
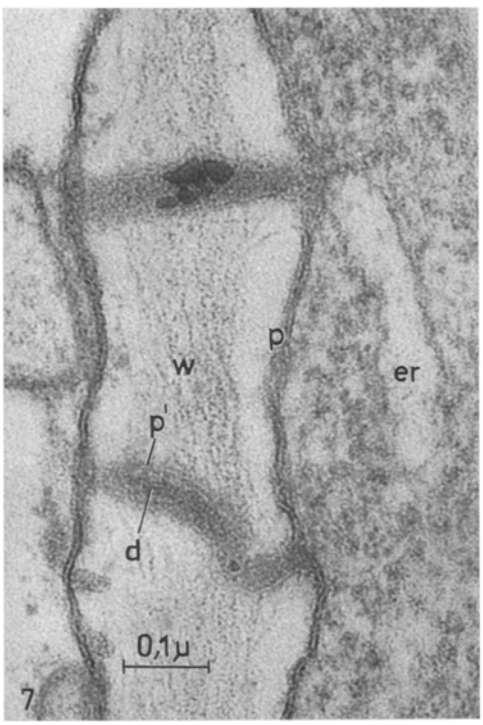



Fig. 6. Reinforcement diagrams prepared from a secondary plate via a positive plate enlarged × 10 from the original. × 1,700,000. The top left micrograph is a direct print; the remainder have been rotated from 9 to 13 times for the completion of each circle. Maximum symmetrical reinforcement is clear when $n = 11$

will be referred to in this paper as the *desmotubule* (*Gk.* desmos — bond) and the *central rod*. Transverse sections of some plasmodesmata indicated the presence of subunits in the desmotubule wall (Figs. 3 and 6). The obvious similarity between the structure of this tubule and that of cytoplasmic microtubules and nuclear spindle fibres suggested that it would be worthwhile attempting to define the substructure of the desmotubules still further by using the method of image reinforcement described by MARKHAM et al. (1963). The results of such a reinforcement series are shown in Fig. 6. The strongest reinforcement usually appeared to be obtained when the photographic paper was moved 11 times to complete a circle (i.e. $n = 11$). Analysis of reinforcement diagrams is partly subjective and requires rigorous attention to the conditions already defined. However, when reinforcement was demonstrated it was normally at $n = 11$, and there seems little doubt that the desmotubule is similar in structure to a normal microtubule (LEDBETTER and PORTER, 1964) but with 11 instead of 13 subunits. To test further the



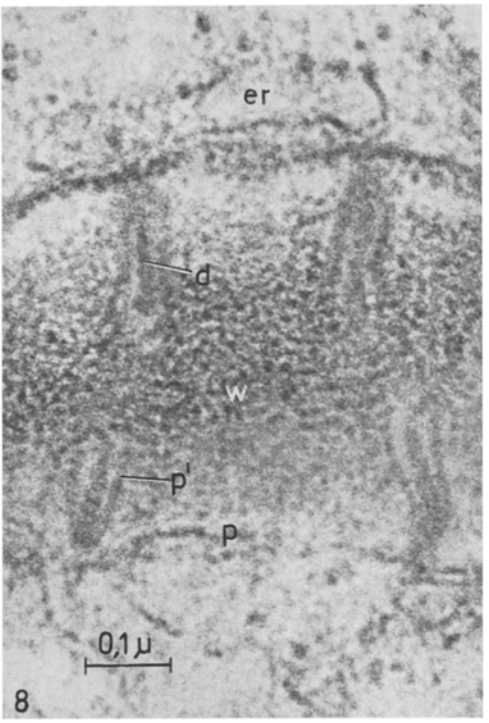




Fig. 7. Longitudinal section of young plasmodesmata in a ray cell wall. The plasmodesmata are continuous from cell to cell. Note the 5-layered plasmalemma (*p*). × 110,000

Fig. 8. Longitudinal section of older plasmodesmata which show discontinuity in the region of the middle lamella. × 110,000

validity of this supposition, cortical microtubules from the same species (Fig. 5) were subjected to the same reinforcement technique. Best reinforcement was obtained at $n = 13$, although $n = 11$ also sometimes produced reinforcement. The desmotubules (Figs. 3 and 4) have a diameter of about 200 Å compared with 250 Å for cortical microtubules (Fig. 5). Assuming that $n = 11$ and $n = 13$ respectively, then the centre-to-centre spacing of the subunits is approximately 46 and 48 Å. An unexpected result of the reinforcement technique was that, when the image was centred using the inner layer of the plasmalemma for alignment (instead of the desmotubule), reinforcement was shown in the opaque layers of the plasmalemma. This was also at $n = 11$, but producing 33 subunits. The outer layer, being essentially less opaque, did not show such strong reinforcement but still appeared to have a

particulate substructure. The opaque central rod has not been shown to have any resolvable structure of its own, although it normally appears attached to the desmotubule by fine filaments (Figs. 3 and 6).

The appearance of simple plasmodesmata in longitudinal section (Figs. 7 and 8) helps to clarify their structure. Through the central part of the plasmodesma the desmotubule is separated from the plasmalemma by a 90—100 Å wide featureless gap. Towards the ends of the plasmodesmata the wall is raised to form small protuberances (Fig. 7) and, in this region, the desmotubule is in close contact with the plasmalemma — as can be confirmed from transverse views (Fig. 4). The desmotubule must terminate in this region for there is no doubt that strands of endoplasmic reticulum contact these plasmodesmata (as they do in most other described cases), and the desmotubule does not continue into the cytoplasm. So far it has not been possible to establish the nature of the contact between the endoplasmic reticulum and the desmotubule. In young cells the plasmodesmata are apparently completely continuous across the wall (Fig. 7), but older plasmodesmata may not be continuous across the region of the middle lamella (Fig. 8).

The interpretation of these results is provided in the form of diagrams (Fig. 1).

## Discussion

AGRAWAL et al. (1965) and RINGO (1967) have stressed the ease with which misinterpretation of structure may arise from using the image reinforcement technique described by MARKHAM et al. However, these authors consider the technique to be of considerable value provided that suitable precautions are taken while using it. It is, for example, thought to be important that the reinforcement images should be compared with the original micrographs. It is also vital that the original structure should be circular and have a clear centre of symmetry. It is considered that the micrographs and reinforcement images presented here fulfill such demands. For this reason it is felt that the demonstration of a tubule with 11 subunits is a positive result not attributable to artifacts. The diameter of these tubules (200 Å) is less than that found for cortical microtubules either in the same tissue or in other plants: it is much closer to that of nuclear spindle fibres (LEDBETTER and PORTER, 1964). As there appear to be 11 subunits in the 200 Å desmotubules and 13 in cortical microtubules of about 250 Å diameter, the inter-subunit spacing (45—50 Å) remains the same, irrespective of tubule diameter. The desmotubules are, therefore, apparently identical with nuclear spindle fibres, and the assumption is that they become embedded in the developing cell plate during cytokinesis and subsequently remain in this position.

While this is the first clear demonstration of tubules within plasmodesmata, it is not the first time that this association has been suggested. As long ago as 1933 JUNGERS questioned whether plasmodesmata might not represent spindle fibres which had become trapped during cell plate formation and subsequently become incorporated into the wall. More recently, O'BRIEN and THIMANN (1967), commenting on the plasmodesmata between apical parenchyma cells of oat coleoptile, stated that 'the dark strand which occupies the center of the pore bears no resemblance to a membrane but looks more like a spindle fibre'. The question thus arises whether a tubule within the intercellular canal is a phenomenon general among plasmodesmata, or whether this condition exists side-by-side with continuity of endoplasmic reticulum from cell to cell. The situation described here has only been found in simple plasmodesmata and it is not implied that it necessarily extends to other, more complex structures. The remarks of O'BRIEN and THIMANN, however, would suggest the probability of some fundamental similarity.

The possibility that the endoplasmic reticulum traverses the cell wall via the plasmodesmata has recently appeared less convincing, despite frequent suggestions to the contrary (FREY-WYSSLING and MÜHLETHALER, 1965; LÓPEZ-SÁEZ et al., 1965). There are a number of reasons for this, the predominant one being that the dimensions normally cited for the supposed tubule of endoplasmic reticulum are below the limit of size in which a unit membrane can adopt a stable configuration (ROBERTSON, 1964). Indeed, the diameter of the plasmalemma lining the canal is itself, at about 600 Å, at the lower end of the range of diameters of membrane-bounded vesicles and tubules normally seen in the cytoplasm. LÓPEZ-SÁEZ et al. interpret the structures seen in their micrographs as tubes of endoplasmic reticulum traversing the plasmodesmata; the inner layer appearing as a central, opaque point. This is improbable, not only on dimensional grounds, but also because interpretation of the central point as a single opaque layer of a unit membrane is unlikely on theoretical considerations: while it is conceivable that the central rod represents a lipid or lipoprotein assembly with the polar, hydrophilic groups directed outwards, the assumption that the polar groups are centrally orientated has no basis for support and would certainly not fulfill a stoichiometric relationship with the supposed outer layer of the unit membrane. An explanation of the results of LÓPEZ-SÁEZ et al. may lie in the use of potassium permanganate as the primary fixative for their material. This fixative does not preserve microtubules and, in this case, the results obtained by these workers, as well as those presented in this paper, may have a common basis: all that is required is the assumption that the desmotubule wall is sufficiently retained when fixed in potassium permanganate to produce

an opaque layer but not to show the typical substructure produced by glutaraldehyde fixation.

The structure of plasmodesmata following fixation in solutions containing osmium tetroxide has been reported by Buvat (1960) and Kollmann and Schumacher (1962, 1963). Such treatment also reveals tubules within the intercellular canal and these, again, have been interpreted as connexions between the endoplasmic reticulum of adjacent cells. The dimensions of the structures seen by Kollmann and Schumacher (1962) are in close agreement with those reported in this paper. However, it seems probable that the fixatives used do not give complete preservation of microtubules. Thus, while the results reported here are interpreted in a different way, there do not appear to be inconsistencies if the effects of different fixatives are taken into account. In passing, it should be noted that the work of Kollmann and Schumacher has been concerned with phloem tissue and, to a large extent, with connexions to sieve elements. As pointed out by other workers (Wooding and Northcote, 1965), such plasmodesmata are relatively complex and often anastamose in the region of the median nodule. The results recorded in the present work, however, apply mainly to the simple connexions between differentiating xylem ray cells.

Further evidence for the continuation of the endoplasmic reticulum across the wall seems to stem from the disposition of strands of endoplasmic reticulum during cell plate formation — with the correlated assumption that these strands remain to form a tubule through the plasmodesmata (e.g. Frey-Wyssling and Mühlethaler, 1965).Whether or not endoplasmic reticulum is trapped in such a way does not invalidate the facts cited above. However, while endoplasmic reticulum certainly does approach the plasmodesmata closely, it does not seem to be in direct continuity with any part of the plasmodesmatal structure (as also emphasized by O'Brien and Thimann, 1967). Such a relationship could also account for the profiles of endoplasmic reticulum commonly seen very close to the plasmalemma but rarely, if ever, shown to fuse with it.

The particulate nature of the plasmalemma through the plasmodesmatal canal could be an artifact, but this is thought unlikely as the plasmalemma around the periphery of the cytoplasm shows a normal, non-particulate, nature. Glauert (1968) has fully reviewed contemporary views on membrane structure and suggests that, in varying circumstances, membranes or parts of membranes may exist either as an extended bi-layer or in the form of globular micelles — a conclusion arrived at independently by Branton (1966) using evidence from freeze etching. The tight configuration of the plasmalemma through the plasmodesmatal canal would impose strain on the structure — especially

if in the form of a bimolecular leaflet (ROBERTSON, 1964), whereas the stability of globular micelles could be less affected.

The nature of the central rod is not established, nor is its function; and unless such a structure exists in normal spindle fibres, the manner in which it arises within the desmotubule is also unknown. It is interesting that, in some cases, published micrographs of cytoplasmic microtubules do show a central core (microtubules in the root tip of *Juniperus chinensis*, Dr. M. C. LEDBETTER in JENSEN and PARK, 1967). Such a variation in microtubule ultrastructure not only reinforces interpretation of the desmotubule as a microtubule, but also stresses structural variation between microtubules — and hence probable functional differences as well. The connexions between the central rod and the inside of the tubule suggest that the whole complex may be such that it will possess high intrinsic strength and stability.

The fact that the plasmodesmata do not appear to be continuous across the walls of more mature ray cells, in the same way that they are across young cell walls, may be an indication of secondary elaboration of plasmodesmatal ultrastructure. The development of the cell wall in the region of the middle lamella may affect this process. The results of O'BRIEN and THIMANN, as well as those cited here, suggest that the simplest form of plasmodesmatal complex — as exemplified by Fig. 1 — may be only a transitory stage. However, it is likely that any further alteration to the structure of the plasmodesmata could only reduce effective intercellular communication rather than enhance it.

The question of a direct connexion across the plasmodesmata is now quite different from that existing when continuity of endoplasmic reticulum was envisaged. In fact, there appears to be no direct link across the plasmodesmata except by way of the plasmalemma. At the ends of the plasmodesmata the pores are securely sealed by the close apposition of the plasmalemma to the desmotubule: the only other possible continuity may be the indirect one through contact of the endoplasmic reticulum with the end of the tubule.

How these findings will affect the interpretation of plasmodesmatal function remains to be elucidated by further work.

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