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PLASMODESMATA IN HIGHER PLANTS

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2.1. INTRODUCTION

The first detailed description of "*offene Communicationen*", or protoplasmic connecting threads is usually attributed to Tangl (1879) although other workers subsequently contested his precedence. However, Tangl was the first botanist to write at length on these structures and his article stimulated a spate of publications which described such connections between cells from all parts of the plant kingdom (for fuller details refer to Meeuse, 1941b; 1957, and Chapter 14). In 1901 Strasburger used the term "*Plasmodesmen*" to describe the protoplasmic connections and, despite numerous other suggestions (see Meeuse, 1957), the word has survived the test of time and is now almost universally accepted (English - plasmodesma - Gk. *plasma*, form; *desma*, bond - Plu. plasmodesmata). Virtually all the early investigations involved treatment of cells to cause swelling of the wall so that plasmodesmata could be demonstrated by optical microscopy. This led to many criticisms and, as recently as 1964, Livingston reconsidered the "*nature of plasmodesmata in normal (living) plant tissue*", so highlighting the problem that has been with us for over 80 years. The advent of electron microscopy has done much to expand knowledge about the structure and variability of plasmodesmata; it has been of rather less value in helping us to understand the true nature and physiological function of these connections. In this Chapter I shall collate some of the information relating to the distribution, structure and possible physiological roles of plasmodesmata. The literature is extremely large and diffuse, and no attempt has been made to provide a comprehensive review.

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B. E. S. Gunning et al. (eds.), *Intercellular Communication in Plants: Studies on Plasmodesmata*

2.2. WHAT ARE PLASMODESMATA?

Most plant cells are joined to each other by *protoplasmic connecting threads* (see 2.3.). They are, therefore, closely linked, and together constitute something approaching a true syncytial structure. This, in itself, creates a fresh problem in defining what is a plasmodesmatal connection and what is merely an intercellular channel. Cytomyctic channels such as those described by Heslop-Harrison between angiosperm meiocytes (1966b) are not plasmodesmata, although they may each have been formed at the original site of a plasmodesma (for recent information on connections between angiosperm meiocytes, see Whelan, 1974; and Whelan, Haggis and Ford, 1974); similarly Weiling (1965) distinguishes between plasmodesmata and plasma channels in the pollen mother cells of *Lycopersicon* and *Cucurbita* on the basis of size (25.3 and 175 nm respectively) and structure (this contrasts with Ciobanu (1969) who refers to such channels as plasmodesmata, and has recorded the passage of organelles through them); Bisalputra and Stein (1966) examined the intercellular connections in *Volvox aureus* and concluded that they were "*cytoplasmic bridges*", not plasmodesmata (but see Dolzmann and Dolzmann (1964) for an opposing opinion). However, structures of intermediate size, which produce difficulties in definition are encountered. Meeuse (1957) attempted to solve the problem by a rather long definition: "*Plasmodesmata are continuous, tenuous threads of protoplasm connecting adjacent protoplasts through substances separating these protoplasts (generally speaking, plant cell walls, but also the mucilaginous intercellular substances in* Volvox *colonies, and, in the analogous case of animal tissues, substances such as bone, dentine and cartilage), thus establishing a correlated entity of interconnecting protoplasts. They are never so wide as to permit a real fusion of protoplasts or migration of protoplasmic inclusions, such as nuclei, plastids, microsomes, granula, under normal conditions, but may be converted into wider protoplasmic connections, or, conversely, arise in special cases by a reduction in diameter of wider protoplasmic strands connecting adjacent cells. They are situated in minute perforations of the cell wall, the plasmodesmoducts or plasmodesma channels (or pores).*"

Meeuse's comparison with animal intercellular connections is interesting because plasmodesmata have generally been considered exclusively a plant phenomenon. However, although the structure may be quite different, some junctions between animal cells pose many of the problems familiar to botanists (c.f. the 'cytodesma' of Komnick and Wohlfarth-Bottermann (1964), and the gap-junction model proposed by Bennett (1973a)).

Open cytoplasmic channels usually have no well defined core (desmotubule) but, unfortunately, nor do some plasmodesmata (see 2.4.), so a distinction cannot be made on this basis. For the moment it is probably both useful and sufficiently accurate to think of plasmodesmata as being small protoplasmic connections through which organelles and large macromolecules cannot normally pass (the special case of viruses is dealt with separately - Chapter 8), irrespective of whether or not a desmotubule is present. Juniper (in press) adopts a more liberal definition of plasmodesmata, finding it impossible to make a distinction on the basis of size alone. While far from satisfactory, it is impossible to produce a definition which will not exclude some of the structures which have been described in the literature and which must clearly be studied along with other plasmodesmata, although they may be distinctly different in structure (for example, the plasmodesmata found in *Bulbochaete* by Fraser and Gunning, 1969). One of the few features that appears common to all plasmodesmata (very few, possible, exceptions have been noted - see Chukhrii, 1971) is the

continuity of the plasmalemma, through the plasmodesmatal canal, from cell to cell.

Despite almost 100 years of study by optical microscopy, the small size of plasmodesmata still needed electron microscopy for real advances in structural knowledge. It is interesting, however, to consider two relatively late papers which deal with plasmodesmata from an anatomical viewpoint. Both authors (Meeuse, 1957 and Livingston, 1964) refer to results from electron microscopy, available at the time that their papers were published. Even in 1964, Livingston considered the presence of plasmodesmata so poorly substantiated (despite an already growing literature from electron microscopy) that he felt it necessary to stress the particular point that his own study "*answers the criticism which has frequently been made in the past that these structures (plasmodesmata) are artifacts ...*".

Meeuse (1957) supported the suggestion of Strasburger (1882), that living cells in (higher) plants are connected by fine strands of protoplasm. The protoplasmic nature of plasmodesmata, assumed from their first discovery, and based mainly on ideas about their involvement in translocation, was not challenged seriously until 1930 when Jungers suggested that they are trapped nuclear spindle fibres[1]; however, Hume (1913) had earlier commented that "*it remains an open question whether they (plasmodesmata) arise from the spindle fibres and penetrate the wall* ab initio *or whether they may be formed subsequently*". (Hume was referring to plasmodesmata across a non-division wall). Meeuse (1957) collated the optical microscope evidence against the viewpoint of Jungers (see also Livingston, 1935, 1964). The main points he listed are: plasmodesmata have an affinity for protoplasmic stains; plasmolysis experiments either leave the plasmodesmata connected to the withdrawn protoplast, or may extract the threads from the wall (Strasburger, 1901; see also Burgess, 1971); enzymes move easily into plasmodesmata (Gardiner, 1897); plasmodesmata are only found where they can actually join protoplasts (e.g. not on outer walls); and plasmodesmata disappear from the walls of dying cells. Meeuse effectively summarizes the arguments and concludes that "*the accumulated evidence seems to be overwhelming and conclusive*" (that plasmodesmata are protoplasmic).

2.3. DISTRIBUTION AND FREQUENCY

The review by Meeuse (1941b) amply illustrates that optical microscopists had described plasmodesmata in most plant species, although many observations were contested. Electron microscopy has made the identification of plasmodesmata more certain but, at the same time, has imposed great problems in scanning sufficient material to obtain statistically useful results. However, it can be stated with certainty that plasmodesmata occur in angiosperms, gymnosperms, pteridophytes, bryophytes, and many algae. The distribution, frequency, and structure in fungi and blue-green algae are less well documented, although plasmodesmata are reported from both of these groups (see Chapter 3).

2.3.1. Distribution in Higher Plants

Statements that plasmodesmata occur between all living cells of higher plants (e.g. Meeuse, 1941b: Fahn, 1967) are now known to be

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[1]Some of the earlier botanists distinguished spindle fibres from protoplasmic components - a separation that would not now be made. The nature of the traversing strand is discussed more fully in 2.4.2.

strictly false. However, it does seem that *all* cells in the early life of a plant are connected to each other (Schulz and Jensen, 1968a), and that the subsequent absence of plasmodesmata from particular walls is brought about by a pattern of loss - an area of investigation worthy of much fuller study. (A full account of the developmental consequences of isolation of cells from tissues is given in 13.4.).

The presence or absence of plasmodesmata, as well as their relative frequency between different cells, must relate to any functional role. This is frequently stated or implied in the literature, particularly where the performance demands very high symplastic fluxes between cells, or where cells would need to be isolated from their neighbours. As examples of the first case (high fluxes) the location and frequency of plasmodesmata seems particularly important in secretory glands (e.g. Cardale, 1971 - salt gland of *Aegiceras*; Hill and Hill, 1973 - salt gland of *Limonium*; Thomson and Liu, 1967 - salt gland of *Tamarix*; Ziegler and Lüttge, 1966, 1967 - salt gland of *Limonium*; [Lüttge, 1971, reviews plant glands - including the role of plasmodesmata]); in root nodule tissue (Pate, Gunning and Briarty, 1969; Sprent, 1972) and in the trigger hair of the Venus's fly-trap (Williams and Mozingo, 1971).

Lack of plasmodesmata between adjacent cells may be as significant as a high frequency, even if more difficult to prove! The relationships of reproductive and embryonic cells with each other have proved especially interesting, and, as a general rule, the structures of separate plant generations are *not* linked by plasmodesmata. Plasmodesmata are said to be absent between the zygote and surrounding cells of *Capsella* (Schulz and Jensen, 1968a,b), of *Quercus gambelii* (Singh and Mogensen, 1975), and of *Hordeum* (Norstog, 1972); also absent between the suspensor and embryo sac of *Capsella* (Schulz and Jensen, 1969), between the nucellus and megagametophyte of *Zea mays* (Diboll and Larson, 1966), between the nucellus and embryo sac and zygote and all other cells in *Myosurus* (Woodcock and Bell, 1968), between cells destined to form pro-embryoids in *Citrus* (Button, Kochba and Bornman, 1974), between adjacent pollen grains at later stages of development (Heslop-Harrison, 1964, 1966a), and from walls separating generative cells from each other and from surrounding tapetal cells (Ledbetter and Porter, 1970). A careful study of plasmodesmatal distribution during oogenesis in *Marchantia* has been made by Zinsmeister and Carothers (1974), who document the presence and absence of connections across different archegonial cell walls. These examples all refer to specific cases, but there can be little doubt that they are indicative of general categories of plasmodesmatal exclusion that further studies will reveal. (The significance of cell isolation in the development of alternating generations during reproduction is considered in 13.4.4.).

A more contentious area is the stomatal guard cell wall, where some authors have stated plasmodesmata to be absent (e.g. Allaway and Setterfield, 1972 - *Vicia* and *Allium*; Thomson and de Journett, 1970 - *Opuntia*; Brown and Johnson, 1962 - 16 species of grass), or 'infrequent' (Tucker, 1974 - Magnoliaceae), while other workers have reported their presence (Burgess and Fleming, 1973 - *Pisum*; Inamdar, Patel and Patel, 1973 - *Asclepiadaceae* [optical microscopy]; Pallas and Mollenhauer, 1972b - *Vicia* [!] and *Nicotiana*). Discrepancies between different observations - sometimes on the same species - might conceivably find an explanation in developmental changes, for example, the loss of plasmodesmata during maturation. If this is so, then it will be most important to determine, and cite, the age or state of development of the particular cells so that comparative observations can be made. (This point is discussed in detail in 13.4.2.).

Early statements relating to the inability to demonstrate plasmod-

esmata in certain walls may often be ascribed to the inadequacy of techniques. For example, Livingston (1935) cites the often quoted absence of plasmodesmata from cambial cells, but electron microscopy reveals their undoubted presence. Similarly, early reports that plasmodesmata are absent from tobacco callus tissue (Kassanis, Tinsley and Quak, 1958) have more recently been revised and plasmodesmata clearly shown (Kassanis, 1967; Spencer and Kimmins, 1969). This is in keeping with the increasing citations of plasmodesmata in different tissue culture and other *in vitro* systems (e.g. Fowke, Bech-Hansen, Constabel and Gamborg, 1974 - dividing cells of soybean; Haccius and Engel, 1968 - callus culture of *Cannabis* [optical microscopy]; Halperin and Jensen, 1967 - carrot suspension culture aggregates).

So far, in this account of distribution of plasmodesmata, I have confined the discussion to plasmodesmata in cell walls which would usually arise during the process of cell division. The formation and development of plasmodesmata are considered fully in Chapter 4, but at this point it is relevant to state that there is good reason to support the view that plasmodesmata can form across *non-division* walls.

To summarize, plasmodesmata are to be found in all higher plants that have been examined, pteridophytes, bryophytes, some algae and some fungi. Some mature cells may have no intercellular connections, while in others they are distributed in a manner which suggests a quantitative relationship with the fluxes of solutes in the symplast. Often different walls of the same cell have different plasmodesmatal frequencies (see the data for *Nicotiana*, *Zea mays* and *Hordeum* in Table 2.1.; and for vein phloem in Table 11.1.). Plasmodesmata are also found in walls which have not been formed during the normal process of cell division; they must therefore be thought of as dynamic structures, capable of secondary elaboration, and not simply as trapped structural remnants from cell plate formation.

2.3.2. Frequencies between Cells

In many cells, particularly when a secondary cell wall has been deposited, plasmodesmata are found grouped together: such groupings correspond to the primary pit fields and, later, to the pits. Much work remains to be done in determining whether the initial distribution of plasmodesmata across a wall is random, so that grouping occurs through selective loss, or whether the original pattern of distribution is already in a grouped form (see Chapter 4). This matter is important, not least because it means that calculations of plasmodesmatal frequencies must be made with great care to include samples from the whole intercellular face and not simply from the pits or pit fields. The range of plasmodesmatal frequencies is large (Table 2.1.). In presenting this information it was, however, a matter of greatest difficulty to select from the literature frequencies which can be regarded as statistically reliable. Counts made by optical microscopy are about an order of magnitude lower than those made by electron microscopy on the same cells (e.g. Krull, 1960, who compared her own data, obtained by electron microscopy, from *Viscum* with those obtained by Kuhla (1900) using optical microscopy on the same species). Even electron microscopy presents difficulties: plasmodesmata are either sectioned longitudinally, and frequencies determined on the basis of the estimated section thickness; or they are sectioned transversely, when it is an exacting task to make sure that the correct wall is being sectioned and that the areas being examined are representative of the whole interface.

Table 2.1. cites some plasmodesmatal dimensions and frequencies taken from the literature. These figures should be self-explanatory,

TABLE 2.1.

DIAMETER AND FREQUENCY OF PLASMODESMATA

(Data determined by electron microscopy except where stated)

1. ANGIOSPERMS AND GYMNOSPERMS

Species and Original Source	Cell Type	Diameter (nm)	Frequency (per μm^2)
Nicotiana tabacum	Various	500	
	Epidermis End walls		0.30-0.36
	Epidermis Side walls		0.18-0.25
Livingston, 1935	Outer cortex End walls		0.21-0.24
	Outer cortex Side walls		0.08-0.09
	Inner cortex End walls		0.09-0.13
	Inner cortex Side walls		0.09-0.11
	Basal septum, hair cells		0.14
	Data determined by optical microscopy from 'mature unswollen tissue'.		
	Callus cells	50-70 300-500*	-----
Spencer and Kimmins, 1969	*This larger diameter derived from optical microscopy; possibly a good example of the relative dimensions recorded from optical and electron microscopy.		
Pinus strobus	Cambium	< 200 (550)*	-----
Sequoia sempervirens	Xylem ray and wood parenchyma	200	-----
Lycopersicon esculentum	Parenchyma	200	-----
Livingston, 1964	Optical microscope measurements. *Diameter of central nodes.		
Lycopersicon esculentum and *Cucurbita maxima*	Pollen mother cells	25.3	-----
Weiling, 1965	Also plasma channels 175 nm diameter.		

Table 2.1 (continued)

Species and Original Source	Cell Type	Diameter (nm)	Frequency (per μm^2)
Metasequoia glyptostroboides	Sieve areas	50-85 (max. 350)	-----
Kollmann and Schumacher, 1963	Median nodule 3.0 μm diameter		
	Phloem parenchyma - radial cell walls	50-60	30-40*
	Sieve cells - tapering radial end walls	50-500	7**
Kollmann and Schumacher, 1962	*Frequency in pit membrane only		
	**Frequency in sieve area only		
Viscum album	Epidermal cells	----	0.02-0.04
	Cortical parenchyma	----	0.01-0.05
	transverse walls	----	0.14-0.38
	Cambial radial walls	----	0.05-0.12
	tangential walls	----	0.12-0.19
	Pith parenchyma	----	0.04-0.22
Kuhla, 1900	All figures rounded from three to two significant places - optical microscopy.		
Krull, 1960	Mature cortical cells of growing internodes	15*	0.6-2.4**
	Figures cited by Tyree, 1970, as *50-60 nm, and **2.0. The plasmodesmata studied by Krull are fine strands through tubes that frequently anastomose. She was unable to give detailed dimensions but referred to 15 nm diameter connections. Because she counted all channels cut transversely, and because she found the mean number of 'arms' per plasmodesma to be three, she divided the number of connections counted by three. The frequencies cited here include this downward adjustment.		
Cucurbita pepo	Root endodermal cells:		
	Inner tangential wall	----	6.2
	Outer tangential wall	----	10.4
Robards, 1975	Radial longitudinal wall	----	6.9

Table 2.1 (continued)

Species and Original Source	Cell Type	Diameter (nm)	Frequency (per μm^2)
Phalaris canariensis López-Sáez *et al.*, 1966a	Root tip cells *Outer diameter	40-50*	-----
Pisum sativum Vian and Rougier, 1974	Parenchyma from root apex: thin (0.2 μm) walls thicker (several μm) Determined from sections of frozen material. The plasmodesmata are reported to have constrictions (necks) at each end	60 40-50	-----
Pinus strobus Murmanis and Evert, 1967	Phloem parenchyma	30-50	-----
Murmanis and Sachs, 1969	Pit membranes of secondary xylem tracheids Plasmodesmatal diameter increases as cells age	30-50	-----
Pinus pinaster Carde, 1974	'Strasburger' cells of pine needle. There is a small constriction at each end of the plasmodesma. The frequency cited (*) applies to the pit field only. Over the whole wall, the frequency is much less (Carde - personal communication).	60	20-25*
Abutilon Gunning, this Volume	Distal wall of stalk cell of nectary hairs *Outer diameter (See Table 2.2.)	44*	12.5
Oenothera Brinckmann and Lüttge, 1974	Mesophyll cells *Assumptions of pore size made by A.W.R. **Frequency derived from my assumptions of pore diameter in conjunction with 0.38% open wall area cited by original authors	40 or 50*	3 or 2**
Dactylorchis fuchsii Heslop-Harrison, 1966b	Archesporial cells	28	>7

Table 2.1 (continued)

Species and Original Source	Cell Type	Diameter (nm)	Frequency (per μm^2)
Salsola kali	Wall between mesophyll and bundle sheath cells	44*	14**
Olesen, 1975	**Internal* diameter of pore **57 μm^{-2} within pit fields		
Zea mays	Transverse walls of cap cells		
Clowes and Juniper, 1968	Meristematic	25*	4.5
	Peripheral		0.81
	*At least during early stages of differentiation		
Juniper and French, 1970 also Juniper and Barlow, 1969	Root cap cells		
	Meristematic Transverse	----	14.87
	Meristematic Longitudinal	----	5.30
	Peripheral Transverse	----	5.13
	Peripheral Longitudinal	----	0.45
	Quiescent cell zone		
	Central	----	5.76
	Peripheral	----	1.52
Hordeum vulgare	End walls of young endodermal cells	46*	-----
Robards *et al.*, 1973; Robards, 1975 and unpublished results	Inner tangential wall of root endodermal cells (distance from root tip) 2 mm		1.2
	4 mm		0.7
	10 mm		0.7
	30-40 cm		0.7**
	Outer tangential wall of root endodermal cell	60-70*	0.4
	Radial wall of root endodermal cell		0.3
	*Outer diameter (see Table 2.2.) **Derived from sections cut in the plane of the cell wall (i.e. plasmodesmata sectioned transversely); in all other cases frequencies were determined from counts of plasmodesmata which had been sectioned longitudinally		
Helder and Boerma, 1969	Endodermis 5 mm from root tip	60-90*	5.0**
	*Measured in the mid-line of the wall. In the neck region the diameter of the pore is given as 30 nm		
	**Derived and cited by Clarkson *et al.*, 1971 by making assumptions for area of endodermal wall		

Table 2.1 (continued)

Species and Original Source	Cell Type	Diameter (nm)	Frequency (per μm^2)
Triticum aestivum Kuo *et al.*, 1974	Inner tangential wall of mestome sheath cells abutting phloem *The diameter is of the conducting pore, and *excludes* the plasmalemma **Derived by A.W.R. from the published figures - frequencies within pit field were within the range 25-38 μm^{-2}; the authors comment on sampling problems involved in such counts	50*	7.7**
Ledbetter and Porter, 1970	Stamen filament cells	40	-----
Allium cepa Strugger, 1957a Strugger, 1957b	Root meristem An interesting case, where the dimensions derived from electron microscopy (30-40 nm) have subsequently been 'corrected' to allow for presumed shrinkage. (*Cited by Tyree, 1970, as 80-100 nm). **The implication from Krull (1960-p.616) is that this frequency range applies to the pit-field only	30-40* 100-200	6-7** -----
Scott *et al.*, 1956	Mature cortical cells of root *Calculated by Tyree, 1970, from micrographs of shadowed cell walls: a dubious method of obtaining plasmodesmatal dimensions, which will almost certainly be overestimated	80-120*	1.5*
Various Buvat, 1957 Buvat, 1963 Buvat, 1969	Various	 25-50 20 <50	 ----- ----- -----
Avena sativa Böhmer, 1958	Cortical cells of mature coleoptile *Derived and cited by Tyree, 1970	60-100*	3.6*
Tradescantia virginica Van Went *et al.*, 1975	Cross walls of staminal hairs *Outer diameter	45*	11

Table 2.1 (continued)

Species and Original Source	Cell Type	Diameter (nm)	Frequency (per μm^2)
Tamarix aphylla	Walls between collecting and secreting cells of salt glands	80	17*
Thomson and Liu, 1967	Anticlinal walls of secretory cells	60	
	*Derived and cited by Tyree, 1970		
Tussilago farfara	Wall between companion cell and sieve element of minor vein (branched channels)		
Gunning *et al.*, 1974	Companion cell side	43	6
	Sieve element side	62	2
Tillandsia usneoides Dolzmann, 1965	Hair cell walls	50-70	-----
Utricularia monanthos	Transverse wall between pedestal and basal epidermal cell of trap hairs*	----	7
Fineran and Lee, 1975	Transverse wall between pedestal and terminal cells of hairs**	----	35
	*These plasmodesmata have four arms on the epidermal cell side but a single arm into the transfer cell (counted as single plasmodesmata)		
	**Simple plasmodesmata		
Impatiens balsamina Jones, this Volume	Wall between giant cells induced by the root knot nematode *Meloidogyne incognita*	50	2.7-7.6*
	*29 μm^{-2} within pit fields		
2. PTERIDOPHYTES			
Dryopteris filix-mas Burgess, 1971	Root meristem young primary walls	50-80*	140
	Pit fields in older primary walls	----	10-20
	*"Pore through the cell wall"		
Polypodium vulgare Fraser and Smith, 1974	Wall between basal and protonemal cells of gametophyte	60*	-----
	*Outer diameter, and containing a 22 nm desmotubule. The plasmodesma is within a 'wall canal' 110 nm wide		

Table 2.1 (continued)

Species and Original Source	Cell Type	Diameter (nm)	Frequency (per μm^2)
Azolla filiculoides Robards, unpublished	Cortical cells of young root *Outer diameter	35*	-----
3. BRYOPHYTES			
Polytrichum commune Eschrich and Steiner, 1968	Leptoid end walls Parenchyma cell cross walls	---- ----	16-20 9-12
4. FUNGI			
Rhizopus sexualis *Gilbertella persicaria* Hawker *et al.*, 1966	Walls between suspensors and young gametangia	7.5-10	-----
Geotrichum candidum	Vegetative cells		
Wilsenach and Kessel, 1965		30-60	-----
Hashimoto *et al.*, 1964		20-70	-----
5. ALGAE			
Egregia menziesii *Fucus evanescens* Bisalputra, 1966	Vegetative cells	37.5	-----
Himanthalia lorea Berkaloff, 1963	Meristematic cells	50	-----
Laminaria spp. Ziegler and Ruck, 1967	Trumpet cell cross walls [1]	60	50-60
Laminaria groenlandica Schmitz and Srivastava, 1974a	Sieve elements[1]	60	42

[1]There is some difficulty in comparing the sizes of sieve plate pores and true plasmodesmata in these algae: the large diameters are generally of sieve plate pores, but have been included here for the sake of comparison, and because there is a range of cited dimensions

Table 2.1 (continued)

Species and Original Source	Cell Type	Diameter (nm)	Frequency (per μm^2)
Pelagophycus spp. Parker and Fu, 1965	Conducting elements	[300-800][1]	4-6
Macrocystis pyrifera Parker and Philpott, 1961	Sieve tubes[1] *Ziegler (1963) considered that these pores were ordinary plasmodesmata rather than sieve plate pores. J. Parker (1964) discussed this interpretation. (See also Chapter 3).	30-50*	-----
Macrocystis pyrifera Ziegler and Ruck, 1967	Sieve tubes *This frequency is cited by Ziegler and Ruck (1967) as from Ziegler (1963), but such a frequency cannot be correct in combination with pores of the quoted diameter, and Ziegler (1963) does not explain the origin of the figure	[2,000-3,000][1]	[1.0]*
Macrocystis pyrifera Parker and Huber, 1965	Sieve tubes *Calculated by A.W.R. from data cited	[2,400-6,000][1]	0.008*
Bulbochaete hiloensis Fraser and Gunning, 1969; 1973	Filament cells *Outer diameter Pores at ends of plasmodesmata 8.5-15 nm diameter. The plasmodesmata are contained within 'wall canals' of about 120 nm diameter	75*	3.2
Oedogonium Pickett-Heaps, 1967	Vegetative cells	130*	-----
Coss and Pickett-Heaps, 1974	Antheridia *Outer diameter, calculated by Dr. H. Marchant from authors' micrographs	77*	-----
Ulothrix Floyd, Stewart and Mattox, 1971	Filamentous cells *Outer diameter, calculated by Dr. H. Marchant from authors' micrographs	28-36*	-----

[1]See footnote on previous page

Table 2.1 (continued)

Species and Original Source	Cell Type	Diameter (nm)	Frequency (per μm^2)
Aphanochaete Stewart, Mattox and Floyd, 1973	Vegetative cells *Outer diameter, calculated by Dr. H. Marchant from authors' micrographs	20*	-----
Coleochaete scutata Marchant, unpublished	Vegetative cells	30-70	-----
Chara spp. Pickett-Heaps, 1967	Meristematic vegetative cells	48-64	-----
Chara spp. Marchant, unpublished	Internode/nodal	82-120	-----
Chara corallina Fischer *et al.*, 1974	Internodal/peripheral cell wall Internodal/central cell wall	100 118	12 14
Nitella translucens Spanswick and Costerton, 1967	Wall between mature internodal and nodal cells Wall between mature nodal cells *Figures in brackets are the *outer* diameters, obtained by adding 2x8 nm, for the thickness of the plasmalemma, to the diameter of the pore **Frequencies are much higher between immature cells: 6.8 μm^{-2} and 67 μm^{-2} for internodal/nodal, and nodal/nodal respectively. Frequencies calculated by A.W.R. from figures cited	55(71)* 52(68)*	2.5** 4.5**

although it is not always clear exactly how the different authors have made their measurements. It is essential, if transport phenomena are to be considered, that frequencies and sizes of connecting channels are precisely determined and stated. From the data available, some general conclusions may be drawn. For example, the plasmodesmata of higher plants have an outer diameter of up to about 60 nm (external diameter of plasmalemma-lined tube). The frequencies range from less than 1.0 to more than 10.0 per square micrometre over whole wall surfaces (i.e. not within pit fields alone). The maximum frequency of face-packed

plasmodesmata of 60 nm outer diameter would be almost 280 per μm^2. Figures of 180 (anticlinal walls of *Osmunda cinnamomum* shoot apex, calculated by Juniper (in press) from micrographs of Hicks and Steeves (1973)), and 140 (primary walls of *Dryopteris filix-mas* root meristem - Burgess (1971)) are very high frequencies indeed. The available data on these two ferns is limited and in view of the *relative* paucity of plasmodesmata in apical (e.g. Bowes, 1965; Havelange *et al.*, 1974) and intercalary (Leshem, 1973) meristems in angiosperms, further documentation is highly desirable (see also 4.5.). Although the frequencies per square micrometre do not appear very dramatic, even the smallest

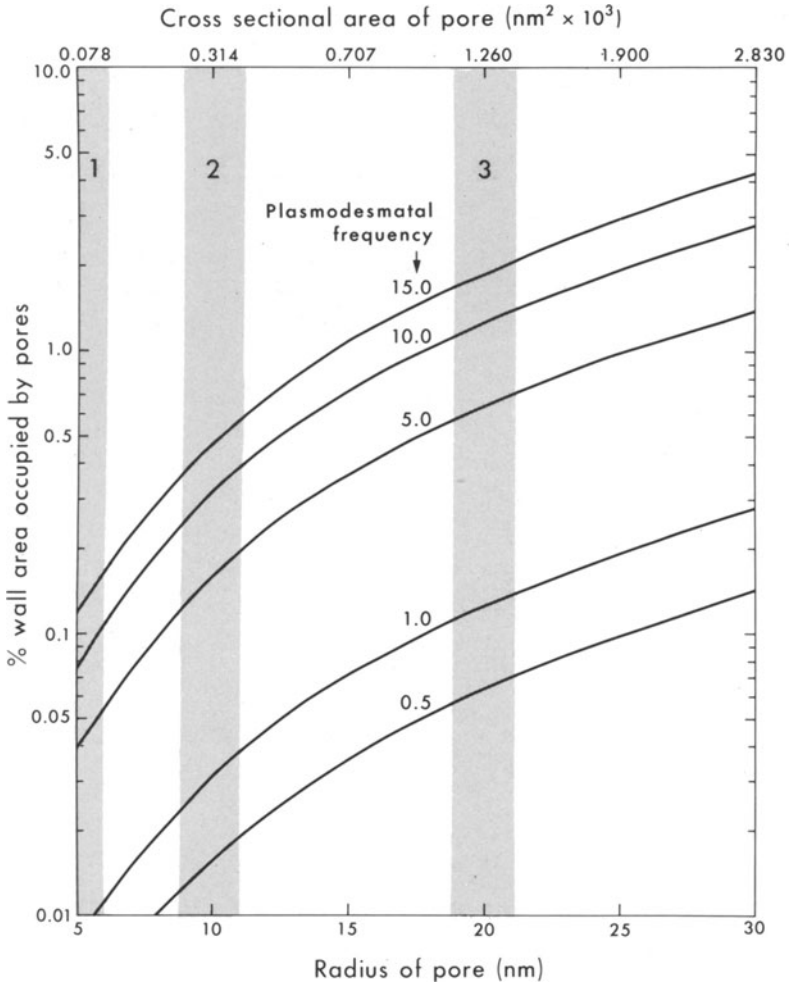

Fig. 2.1. Diagram relating the frequency and pore dimensions of plasmodesmata to the proportion of wall area occupied by pores. The shaded vertical columns show the wall area occupied at different frequencies for pore radii centred about: 1) 5 nm (e.g. the desmotubule); 2) 10 nm (e.g. the plasmalemma-lined pore); 3) 20 nm (e.g. a plasmalemma-lined pore without restrictions at the ends). It can be seen that the wall area occupied by pores is rarely likely to be much above 1%, and is usually much lower than this

meristematic cells will have between 1,000 and 10,000 connections with their neighbours (Clowes and Juniper, 1968).

Of more importance to the physiologist is the area available for transport from cell to cell. This parameter requires knowledge of the nature and size of the conducting pore - something that remains debatable. It is becoming increasingly popular to refer to the proportion of wall occupied by pores. That is, the total surface area of the presumptive conducting channels expressed as a proportion of the total wall area. Although no definitive figures can be cited, some limiting cases can be demonstrated (Fig. 2.1.). Using different pore sizes and frequencies it is seen that the open channels from cell to cell are unlikely ever to account for much more than 3% of the interface area and, in most cases, the proportion will be very much lower than this. (The figures cited apply to higher plants. In some algae - e.g. *Chara*, the plasmodesmatal area may occupy 5% or more of the wall between nodal and internodal cells - Table 9.1., see also Fischer, Dainty and Tyree, 1974). Finally, a neglected aspect of plasmodesmatal size and frequency is the amount of plasmalemma 'trapped' in plasmodesmata, as opposed to that which lines the cell surface. Some authors (e.g. Carde, 1974; Vian and Rougier, 1974) have commented on apparent ultrastructural differences between the plasmalemma within the plasmodesmata and that over the rest of the wall. In a wall 0.5 μm thick, having a plasmodesmatal frequency of 5 per μm^2 with a diameter of 60 nm, almost half as much plasmalemma is within the plasmodesmata as lines the rest of the wall; if the wall is 1.0 μm thick, then the amounts are approximately equal.

2.4. STRUCTURE

If anything meaningful is to be said about the possible functions of plasmodesmata, it is important that the details of fine structure should be well understood. It is also useful to have a uniform approach to the citation of dimensions and the terminology relating to these, sometimes complicated, structures. The cited dimensions vary greatly (Table 2.1.). From a structural viewpoint this might not be too significant. However, when measurements are related to theories of symplastic transport, as pointed out in Section 1.2.1., where it was shown that the carrying capacity of plasmodesmata can vary in relation to the *fourth* power of the radius, even small differences can have relatively profound effects. The system of nomenclature that I shall use is outlined in Fig. 2.2.

2.4.1. Optical Microscopy

The direct observation of plasmodesmata in untreated specimens by optical microscopy is restricted to relatively few cases: for example, the endosperms and cotyledons of seeds, such as those of *Strychnos nux-vomica* (Frontispiece) and *Aesculus hippocastanum*. Other than in such situations, plasmodesmata generally need to be stained, swollen and stained, or impregnated with a soluble salt of a metal (often silver) to render them visible. One of the simplest stains for plasmodesmata is iodine (Tangl, 1879), used either in simple solution, vapour form, or one of the often-cited complex forms such as aqueous or alcoholic Lugol solution (1% iodine in a 2% solution of potassium iodide). Applied following swelling agents such treatments show plasmodesmata, and this fact has been used to support the contention that plasmodesmata are protoplasmic in nature. A wide variety of other stains has been used to demonstrate the protoplasmic connections: aniline dyes, haematoxylin, methyl violet, etc.; no doubt individual users have each

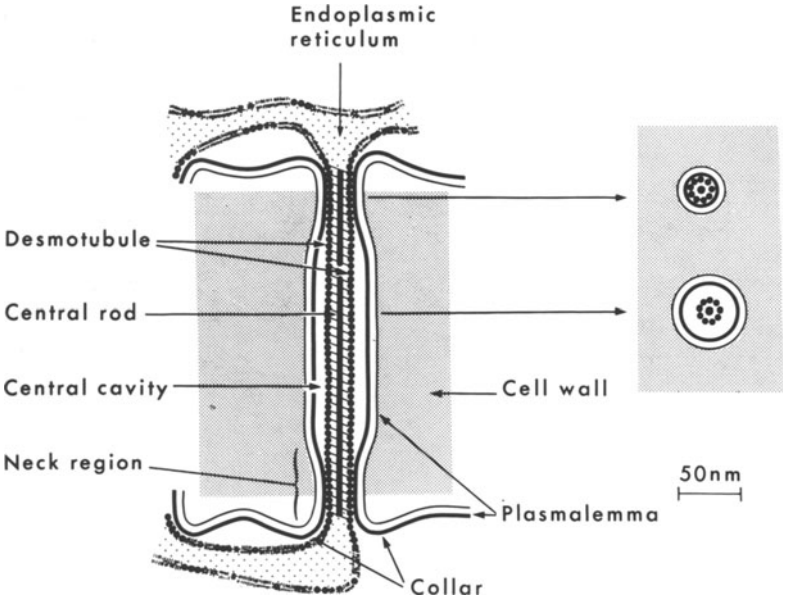

Fig. 2.2. The components of a simple plasmodesma. This diagram shows the various plasmodesmatal features seen in electron micrographs. It is not meant to imply any general uniformity of structure, nor specific features

found their particular recipe successful, but no clear picture emerges of any specificity. Swelling agents for demonstrating plasmodesmata have usually been sulphuric acid (followed, or accompanied, by iodine) or chlor-zinc-iodine (Schultz's solution). In either case, the cell wall framework swells and produces a blue or purplish colour while the plasmodesmata are yellow to brown. Part of the virtue of these methods appears to reside in the swelling of the wall, so allowing more space for the penetration and reaction of iodine molecules with the protoplasmic connection. Still more contrast may be obtained if sections, which have been stained with iodine after swelling, are further treated with a stain which reacts with the iodine: this is the basis of the pyoktanin method which uses methyl violet (see Meeuse, 1957 for further details). Even here, however, there appear to be no grounds for optimism relating to the specificity of the reaction, although several authors have suggested it (Jungers, 1930; Livingston, 1935). Impregnation of plasmodesmata with silver salts relies upon more rapid absorption and/or reduction of the reagent by the plasmodesmata than by other components - something that should possibly be kept well in mind when evaluating cytochemical precipitation methods involving silver salts (see Chapter 7). While having a useful place in the range of techniques for optical microscopy (e.g. Mühldorf, 1937), again, it may not be regarded as a specific reaction.

The conclusion to be drawn from techniques for optical microscopy is that, while a whole host of methods has been described (see Mühldorf, 1937; Meeuse, 1941b, 1957; Livingston, 1935, 1964), and each probably has its own usefulness, there is no single method that can be relied upon to demonstrate plasmodesmata specifically. Therefore, the usual precautions must be taken in analysing results from such methods: there is clearly a considerable risk of mis-identification,

as well as the certainty that many are never seen at all by optical microscopy.

Optical microscopy showed that plasmodesmata comprised thin threads of protoplasm running through a pore in the cell wall. Beaded threads were ascribed to swelling artifacts (Mühldorf, 1937) or precipitation phenomena (Livingston, 1964). A thickening at the centre of a plasmodesma was thought by Meeuse (1957) to be another swelling artifact, but Livingston, from his examination of unswollen material, concluded that this 'node' is a constant morphological feature and not artifactual. Optical microscopy also demonstrated anastomosing plasmodesmata.

Although optical microscopy allowed real and important progress towards understanding the nature and distribution of plasmodesmata in plant cell walls (often confirmed subsequently by electron microscopy), it must be emphasised that, whatever the actual diameter of plasmodesmata, it always lies close to the limit of resolution of the optical microscope. Perhaps, therefore, it is not surprising to find that such measurements, when made from optical observations, are usually in the range of 0.1-0.5 μm (Table 2.1.). It has been suggested by some authors that the measured diameter is smaller than the *in vivo* condition (Münch, 1930; Livingston, 1935, 1964; Strugger, 1957a,b) because the techniques used to demonstrate plasmodesmata usually involve the use of wall swelling agents. Thus, Livingston (1935) suggested that mature, unswollen plasmodesmata of *Nicotiana* would have a diameter of 0.5 μm as opposed to the 0.2 μm measured after treatment with chemicals. Similarly Strugger (1957a) measured plasmodesmata to be 30-40 nm in diameter (electron microscopy), but he later corrected this to 100-200 nm (1957b), allowing for his estimate of shrinkage during processing. Although Strugger was using the electron microscope as his tool, his observations are clearly relevant to this section as they are among the earliest results from the electron microscope in this field of study, and his considerations were clearly influenced by the accumulated weight of evidence from optical microscopy. It appears to me probable that the optical microscope is a misleading instrument in this matter: plasmodesmata, as already stated, are usually demonstrated in tissue swollen in some harsh reagent, after which they appear as fine filaments close to the very limit of optical microscope resolution; under such circumstances, and with grouped plasmodesmata, one would expect diffraction phenomena to occur (particularly in the relatively thick sections usually studied) and, therefore, it would be unwise to regard these results as other than indicative of the uppermost limits of size, while not excluding much smaller structures.

2.4.2. Electron Microscopy

The electron microscope quickly confirmed the presence of plasmodesmata in material fixed either in osmium tetroxide (Buvat, 1957; Strugger, 1957a,b; Kollmann and Schumacher, 1962, 1963), or potassium permanganate (Porter and Machado, 1960a; Whaley, Mollenhauer and Leech, 1960; Esau, 1963). A detailed study by Scott, Hamner, Baker and Bowler (1956) of macerated, cleaned and shadowed onion root cell walls demonstrated the pores through the walls of pit-fields through which it was assumed that plasmodesmatal strands passed. Although the electron microscope established the morphological reality of plasmodesmata, it soon raised further problems largely attributable to the small size of the structures (or, at least, their components) even judged by electron microscope standards. In general, plasmodesmata seen in the electron microscope are about one tenth the size of those seen by optical microscopy. This led Clowes and Juniper (1968) to suggest that there may be two types of plasmodesma, a view that lacks

much support. The discrepancies are largely related to the *apparently* greater size of plasmodesmata viewed by optical microscopy. This, in turn, may be contributed to by: diffraction effects of closely adjacent structures; branched plasmodesmata; and the possibility that, not only the plasmodesma, but also a restricted area of the wall around it, may stain differently from the remainder of the cell wall, and so increase the apparent size. (Both Taiz and Jones (1973) and Vian and Rougier (1974), cite electron microscopical evidence that would support this last possibility).

Examination of frozen-etched plasmodesmata with minimum pretreatment and no dehydration shows structures of general dimensions very similar to those obtained by conventional electron microscopy (own unpublished results); the same is true for plasmodesmata prepared by freeze-substitution (A.J. Browning, unpublished results). In ultrathin sections cut from frozen blocks of fixed plant tissue, plasmodesmata are of much the same size-range (Vian and Rougier, 1974) and, more significantly, so are those in frozen-cut sections of chemically untreated material (Roland, 1973; Vian and Rougier, 1974, Plate IId).

In the electron microscope the very small size of the plasmodesmatal canal, together with the included protoplasmic thread, has made it difficult to determine the true nature of the connection. From the very earliest micrographs of fixed and sectioned material it was noticed that the plasmodesmata were usually contacted at either end by a strand of endoplasmic reticulum. It therefore became a generalisation that the plasmodesma contains a strand of endoplasmic reticulum running through the wall from cell to cell (Wardrop, 1965; Frey-Wyssling and Mühlethaler, 1965; and many others). Esau (1963), with her usual care, avoids the unsupported view that endoplasmic reticulum necessarily traverses the wall: "....*endoplasmic reticulum is typically connected to the plasmodesmata; in fact, many investigators think that tubules of endoplasmic reticulum are structural elements of plasmodesmata*". This is a fair statement of fact, but there is little further *direct* evidence to clarify the nature of the connection. Porter and Machado (1960a) and Falk and Sitte (1963) were, like Esau, not prepared to accept direct endoplasmic reticulum continuity without reservations. It is ironic that, for many years, optical microscopists struggled with the inherent limitations to the resolution of their instruments in an effort to determine plasmodesmatal structure: then, as soon as detailed observations were made of thin-sectioned material in the electron microscope, it was seen that, once again, instrumental resolution (in association with specimen effects) imposes limitations in elucidating the crucial features of the connecting strands. Part of the resolution problem is outlined in Fig. 2.3. Further, the normal practice of presenting very slightly under-focused images may cause misleading phase and amplitude changes, which could affect image interpretation (Helder and Boerma, 1969; Robards, 1971). Chromatic loss due to the specimen itself will also adversely affect resolution. Still thinner sections can be cut, but it is then very difficult to obtain sufficiently high contrast in the structure of the desmotubule and endoplasmic reticulum to allow them to be seen and recorded at high resolution. The remarks above apply, in the main, to longitudinal sections of plasmodesmata. Transverse sections involve many of the same problems; they are, however, often easier to interpret as there is a greater possibility that the material (plasmalemma, desmotubule, etc.) is homogeneous throughout the thickness of the section (Fig. 2.3.).

Having mentioned these limitations to the ultrastructural study of plasmodesmata, I can now consider the results that are, in fact, available. The diversity of plasmodesmatal form is such that it will be considered separately later (Chapter 4). For the present I shall

confine myself to *basic-type* plasmodesmata; these are simple plasmodesmata with no anastomosing desmotubules or complicated median nodules (Fig. 2.4.).

The ultrastructural demonstration of cytoplasmic continuity (Buvat, 1957; Strugger, 1957a) in cells fixed by osmium tetroxide was followed by studies in which potassium permanganate was used as the fixation agent, allowing membranes to be seen with clarity previously unobtainable. It was thus possible for Whaley, Mollenhauer and Leech (1960) to suggest the continuity of a strand of endoplasmic reticulum through the plasmodesmatal canal, and for Porter and Machado (1960a) to associate plasmodesmatal formation with cell plate deposition during mitotic telophase. In these and a large number of supporting papers, it

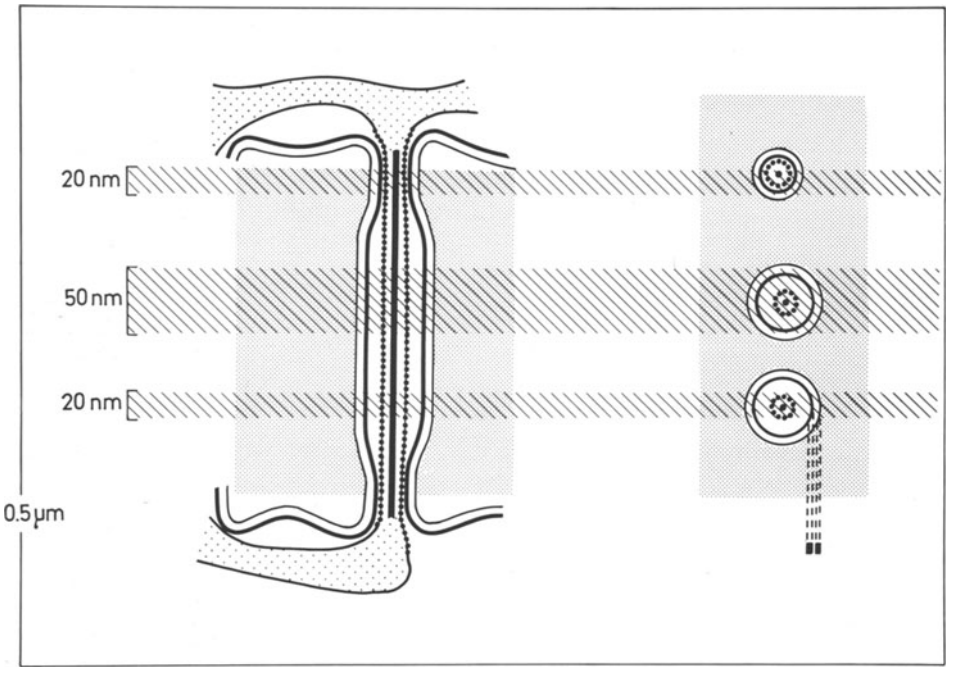

Fig. 2.3. Diagram illustrating the relationship of plasmodesmatal dimensions to section thickness. The height of the frame is equivalent to a section thickness of 0.5 µm (as, for example, might be used in high voltage electron microscopy): a complete plasmodesma could be contained vertically, while eight or more could be stacked horizontally upon one another within such a thickness. Resolution *within* the plasmodesma would be poor. Indeed, it is often impossible to see plasmodesmata orientated in the plane of a thick (0.5 µm - 1.0 µm) section viewed at high voltage (0.4 - 1.0 MeV) because the contrast of a thin component in a thick section is so low. A 'conventional' thin section (approx. 50 nm) includes the whole of a plasmodesma lying in the plane of a section, and gives reasonable resolution across parallel membranes. In the neck-region the image of such a section superimposes too much information for all details to be resolved. Even a *very* thin section (20 nm) encompasses half a plasmodesma cut vertically through the neck region. Longitudinal sections of plasmodesmata showing unit membrane structure of the plasmalemma with maximum clarity need to be of the order of 20 nm thick (lower right diagram)

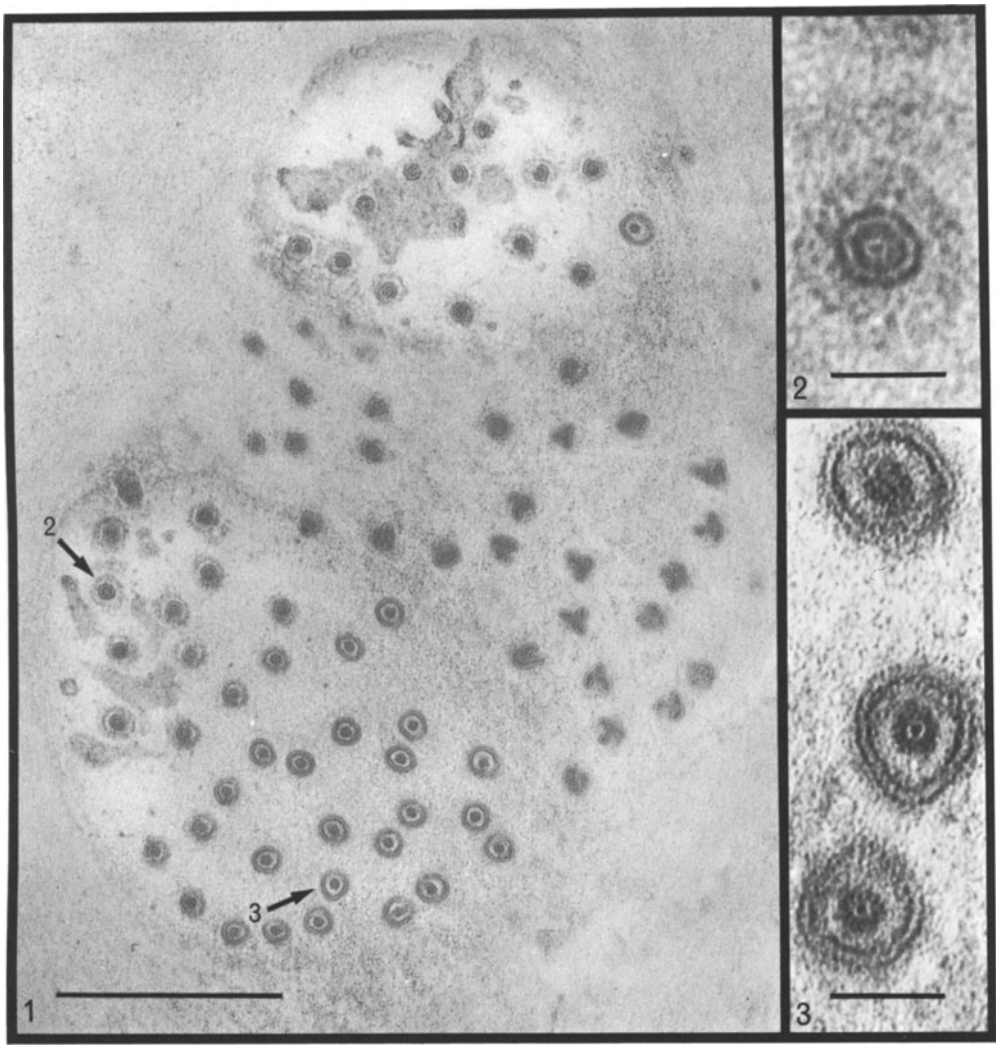

Fig. 2.4.1. Micrographs of plasmodesmata in the longitudinal radial walls of xylem ray cells of willow (*Salix fragilis*, L.) fixed in glutaraldehyde and osmium tetroxide. The section was slightly oblique to the plane of the wall so that plasmodesmata were cut at different levels. Those sectioned through the mid-line show a clear gap between the desmotubule and the plasmalemma (type indicated by '3'), whereas those cut through the neck region (Fig. 2.2.) have no such space ('2'). Such pictures provide the best evidence for a tight seal between plasmalemma and desmotubule in the neck region of some species (c.f. Fig. 1.1.). A desmotubule and central rod appear to be universally present. Observations on such plasmodesmata were used to construct the models illustrated in Figs. 2.6. and 2.7. These micrographs were first published in Robards (1968b). Scale markers = (1) 0.5 μm; (2) and (3) 50 nm

was demonstrated that profiles of endoplasmic reticulum become trapped as the vesicles converging upon the cell plate fuse with each other. Subsequently, López-Sáez, Giménez-Martín and Risueño (1966a), also using permanganate fixation, concluded that the plasmodesmatal canal is traversed by a strand of endoplasmic reticulum, tightly curved into a tubule, so that the inner opaque layer of the membrane appears as a central rod (Fig. 2.5.). López-Sáez *et al.* do not dwell on the possible function of plasmodesmata, merely stating that an *inter-tubular gap* (the gap between the plasmalemma and the endoplasmic reticulum strand [desmotubule] through the wall) would allow some connection between the hyaloplasm of adjacent cells whereas, if there is no continuous intertubular gap, "*the plasmodesm plays no physiological role in intercellular transport*". A very similar model has been proposed by Semenova and Tageeva (1972) who believe that the dark, axial, structure is a solid rod formed from a tightly constricted endoplasmic reticulum membrane tubule.

It is important to realise the implications of the statements by López-Sáez *et al.*, or Semenova and Tageeva: that communication, if it does occur, cannot take place through the supposed strand of endoplasmic reticulum (desmotubule), but rather through a possible *leaky seal* between the neck and the desmotubule (a point not clarified in these papers); if this channel, too, were closed, cytoplasmic continuity could not exist except in the form of a solid structure.

Improved methods of fixation for electron microscopy, such as the use of glutaraldehyde (Sabatini, Bensch and Barrnett, 1963), together with a basic dissatisfaction over the gap existing between structural and physiological approaches to the intercellular movement of ions and molecules, led me to carry out an investigation of plasmodesmatal ultrastructure using glutaraldehyde/osmium tetroxide fixed cells (Robards, 1968b). As I was working on differentiating xylem cells, and transport of solutes between thick-walled ray cells represents an area of particular interest, the plasmodesmata between ray cells were

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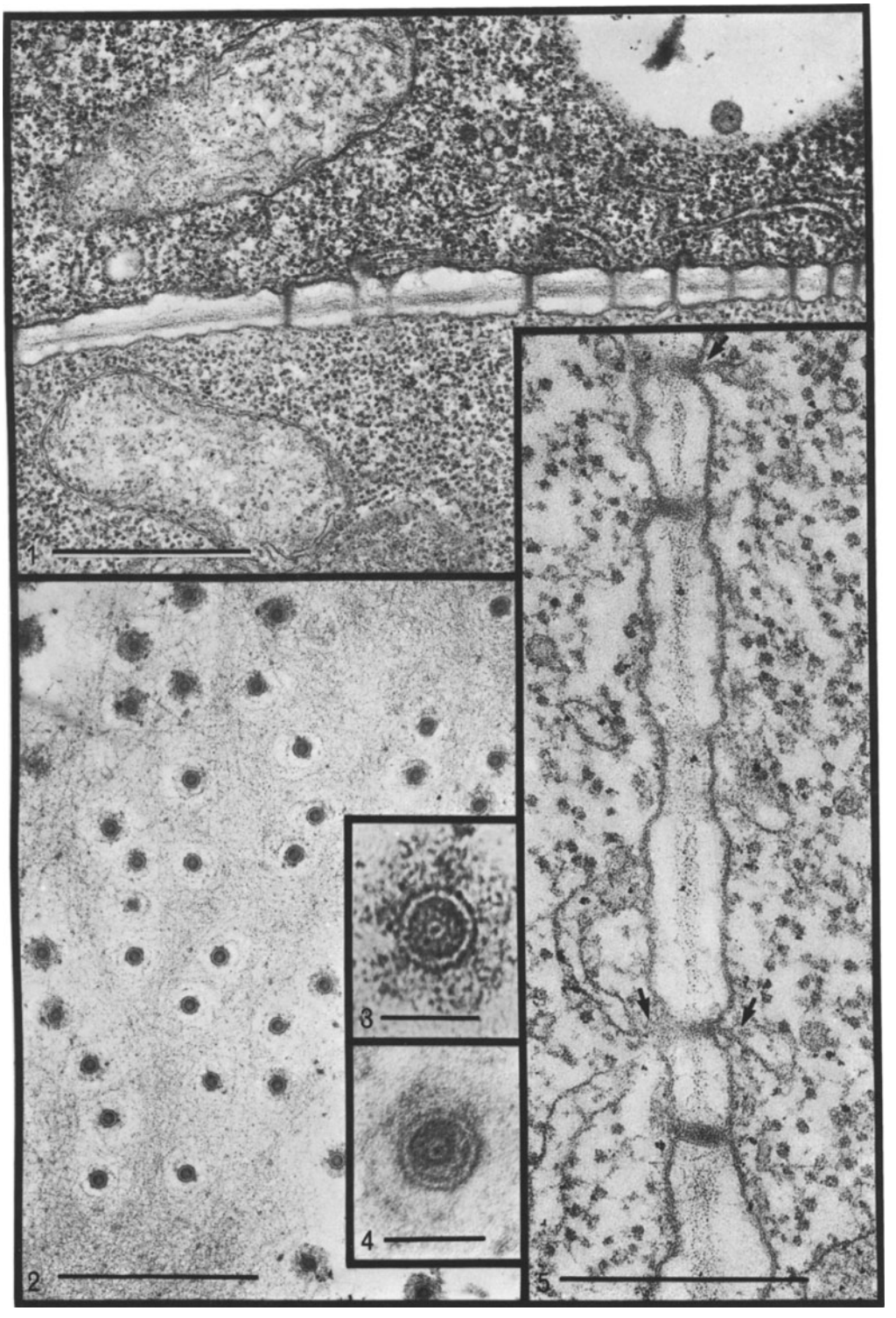
Fig. 2.4.2. Plasmodesmata in the root of the water fern *Azolla filiculoides* (see also Fig. 4.4.). Fixation was in glutaraldehyde and osmium tetroxide. In the case of (2) and (3) tannic acid was added to the primary fixative solution. ▶

(1) Longitudinal sections of plasmodesmata through the outer tangential endodermal wall.

(2) Transverse sections of plasmodesmata fixed in a solution containing tannic acid.

(3) Detail of a plasmodesma from (2): the cavity between the desmotubule and plasmalemma is electron-opaque, due, presumably, to the negative staining effect of the tannic acid. The wall of the desmotubule appears particulate; there is a central rod. The same features are seen in (4), which was not treated with tannic acid; the desmotubule wall is less clearly contrasted. Note that these plasmodesmata appear narrower than those illustrated in Fig. 2.4.1. This contributes to the difficulty of showing continuity of the desmotubule through the wall (5). Such longitudinal sections indicate the relationship of the endoplasmic reticulum with the plasmodesmata (arrowed). In these rather narrow plasmodesmata there seems to be no neck, but simply a narrow space around the desmotubule throughout the pore (see Table 2.2. for dimensions). Scale markers = (1) 1 µm; (2) 0.5 µm; (3) and (4) 50 nm; (5) 0.5 µm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examined in both longitudinal and transverse sections. In general, my observations confirmed those of many other authors. The outer diameter of the plasmodesmata in the middle lamella region was about 60 nm; the core, in transverse section, comprised an opaque ring about 20 nm in diameter with a 4 nm thick wall; this tube contained a further opaque rod, roughly 4 nm in diameter. Transverse sections clearly demonstrated that the desmotubule[1] was closely encompassed by the plasmalemma in the collar region at either end of the pore (Fig. 2.4.1.). Thus, if the interpretation by López-Sáez *et al.* of their similar structures was correct, no intercellular translocation seemed possible. The wall of the 20 nm diameter tubule was strongly reminiscent of a microtubule dimension and structure, and this, together with the doubt that a lipoprotein bilayer could behave as suggested by López-Sáez *et al.* (particularly in curving about such a small radius, see Robertson, 1964, but also see 4.2.1.), led me to suggest that the desmotubule in fact had a structure similar to that of a microtubule. Rotational image reinforcement experiments suggested that the desmotubule had 11 subunits when seen in transverse section and, in the absence of other information to the contrary, I depicted the endoplasmic reticulum as abutting the desmotubule without being continuous with it (Fig. 2.6.). (Zee (1969), studying sieve plate pore initials, also used rotational reinforcement and concluded that the central core has a 14-fold subunit arrangement. He did not observe direct connections between the endoplasmic reticulum and sieve plate pore initials at this early stage of development. See 2.4.4.). This proposal, that the desmotubule has a microtubular structure, reflected earlier observations of a similar nature (e.g. Bajer, 1968a; Hepler and Jackson, 1968; O'Brien and Thimann, 1967a), as well as other comments in the literature concerning the possibility that an unmodified strand of endoplasmic reticulum passed through the canal. For example, Ledbetter and Porter (1970): "*If derived in the first instance from endoplasmic reticulum, it has come to be compressed by the narrowness of the pore into something no longer recognisable as endoplasmic reticulum membrane or tubule*".

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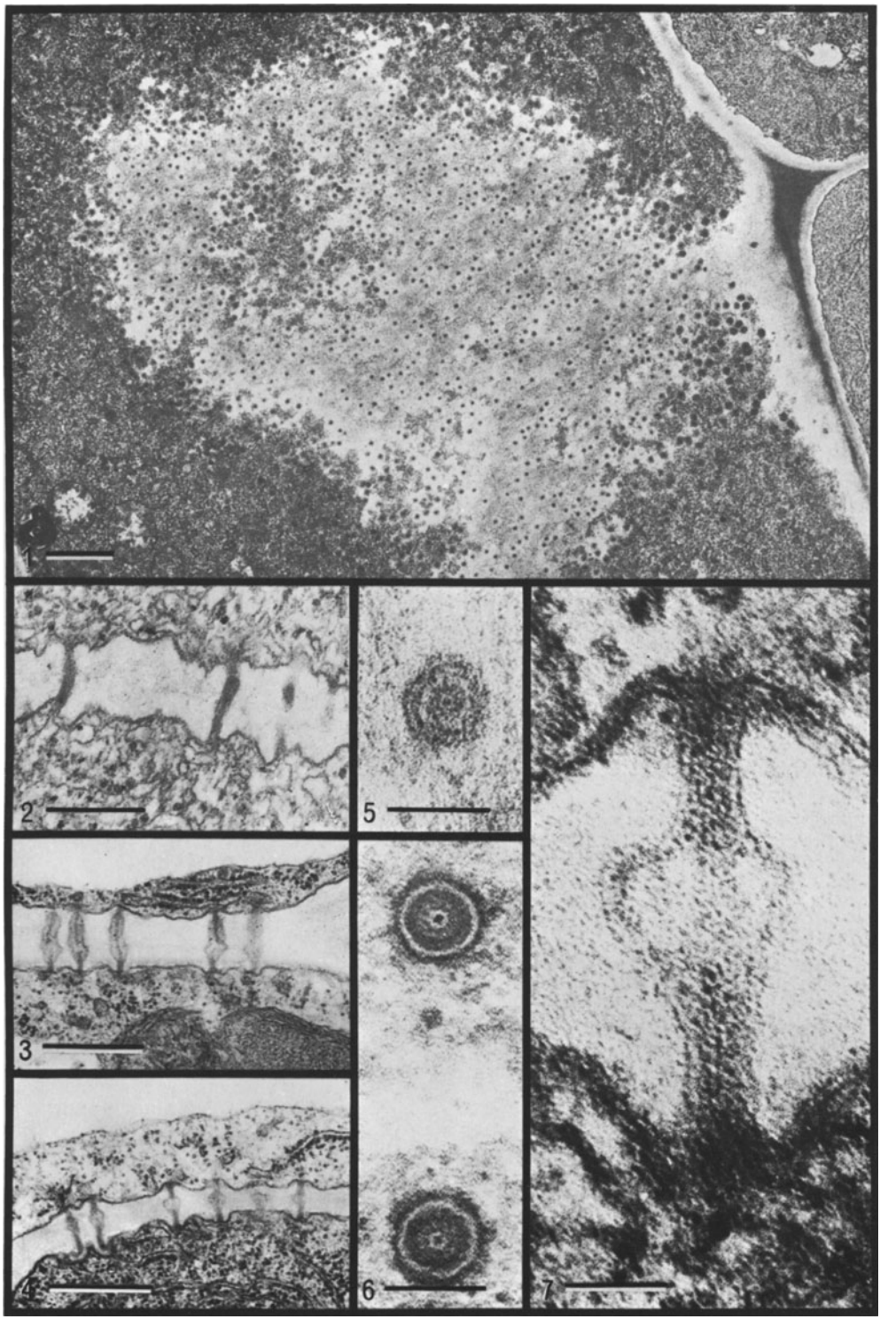
[1]I first used the term *desmotubule* in 1968(a). The derivation from the Greek *desmos* (bond) seemed, and seems, appropriate and compatible with other terms such as plasmodesma and desmosome. My intention was that the word should refer unambiguously to a particular component of plasmodesmata, without pre-empting discussion about its nature.

Fig. 2.4.3. Plasmodesmata in the root of barley (*Hordeum vulgare*, L.). Fixation was in glutaraldehyde and osmium tetroxide. In the case of (1) and (6), tannic acid was added to the primary fixative solution. (1) A pit field in the transverse end wall of a young endodermal cell showing the extremely frequent plasmodesmata. (2) Plasmodesmata through very young cortical cell walls - the plasmalemma-lined pores are relatively straight-sided, and endoplasmic reticulum appears to traverse the connection. (3) and (4) Plasmodesmata through young (State I) inner tangential endodermal walls showing different forms of dilation of the plasmalemma-lined cavity. (5) Transverse section of a plasmodesma through the wall of adjacent cortical cells: plasmodesmatal cavity, the desmotubule, and central rod can all be distinguished. (6) Detail from (1) showing the desmotubule negatively stained, presumably by tannic acid or its derivatives, which fills the space between desmotubule and plasmalemma. (7) Detail from (3). Scale markers = (1) 1 μm; (2) 0.25 μm; (3) and (4) 0.5 μm; (5), (6) and (7) 50 nm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Later (Robards, 1971) I suggested a scheme whereby the continuity of endoplasmic reticulum and desmotubule can be accommodated while still retaining the essential structural features of the earlier proposal. This model is presented in Fig. 2.7.: it will be seen to comprise a direct continuity of endoplasmic reticulum from one cell to the next *via* the desmotubule, which is merely a modified form of membrane composed almost entirely of protein subunits in the form of a tubule, but *not* to be equated with a microtubule *sensu stricto*. The full evidence for proposing such a model is reviewed in the relevant paper. It is adequate for the present purposes to point out the salient features: that the cisternal cavity of the endoplasmic reticulum in one cell is continuous with that of its neighbour through the pore of the desmotubule; that the central rod seen in micrographs (Figs. 1.1., 2.2., 2.4.) is considered to be probably an artifact; and that there is no other open channel of communication through the plasmodesmatal pore (Robards, 1971). Such a model satisfies the probability that the desmotubule is in direct continuity with the endoplasmic reticulum, as suggested by so many authors; it explains why other workers have, in agreement with my own observations, remarked upon the similar appearance of the cross-section of a desmotubule to that of a microtubule; and it overcomes the objections to the desmotubule comprising a tubule of unmodified lipoprotein bilayer membrane. Molecular mechanisms whereby a lipoprotein bilayer could become modified to a largely proteinaceous tubule are discussed in 4.2.1.

The real nature of the central rod is a particularly difficult problem: I have provided one possible explanation for its artifactual presence on the basis of negative staining (Robards, 1971), but there is little evidence for or against such an argument. The belief of López-Sáez *et al.* (1966a), Semenova and Tageeva (1972), and others, that the central rod represents the inner lamina of a tightly furled endoplasmic reticulum unit membrane (or a derivative from it), should not be dismissed out of hand. However, such a conclusion effectively precludes any function of the desmotubule as an open channel of intercellular communication. If the central rod *is* present within a tubular connection from cell to cell, then it will have a profound effect upon the carrying capacity of this particular symplastic channel (Section 1.2.1.).

Criticisms of the protein subunit desmotubule have centred upon doubts that such a structure could branch/anastomose, or be in direct continuity with endoplasmic reticulum membranes (Wooding, 1968); the

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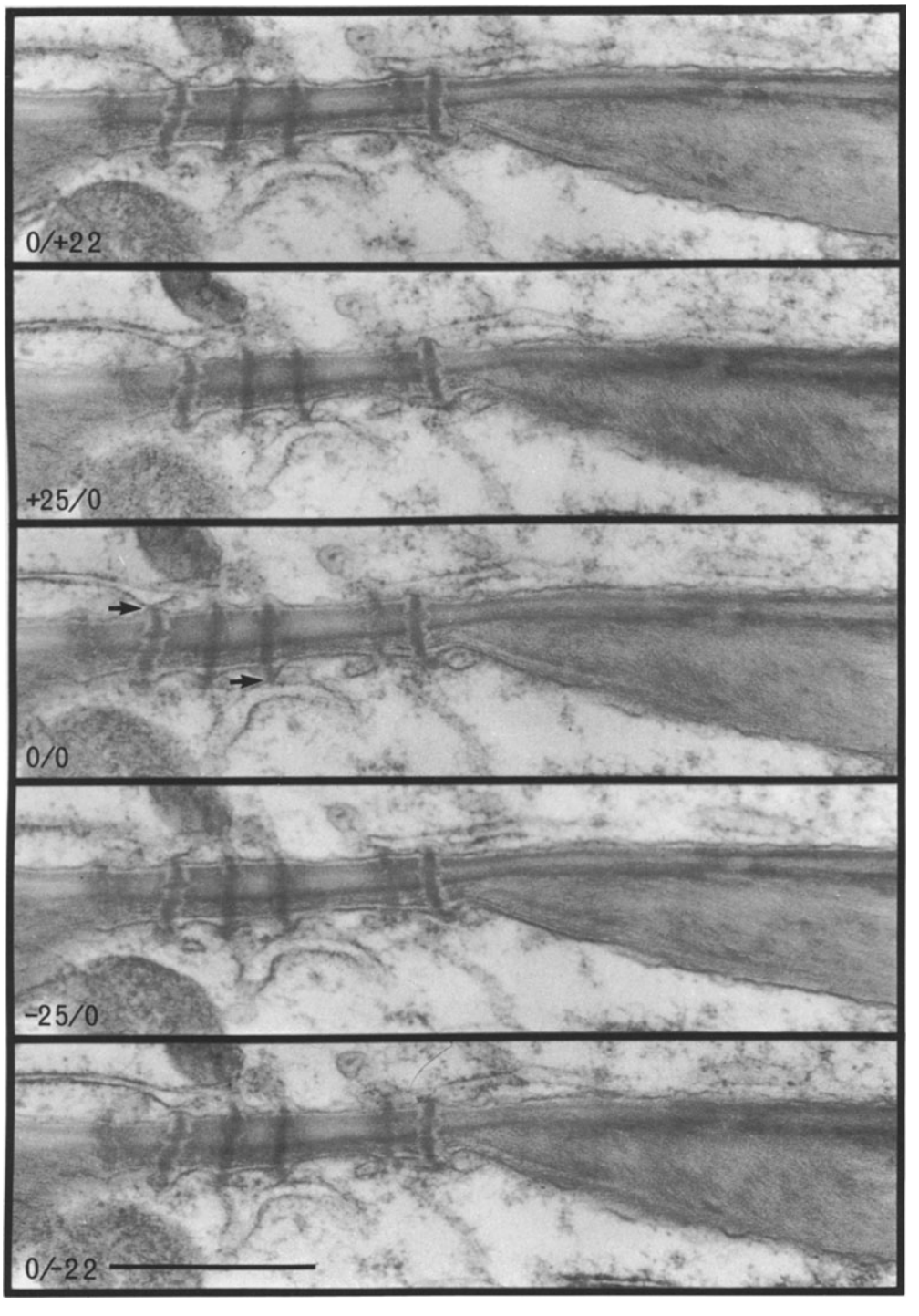
Fig. 2.4.4. Thick (200 nm) sections of plasmodesmata through the inner tangential endodermal wall of barley (*Hordeum vulgare*, L.), fixed in glutaraldehyde and osmium tetroxide, viewed at 200 keV using a high voltage electron microscope (AEI EM7) at the British Steel Corporation Swinden Laboratories, Rotherham. Different tilt angles (indicated in the form X/Y on each micrograph) allow 3-dimensional arrangements to be studied (especially in combination with stereoscopic viewing). There are considerable problems in viewing plasmodesmata by this method: any section thickness greater than that of the structure being observed (in this case about 50-60 nm) will reduce clarity of that structure. Indeed, unless plasmodesmata are specifically heavily stained, they are invisible in 1.0 µm thick sections viewed at 500-1,000 keV (A.W.R. - unpublished observations). The micrographs presented here illustrate the manner in which tilt angle affects the apparent relationships between endoplasmic reticulum and plasmodesmata: check, for example, the points marked with arrows in the central (0/0) micrograph. Scale marker = 1.0 µm ▶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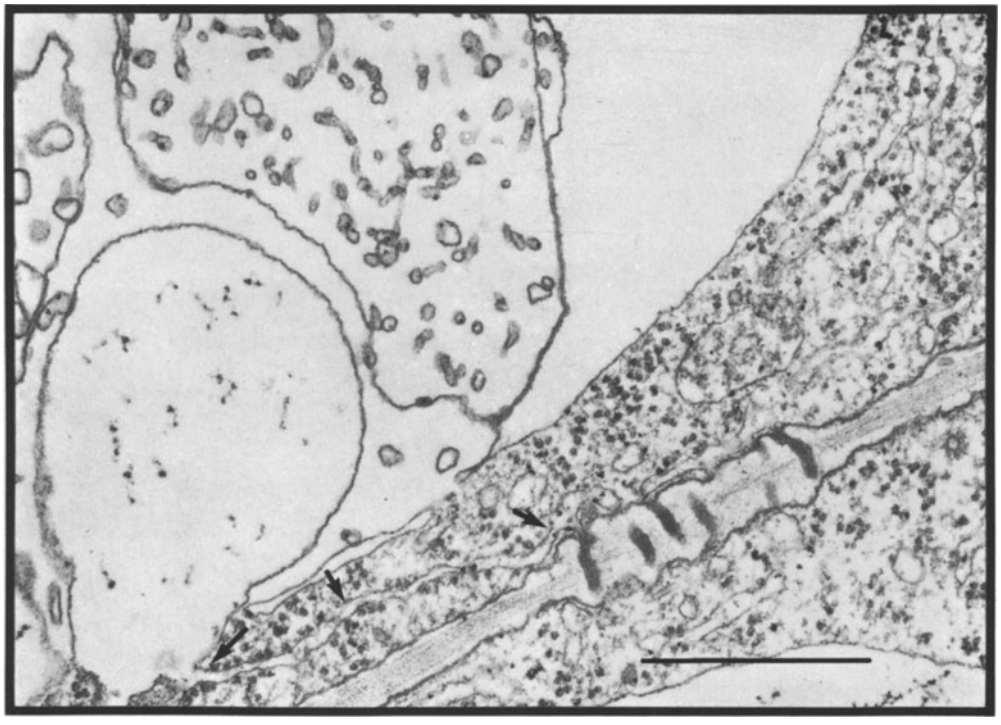

Fig. 2.4.5. Plasmodesmata traversing the wall of adjacent barley root cortical cells fixed in glutaraldehyde/formaldehyde followed by osmium tetroxide. There is clear continuity of what appears to be a strand of rough endoplasmic reticulum with a plasmodesma at one end, and a dilated vacuole-like cisterna at the other. If the endoplasmic reticulum is indeed an intercellular symplastic compartment (see Fig. 10.2B.), then arrangements of the type shown here might be expected. Scale marker = 0.5 μm

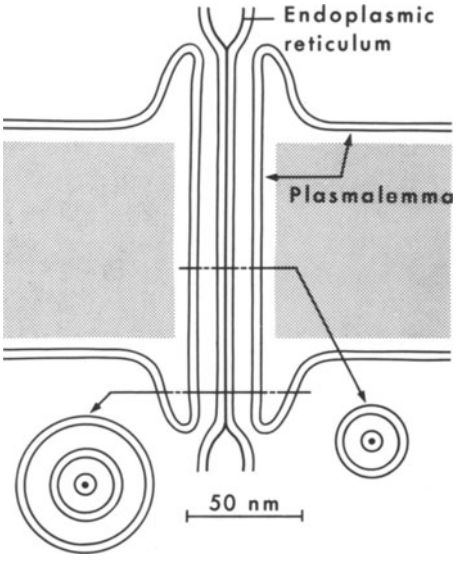

Fig. 2.5. Redrawn model of a plasmodesma derived by López-Sáez *et al.* (1966a) from observations on *Phalaris canariensis* after potassium permanganate fixation. The central spot (in transverse view) is considered by the authors to be the inner layer of the membrane of an endoplasmic reticulum tubule. An *inter-tubular gap* extends along the whole length of the plasmodesma between the supposed endoplasmic reticulum strand and the plasmalemma

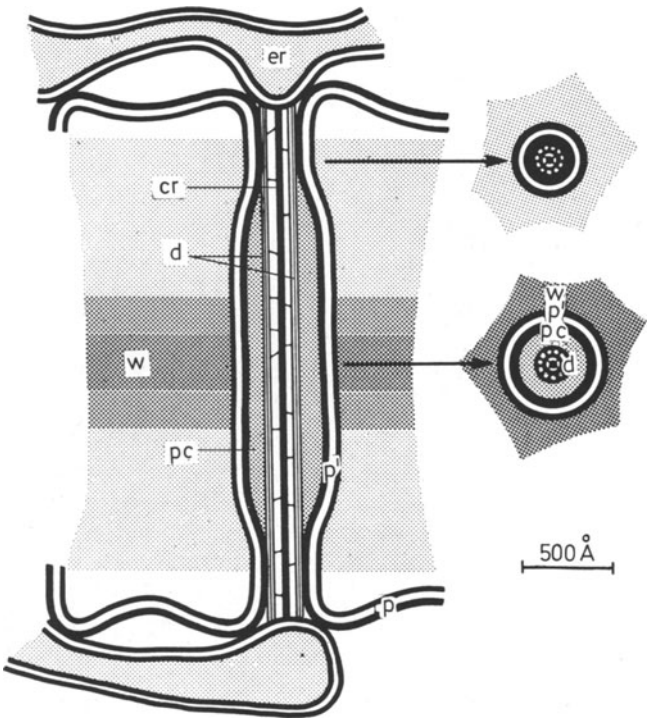

Fig. 2.6. Diagram reproduced from Robards (1968b) representing a simple plasmodesma viewed in longitudinal and transverse section. The diagram closely reflects the image as *seen* in the electron microscope. (This model was constructed from observations on plasmodesmata in the walls of xylem ray cells of *Salix fragilis*, but it is very similar to the situation in other plants - e.g. barley root plasmodesmata). *cr* - central rod; *d* - desmotubule; *er* - endoplasmic reticulum; *p* - plasmalemma; *p'* - plasmalemma through wall; *pc* - plasmodesmatal cavity (central cavity of Fig. 2.2.); *w* - cell wall

unlikely possibility that such a structure could stretch (Burgess, 1971); the fact that the desmotubule structure is largely preserved by permanganate fixation (Burgess, referring to the work of López-Sáez *et al.*); and the possibility that, whatever structure is finally seen, it represents a considerably shrunken representation of the original (Burgess, 1971). I have dealt with the problem of continuity and branching in a previous publication (Robards, 1971); suffice it to repeat here that even microtubules themselves are still at a relatively early stage of study and understanding, and that there are frequent new reports of them linking with each other or with membranes. Similarly, the extensibility of proteinaceous tubules is not known, and the effects of plasmolysis observed by Burgess (1971) would not themselves exclude the model proposed here. The ready, and spontaneous, formation of cylindrical structures from protein subunits in solution, ranging from viral particles to catalase, is another line of evidence that would be consistent with the desmotubule hypothesis. Preservation of the desmotubule (but not in such detailed structure) by permanganate fixation certainly does not preclude a protein subunit form: one has only to consider the case of flagellar fibrils to appreciate that, while many such structures are lost in permanganate fixation, others

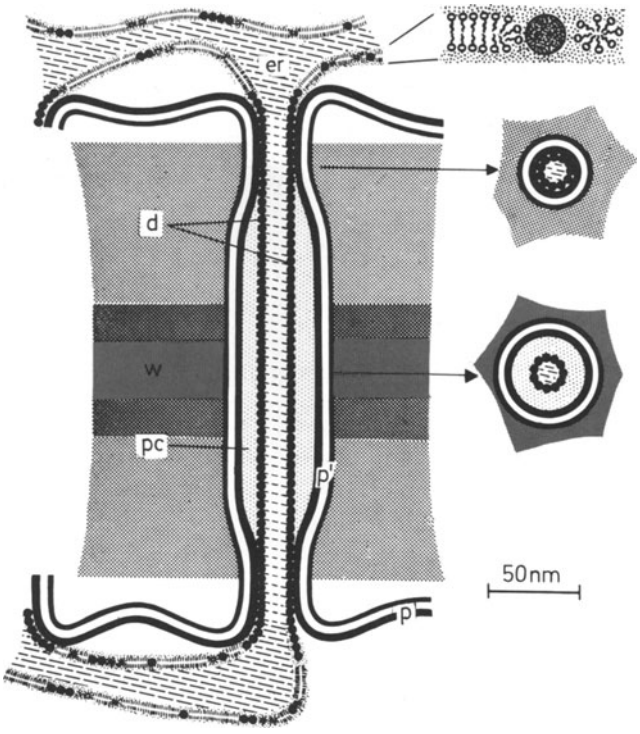

Fig. 2.7. Diagram reproduced from Robards (1971) showing an interpretative model derived from the previous illustration (Fig. 2.6.). Open continuity is shown between desmotubule and endoplasmic reticulum. The endoplasmic reticulum membrane was depicted as an expanded lipoprotein bilayer with included micelles (Lucy, 1964; and Glauert, 1968), although this is not an essential feature of the model. The reasons for deriving this model are given more fully in the text and in Robards (1968b; 1971). Labelling as in Fig. 2.6.

may still be preserved. The shrinkage problem is a severe potential artifact to interpret in the current context; it has already been touched upon in connection with plasmodesmatal size. Plasmodesmatal components, in common with other cell structures, may shrink during processing; how much, if at all, and what the effect will be, remains to be determined. Sections of frozen material, however, gratifyingly indicate plasmodesmata of much the same dimensions as from conventionally prepared material (Roland, 1973; Vian and Rougier, 1974) although the latter paper cites a rather narrow cavity (2-3 nm) within the desmotubule of chemically untreated plasmodesmata.

Burgess (1971) cites the presence of radiating spokes between the desmotubule and plasmalemma and considers that they may arise from processing damage. Similar spokes are evident in the earlier micrographs of Dolzmann (1965), which also show desmotubules appearing to open into the plasmodesmatal canal (and thus appear C-shaped in transverse section); similar observations have been made in my own laboratory, and they might quickly be dismissed as further processing artifacts if it were not for reports of similar structures in cytoplasmic microtubules (Cohen and Gottlieb, 1971).

Two recent reviews of plasmodesmatal ultrastructure have been provided. One is in Polish, by Wozny and Mlodzianowski (1973); in the other, Brighigna (1974) has studied the plasmodesmata of the water-absorbing scale of *Tillandsia* and, using arguments similar to those described above, arrives at the structural model depicted in Fig. 2.8., where the desmotubule is apparently sealed, and few unambiguous comments are made about its functional possibilities. An earlier review (in Russian - Sukhorukov and Plotnikova, 1965) considers the structure and function of both plasmodesmata and ectodesmata.

Some workers have stated that they find specific associations between paramural bodies (Marchant and Robards, 1968) and plasmodesmata (Kurkova, Vakhmistrov and Solovyev, 1974; Vakhmistrov, Kurkova and Solovyev, 1972), although whether this relationship is real or artifactual remains to be demonstrated. (See also the induction of paramural bodies near plasmodesmata by virus infection - 8.3.).

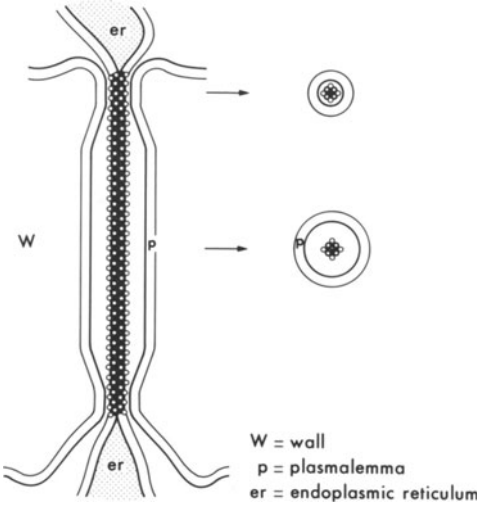

Fig. 2.8. Redrawn from Brighigna's (1974) interpretation of the plasmodesmata in the walls of the absorbing hairs of *Tillandsia usneoides*. *Two* globular components - of 7.0 and 4.5 nm diameter - are said to comprise the desmotubule. Functional capabilities *through* the desmotubule are not clear

One point is certain: *none* of the models described adequately depicts the structure of either a specific or a generalised plasmodesma. The model shown in Fig. 2.7. has at least two unresolved features critical to an understanding of its function: the presence or absence of a central rod through the desmotubule; and the 'tightness' of the neck on the desmotubule. The central rod is a common feature of plasmodesmata from widely different species (e.g. *Salix*, *Hordeum* and *Azolla* - Fig. 2.4.; *Abutilon* - Fig. 1.1.). Whether, as implied in Fig. 2.7., it is an artifact, remains to be determined. In *Hordeum* and in *Salix* (for example) the desmotubule is tightly invested by the plasmalemma at either end of the plasmodesma. If this seal is 'leak-proof', then the only opportunity for transport is through the desmotubule itself. If there is not a tight seal, then not only are two potential pathways opened up, but there exists the possibility for simultaneous bi-directional transport (Fig. 2.10.). Such plasmodesmata occur in *Abutilon* mature nectary stalk cells (Chapter 11), where there is a clear gap along the *whole* length of the plasmodesma between the plasmalemma and the desmotubule (Fig. 1.1.). There seems to be increasing evidence that plasmodesmata have a number of different basic structures or dimensions (contrast *Laminaria; Chara; Bulbochaete;*

Hordeum; Abutilon; Azolla, as examples of 'simple' plasmodesmata - see Table 2.1., and also Table 2.2. for detailed dimensions).

So far as the wall structure through which the plasmodesmatal pore runs is concerned, we have very little information. The early work of Scott *et al.* (1956) pointed to the primary pit fields as probable sites for the intercellular connections. Pit fields may give rise to pits traversed by plasmodesmata in the primary cell wall. It appears that most plasmodesmata traverse the walls of higher plants in these areas. The paper of Kollmann and Dorr (1969) implies that callose is found surrounding the plasmalemma of all plasmodesmata but this remains to be substantiated, both in higher plants and in algae. Callose has been found associated with algal plasmodesmata, although whether as an artifact or not remains contentious (Chapter 3); it is also found around phloem plasmodesmata; and, perhaps, around aging plasmodesmata in a moss (Eschrich and Steiner, 1968). Carde (1974) refers to a callose sheath, which he depicts as a cylinder of material in the neck region, around plasmodesmata, while Vian and Rougier (1974) and van Went *et al.* (1975) are among recent authors to comment on the different

TABLE 2.2.

DIMENSIONS OF PLASMODESMATA (nm)

	Outer diameter of plasmalemma	Inner diameter of plasmalemma	Outer diameter of desmotubule	Inner diameter of desmotubule	Central rod
Azolla young root cortical cells	35	25	16	7	3
Hordeum young (4 mm from tip) root endodermal cells	46	33	20	9	3
Hordeum older (120 mm from tip) root endodermal cells	60	44	20	10	4
Abutilon Distal cross wall of stalk cell of nectary hair (see Chapter 11)	44	29	16	10	3

All dimensions cited here are means of multiple measurements taken from micrographs of calibrated magnification. Measurements were made across the mid-line of plasmodesmata (not the neck region). The main feature contributing to the wider plasmodesmata of the older barley roots is the increased width of the gap between the plasmalemma and desmotubule.

appearance of the cell wall immediately surrounding plasmodesmata. Clowes and Juniper (1964) referred to a changed carbohydrate deposition around plasmodesmata. Taiz and Jones (1973) have demonstrated a component of the cell wall around barley aleurone plasmodesmata that is highly resistant to cellulase enzymes, so that 'wall tubes' are left after enzyme digestion of walls: these tubes enhance the apparent size of plasmodesmata seen in the light microscope. (See also *Abutilon* plasmodesmata, Chapter 11).

2.4.3. Variation in Structure

2.4.3.1. Gross structural differences From the preceding section it will be noted that even 'simple' plasmodesmata from different groups have different structures. More elaborate forms occur, some of which reflect development from the simple state and are considered in Chapter 4. Many plasmodesmata have some form of 'node' (median nodule) in the mid-line of the wall (Mittelknote - Krull, 1960; Cox, 1971) (Fig. 2.9.); others have anastomosing arms (Fig. 2.9.), such types being frequently cited from phloem cells (e.g. Kollmann and Schumacher, 1962; Northcote and Wooding, 1966; Murmanis and Evert, 1967) as well as other sites (e.g. *Viscum* - Krull, 1960; *Tamarix* wood fibres - Fahn, 1967; secretory cells of *Nepenthes* - Clowes and Juniper, 1968; hairs of the trap of *Utricularia* - Fineran and Lee, 1975; Chapter 11); sometimes the median nodules are linked to each other by a large median cavity. The median nodule (Mittelknote) is usually seen as an enlargement of the plasmodesmatal cavity, together with a more complex 'knot'

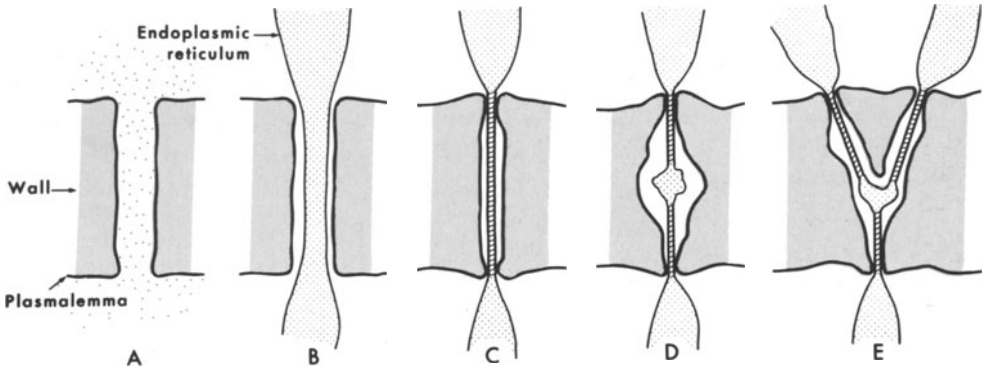

Fig. 2.9. Diagram illustrating plasmodesmatal variation.

A. Simple plasmalemma-lined pores with no desmotubule. A situation found mainly in the algae, but also occasionally reported from higher plants.

B. Loosely bound strand of endoplasmic reticulum. Such a profile is often seen during late stages of cell plate formation, but may persist in some cases. Two separate opportunities for symplastic transport are clearly available.

C. Tightly bound desmotubule. A constriction ('neck') appears to block any possible pathway between the plasmalemma and desmotubule.

D. A median nodule may form in the mid-line of the wall. Such regions often fuse to give a median cavity.

E. Desmotubules may anastomose, often with multiple connections on one side leading to a single channel on the other

structure of the desmotubule in the mid-line of the wall (Fig. 2.9D.). Median cavities arise when there is fusion of the central cavities of adjacent plasmodesmata, so forming large lacunae within the cell wall (Chapter 4).

Although there are few reports of plasmodesmata without desmotubules in higher plants (the micrographs of plasmodesmata through secretory cell walls of *Tamarix* published in Thomson and Liu, 1967 do not show clear desmotubules through all pores; also see Burgess, 1971 and Mueller, 1972), such 'open' pores are common in the algae, and increase the difficulty of relating the physiology of algal plasmodesmata to that of higher plants. In some cases (e.g. Dolzmann, 1965) the desmotubule may appear to be incomplete. Most of the variations described above have also been seen in varying degrees of elaboration in a relatively narrow sample of specimens studied in my laboratory, and it may thus be concluded that they are all of common occurrence. Indeed, the salient problem relating to structural variability is in determining whether the modified structures are indicative of the natural condition, or whether processing artifacts may lead to modifications of one type or another. In the case of plasmodesmata which possess a median nodule, as well as those which have anastomosing arms, there can be no doubt that this is how they exist in nature; these are relatively gross variations which we would not expect to be altered to any great extent by artifacts.

2.4.3.2. Minor structural variations In considering the nature of the desmotubule itself, the structure is closer to the realm of molecular dimensions, and it seems quite probable that artifacts could be readily induced. The complete absence of the desmotubule from a plasmodesma remains to be explained in acceptable functional terms (or *vice versa*!). An opening of the desmotubule into the central cavity (as in some of Dolzmann's plasmodesmata, 1965) need presumably have little effect upon translocatory function so long as the seal between the desmotubule and plasmalemma through the neck remains a good one.

The median nodule is apparently a secondary development from a 'simple' plasmodesma. It could be that this nodule is important in controlling translocatory fluxes or in completely stopping them; it may also be involved in enzyme-mediated processes initiated within the plasmodesmatal cavity. This idea is supported by the reports of enzyme activity associated with the plasmodesmata (Hall, 1969 - ATPase; Robards and Kidwai, 1969 - phosphatase; Ashford and Jacobsen, 1974a,b - phosphatases; also see Chapter 7), as well as the established hydrolytic activities during sieve plate pore formation. The anastomosing of desmotubules may occur with many tubules on either side linking into a relatively large central cavity or median nodule or by many desmotubules on one side fusing into relatively few, or only one, on the other (Fig. 2.9.). In such cases it is often found that the diameter of the single tubule is greater than that of the multiple ones (Wooding, 1968), although what significance this has for the molecular structure of desmotubules is unknown. Once again, one can only guess at the precise functional significance of such arrangements and speculate that they might be related to special requirements for the control of translocation.

2.4.4. Plasmodesmata in Phloem

Plasmodesmata in phloem tissue are of particular interest for their distribution: for example, as summarized by Shih and Currier (1969) in their contribution on cotton phloem: "*plasmodesmata connected parenchyma to parenchyma, parenchyma to companion cells, and companion cells to sieve elements. Their general absence between parenchyma*

cells and sieve elements points to a specific role of companion cells in sieve tube functioning". This finding is in agreement with that of many other workers (e.g. Evert and Murmanis, 1965; Wooding and Northcote, 1965; Northcote and Wooding, 1968; Wark, 1965), with occasional reports to the contrary (Zee and Chambers, 1968 - *Pisum* primary phloem). Wooding and Northcote (1965) describe the common situation of complex plasmodesmata between the sieve elements and companion cells: from a single plasmodesma eight to fifteen arms develop on the companion cell side, but only a single tube, approximately twice the size of the others, is found on the sieve element side; similar reports have been widely published (e.g. Esau, 1973 - *Mimosa*). Behnke states that the desmotubule diameter of phloem plasmodesmata may be increased by up to six times compared to normal (cited by Kollmann and Dörr, 1969); as the radius of a narrow tube is an important parameter affecting flow-rate, such an observation is particularly germane. A median cavity is commonly associated with phloem plasmodesmata (Esau and Gill, 1973). Behnke and Paliwal (1973) have commented (in relation to *Gnetum*) that this may represent a phylogenetic stage prior to specialized connections between sieve tube members and companion cells. In *Isoetes*, Kruatrachue and Evert (1974) reported that the end walls of the sieve elements could have sieve plate pores and/or plasmodesmata, while the side walls have plasmodesmata only.

So far as the sieve plates are concerned, the plasmodesmata which initially traverse the wall appear to be quite normal. They may subsequently develop a median nodule, and from this general area hydrolysis of the wall occurs prior to the deposition of callose to form the completed sieve plate pore (e.g. Esau, Cheadle and Risley, 1962; Deshpande, 1974).

A detailed analysis of the structure of plasmodesmata (sieve plate pore initials) in differentiating sieve elements of *Vicia faba* was made by Zee (1969). The central core (desmotubule) is closely bounded by the plasmalemma, so that the inner, dark, layer of the latter appears to have fused with the central core. Zee's interpretation of the 25 nm diameter desmotubule substructure is similar to that of Robards (1968a, b), except that maximum rotational image reinforcement occurred where n = 14 instead of 11 (see 2.4.2.). In addition, Zee suggested the presence of seven further subunits at the inner margin of the desmotubular ring, these surrounding, and linking with, a central rod of about 15 nm diameter. Although such results are equivocal, and the image reinforcement technique requires most circumspect interpretation, they do strengthen the belief that the traversing strand of plasmodesmata, in widely different situations, is a structure with its own special organization, and not a simple tubule of unmodified endoplasmic reticulum membrane.

Phloem plasmodesmata, from their distribution and complexity, are clearly important in translocation and, presumably, especially in the loading and unloading of sieve elements *via* companion cells (Chapter 11). As in all other cases, however, this area requires intensive investigation to understand the mechanism of symplastic transport.

2.5. SOME CONCEPTS OF PLASMODESMATAL FUNCTION

Much of this Volume is concerned with the possible function of plasmodesmata in a variety of different situations. At the cost of a little duplication, it is useful briefly to mention some of the roles that have been envisaged, and some of the relevant evidence.

The very earliest papers on plasmodesmata assumed that they were involved in the translocation of material between cells. This point of view has been held more or less strongly with relatively few exceptions (see, e.g. Pfeffer, Vol. 1 p. 602, 1897). Where it has not been considered that translocation is the main function of plasmodesmata, then the transmission of stimuli has sometimes been suggested as an alternative possible function. Haberlandt (1914) summarized his ideas thus: "*the fact that protoplasmic connecting threads serve for the transmission of stimuli does not exclude the possibility that these structures may in certain cases be partly or entirely engaged in translocation of plastic materials*". This statement reflects the attribution of translocation as a subsidiary role in plasmodesmatal function and Haberlandt's views were probably coloured by the work of Pfeffer on the transmission of stimuli along plant parts (see Pfeffer, 1906). A similar sort of function is considered by Ledbetter and Porter (1970) who suggest that plasmodesmata may be important for the equilibration of membrane potentials and transfer of membrane-supported excitations; a possibility that is receiving increasing attention. The whole field of the application of electro-physiological techniques to the study of plasmodesmata is potentially a most rewarding one, and is well reviewed by Spanswick (1974; 1975), and is fully discussed in Chapter 6.

Most authors who deal with the question of function have speculated on a possible translocatory role of the intercellular connections. There is no *direct* proof that these structures serve such a purpose, and Clowes and Juniper (1968) state that "*we cannot assign any role to the plasmodesmata with any confidence*". Spanswick (1974) aptly cites a recent plant physiology textbook "...*the extent and importance of the role of plasmodesmata in intercellular transport is not known*" (Greulach, 1973); and Ziegler in a short article on "*What do we know about the function of plasmodesmata in transcellular transport?*" (1974) concludes "...*our knowledge of the function of plasmodesmata in transcellular transport is at present very poor and circumstantial*".

The main problem in attributing translocatory function to plasmodesmata is this: young cell walls, although they may contain plasmodesmata, are usually freely permeable and constitute no severe barrier to the passage of ions and small molecules, *provided that* such solutes can move easily across the plasmalemma: plasmodesmata at these sites are not, therefore, obviously obligatory for intercellular translocation of such solutes. (Quantitative treatments of plasmodesmatal transport *versus* trans-plasmalemma transport are given by Tyree (1970) and in Chapter 5.) Movement of materials from cell to cell across *thick* intervening walls could be assumed to take place *via* the plasmodesmata if the walls were impermeable, but it is difficult to show that they are; if it can be shown that *thick-walled* cells can communicate through their plasmodesmata, then it seems possible that the same channels serve for transport between younger cells also. Consequently, many experiments investigating plasmodesmatal function have been concerned with showing that these structures can be the only route for translocation and, secondly, that they are actually capable of allowing fluxes at high enough rates to account for experimental data (see, for example, Chapters 10 and 11).

Before the recent experimental approaches to the study of plasmodesmata are considered in later Chapters it is important to dwell briefly on the less direct, and often more circumstantial, evidence. While none of this is conclusive, when put together it makes a strong enough case to justify the devotion of further energies to the investigation of this problem. I have already made it clear that the distribution of plasmodesmata reflects the anticipated capacity for the passage of

materials between cells. Some cells, where other evidence suggests the need for complete isolation, have no plasmodesmata at all; others have numerous plasmodesmata in the walls across which high flow rates would be expected; plasmodesmata with branching desmotubules are often found at very active sites of solute movement (such as sieve tubes/companion cells; secretory cells in the *Nepenthes* pitcher; salt glands; etc.) and, in extreme cases, may even show desmotubules enlarged to as much as six times their normal diameter. The stamen filament cells of wheat elongate at 2.0-3.5 mm min^{-1} and, according to Ledbetter and Porter (1970) are "*doubtless supplied by the abundant plasmodesmata*". Plasmodesmata are also present between cells with very thick walls which are considered to be impermeable (e.g. xylem ray cells; thick-walled endodermal cells) or to have barriers to apoplastic transport (e.g. the Casparian strip of young endodermal cells).

Just as there are features which favour acceptance of the idea that plasmodesmata are sites of intercellular communication, so also there are aspects which are difficult to reconcile with such a role. I mention only two: one is that neighbouring interconnected cells can exhibit morphological and/or biochemical differentiation one from another, hence plasmodesmata (or the membranes bounding the endoplasmic reticulum cisternae if this comprises a symplastic compartment) must be selective (as they commonly separate cells of quite different kinds [e.g. tannin cells and tannin-free cells, Esau, 1965; non-articulated laticifers and parenchyma cells]); the other difficulty is that, individually or together, plasmodesmata need to be capable of sustaining bidirectional fluxes (see Chapters 10 and 11). In the first case, even though the *manifestation* of a genetic dissimilarity (e.g. tannin) may be produced in, or sequestered into, a compartment of one cell, but not another, the fact remains that the plasmodesmata presumably allow a degree of intercellular continuity without at the same time jeopardising the individual genetic identity of adjacent cells. The second case arises from the consideration that, while we may envisage a unidirectional volume flow through some plasmodesmata where high flux rates occur, it is difficult to equate this with the certain necessity for at least some transport in the opposite direction. The possibility that plasmodesmata may be polarized to work in one direction only, and that different plasmodesmata through the same wall or barrier allow fluxes in different directions, is one that would be extremely difficult to test (see Kuo, O'Brien and Canny, 1974; also Chapter 12). It would, however, provide a simple means for controlling intercellular flow. A different concept is that single plasmodesmata may be able to carry fluxes in two opposing directions simultaneously through two different symplastic compartments (Fig. 2.10.). O'Brien and Thimann (1967a) suggested that the endoplasmic reticulum may act as a pathway for the intracellular transport of auxin, and that polarity of transport may in some way be associated with the evident connections of the endoplasmic reticulum to the complex plasmodesmata. Another alternative is that some molecules and ions would move through symplastic pathways while others would cross apoplastic ones - at least in part.

It seems to me that the current weight of *circumstantial* evidence is heavily inclined towards the view that plasmodesmata are functional in symplastic transport. Assuming that cells connect with each other through the plasmodesmata, what sort of connection exists? The structure has been discussed; we now look at the problem from a functional viewpoint. It must be stated at once that most physiologists have contemplated intercellular connections as small, but open, channels linking cytoplasm with cytoplasm (Fig. 10.2A.) - a situation apparently common in algae (see Chapter 3); this is different from the concept of a sealed strand of endoplasmic reticulum running from cell to cell.

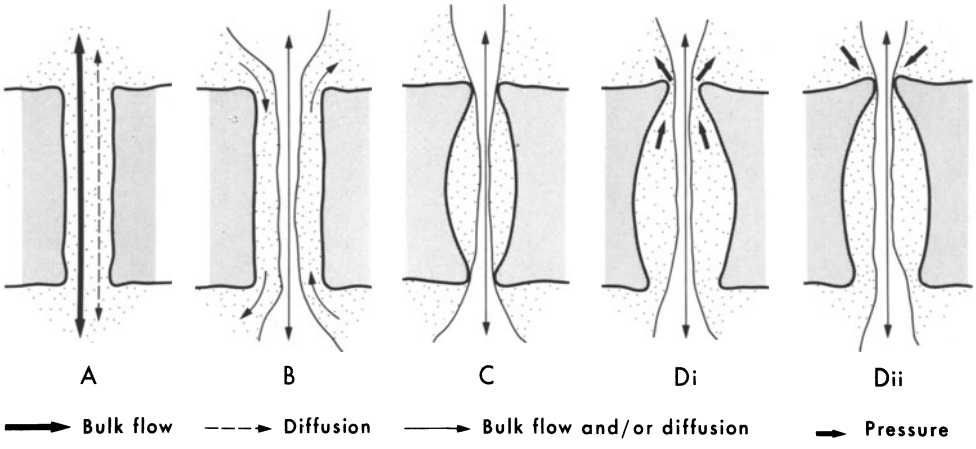

Fig. 2.10. Some theoretical and hypothetical possibilities for plasmodesmatal function.

A. An open tube, without desmotubule. Bulk flow or diffusion could occur in either direction so long as the rate of bulk flow in one direction was not so high as to reduce diffusion against it to negligible proportions. This possibility - bulk flow and/or diffusion - applies to all pathways depicted here, so long as the conducting pore is not too long, in which case the diffusive flux would be greatly reduced (see 1.2.1.).

B. An open tube containing a desmotubule, so allowing two possible pathways: through the desmotubule from endoplasmic reticulum cisterna to endoplasmic reticulum cisterna; or through the cytoplasmic annulus around the desmotubule (the *Abutilon* situation - Fig. 1.1.).

C. A desmotubule tightly sealed in the tube through the wall. The only apparent channel of intercellular communication appears to be through the desmotubule (the *Salix* and *Hordeum* situation - Fig. 2.4.).

D. A hypothetical concept for the operation of plasmodesmata as valves. If the plasmodesmata were permanently, or temporarily, asymmetrical - as shown - greater pressure from the cell on one side could keep the cytoplasmic annulus open (Di); alternatively, a reversal of pressure could close the cytoplasmic annulus, so leaving the desmotubule as the only conducting channel (Dii)

Functionally, the differences are important. In the former case the plasmodesmata may serve as no severe barrier for anything but the largest molecules (although open, but 'necked' plasmodesmata as found in *Bulbochaete* could modify this); in the latter, not only is space more restricted but the channel of communication is, at least in part, from the cavity of the endoplasmic reticulum in one cell to that of the next. A theoretical (Chapter 11) possibility is a *two* compartment connection, with an endoplasmic reticulum tubule passing through an *open* plasmodesmatal canal (Fig. 1.1., 2.10B.; see also Bräutigam and Müller (1975b - Fig. 12.), who propose a similar model). Buvat (1969) encourages the open channel view (although he has always considered that endoplasmic reticulum runs from cell to cell) by putting his concept of the 'plasmodia': the wall being a skeletal support for an internal medium which permeates the entire plant and through which circulate metabolites, water, ions and the products of cellular activ-

ity. Sutcliffe (1962) assumed that plasmodesmata function in the intercellular transport of ions, and "*small vacuoles may move through the protoplasmic connections and could act as vehicles in salt transport*", although we now know that this would be impossible through normal plasmodesmata. Brouwer (1965) and Minchin and Baker (1970) are among the authors who have considered models depicting plasmodesmata merely as open tubes through cell walls, but the evidence is clear that such models are much too simplistic and can only apply to a minority of cases in higher plants. Gamalei (1973) reports having followed the movement of osmiophilic droplets along plasmodesmata between ray parenchyma cells of spruce, and notes that the droplets are often larger than the diameter of the plasmodesma; a situation similar to that sometimes found in the intercellular movement of viruses. Gorin (1969) considers that enzymes can move through plasmodesmata - even of dead cells. Unfortunately, neither of these two papers provide the critical data that would allow an impartial and independent evaluation of the conclusions which are, therefore, still very much *sub judice*. Although *direct* experimental evidence is still required, there appear to be good reasons for believing that plasmodesmata act as pathways for symplastic transport and electrotonic coupling of cells. That is not to say that all plasmodesmata are functional in this way. There also seems to be abundant evidence showing that by secondary modification and/or occlusion, the function of plasmodesmata can be changed.

ACKNOWLEDGEMENT

I am grateful to the Agricultural Research Council and Science Research Council for financial assistance in support of some of the work described in this Chapter.

2.6. OPEN DISCUSSION

Most of the discussion centred around questions of fixation, staining and possible artifacts.

What was it that the early light microscopists saw? asked CARR, noting that plasmodesmata are below the theoretical limit of resolution. Did some diffraction phenomenon make them visible, or were they only seen when several were superimposed or side by side, or was it possible that it was sleeves of special wall material that made the plasmodesmata apparent? (see 2.4.2.). ASHFORD confirmed that wall sleeves do occur (see 4.9.) and that while some of the stains the early workers had used react with protoplasm, others are indeed stains for cell wall constituents. HUGHES and GUNNING described their efforts to repeat some of Tangl's work, by embedding date endosperm in both glycol methacrylate and Spurr's resin and observing sections using a Zeiss Photomicroscope III; with luck and dedication they hoped one day to be able to approach the quality of Tangl's 1879 drawings (see Frontispiece). Electron microscopy did at least confirm that the structures that are visible by light microscopy are indeed plasmodesmata!

GUNNING then made an extended comment about errors that arise when attempts are made to count plasmodesmata using micrographs showing transversely sectioned cell walls, i.e. with plasmodesmata sectioned longitudinally. The answer that is obtained depends very much on the thickness of the section and the diameter of the plasmodesmata in question (see also Spanswick and Costerton, 1967). The section includes

within it parts of plasmodesmata as well as (if it is thick enough) whole plasmodesmata, and if *all* are counted then the sample volume is in fact a volume that is greater than that represented by the thickness of the section multiplied by area (or length of cell wall). Limits for the errors can be set, thus if R is the radius (μm) of the plasmodesma (outermost dimension), T the thickness of the section (μm) and F the frequency of the plasmodesmata per square micrometre:

(i) if *any* portion of a plasmodesma, however small, that is included within the section is detectable and is therefore counted then:

$$F = \text{count per } \mu m \text{ of wall length}/(T + 2R)$$

(ii) if *at least* half a radius of a plasmodesma has to be present in the section for that plasmodesma to be detected and counted then:

$$F = \text{count per } \mu m \text{ of wall length}/(T + R)$$

The true situation is likely to fall between these limits, with a plasmodesma being detectable when about one quarter of its radius lies within the section, in which case:

$$F = \text{count per } \mu m \text{ of wall length}/(T + 1.5R)$$

Using the latter approximation to estimate F for the distal wall of the stalk cell of *Abutilon* nectary trichomes, and assuming a section thickness of 40 nm (this not measured accurately but merely a guess based on the resolution obtained in high magnification views such as Fig. 1.1.), the answer is F = 11.6, well within the standard error of direct counts based on face views of thewall in question, which gave F = 12.6, as described in 11.3. Had the correction for plasmodesmatal dimensions not been used (i.e. the 1.5R value), an erroneous answer of 30.7 would have emerged from the views of transversely sectioned walls.

For the above correction factor to be accurate, another condition must be met. The longitudinal axis of the plasmodesmata must not be at an angle to the plane of the section. If there is an angular deviation, the plasmodesmata will be sectioned obliquely. Because of this, parts of plasmodesmata that would not, with accurate orientation, be sectioned are in fact included, thus again increasing the apparent volume of the sample. The maximum increase in effective volume is given by $(\sec \theta + L/T \tan \theta)$ where θ is the angle by which the plasmodesmatal axis deviates from the plane of the section and L is the length of the plasmodesmata. The effect is well illustrated in the micrographs of Hicks and Steeves (1973) which have been used to obtain such unusually high counts (see Table 2.1. and 2.3.2.), and where if L is taken as 0.2 μm and T as 0.05 μm, a θ of as little as 15° gives an effective doubling of the sample volume. Since the tilt is fairly clearly >15°, the plasmodesmatal frequency, while admittedly high, is probably not as high as has been suggested.

GIBBS, aware of long-term changes in plasmodesmatal frequency (Chapter 4), wondered whether short-term, even day-to-day, changes might occur, thereby contributing further to errors in estimating numbers per unit area. Possible, but no evidence as yet, was the verdict on this suggestion.

ROBARDS then took up the problem of estimating the cross-sectional dimensions of plasmodesmata from thin sections. If the sides of the pore and desmotubule are parallel (e.g. Fig. 1.1.), then the mean of a reasonable number of measurements will provide a good estimate of the various parameters. However, the plasmalemma-lined pore and sometimes even the desmotubule may vary in diameter through a single plas-

modesma (see Fig. 2.4.3.). If this is the case, then citation of a mean from multiple measurements will have neither structural nor physiological significance.

A useful procedure under such circumstances is to record separately dimensions falling within specific ranges from all plasmodesmata under observation; if the section thickness can be reasonably estimated (T_s), and if total wall thickness (T_w) can also be chosen (e.g. from transverse sections of cell walls), then the number of section thicknesses through a wall (N_t) will be approximately known. The number of ranges of dimensions measured (R_x) should be $<N_T$. It is then possible to obtain a rough idea of the dimensions *and* shape of the plasmodesma as follows (this assumes: (a) all plasmodesmata being studied are similar, and (b) there is a continuous variation of size in one direction from mid-line to extremity of the pore).

Let us assume that the inner radius (R_i) of the plasmalemma-lined pore is the parameter of interest. The wall thickness (T_w) = 0.5 μm (500 nm); section thickness (T_s) = 50 nm; therefore N_T = 10. 100 measurements (ΣN) are made: they range between R_i = 25 nm and R_i = 10 nm. Therefore the measurements are each allocated to size classes at 1.5 nm intervals between 10 and 25 nm. They distribute as:

	a	b	c	d	e	f	g	h	i	j
R_i	10-11.5	11.5-13	13-14.5	14.5-16	16-17.5	17.5-19	19-20.5	20.5-22	22-23.5	23.5-25
N	12	11	9	5	6	7	3	15	15	17

Assuming also that the plasmodesmata are symmetrical about the mid-line, then the mean length of plasmalemma-lined tube of radius $R_{i(a-j)}$, over one half of a plasmodesma, $= \frac{N_{(a-j)} \times T_w}{\Sigma N}$

e.g. for $R_{i(a)}$, $0.5 \frac{12 \times 500}{100} = 30$ nm

Similarly, (b) = 27.5, (c) = 22.5, (d) = 12.5, (e) = 15.0, (f) = 17.5, (g) = 7.5, (h) = 37.5, (i) = 37.5, (j) = 42.5. These values then represent the length over which the radial dimension is at each given size class, and realistic estimates of maxima and minima can be determined. Longitudinal sections are, however, still required in order to check on the appearance and number of constrictions, swellings, etc.

BAIN asked which is the best fixative to use for observing plasmodesmatal structure. No special recommendations can be made, said ROBARDS, although systematic studies are needed to check on dimensions. Various observations were offered from amongst those present - that glutaraldehyde prefixation gives very similar results to para-formaldehyde-glutaraldehyde mixtures (when the same cell wall is compared (HUGHES)); that freeze-substituted plasmodesmata are very like chemically-fixed ones (BROWNING); that fixatives incorporating tannic acid enhance certain aspects of ultrastructural detail (ROBARDS, Fig. 2.4.). It was also noted that Roland (1973) and Vian and Rougier (1974) have published a micrograph[1] of a cryosection of *unfixed* tissue showing plasmodesmata containing desmotubules; and that the cryosections of *fixed* material

[1] The same micrograph appears in both papers.

illustrated by Vian and Rougier (1974) also show desmotubules (though very narrow ones), neck constrictions, and tripartite plasmalemma. ROBARDS confirmed for QUAIL that the plasmalemma of the plasmodesma does stain with the phosphotungstic acid-chromic acid 'Roland' procedure (see Roland and Vian, 1971), and also with the periodic acid-thiocarbohydrazide-silver proteinate Thiéry procedure (Vian and Roland, 1972) and GUNNING reported a personal communication from Dr. M.E. McCully stating that it also stains like the rest of the plasmalemma when periodic acid - thiosemicarbazide - osmium vapour is used.

MARCHANT asked whether low temperature treatment or treatment with drugs such as colchicine, which disrupt microtubules, have any effect on the desmotubule. ROBARDS commented that, while both colchicine and vinblastine sulphate had been used, no clear effect on plasmodesmatal structure had been established. He added the caution that the effect might be quite different within the confined space of the plasmodesmatal canal, likening the situation to flagellar protein tubules which are relatively resistant to such disruptive agents.

There was considerable debate between the ultrastructuralists and the biophysicists about the structural complexity of plasmodesmata as compared with the relative simplicity of the models that have thus far been used. Are the complications really necessary? GOODWIN stressed that he had not seen a good longitudinal section showing a clear opening through a desmotubule. ROBARDS explained the technical difficulties of cutting sections sufficiently thin to reveal any such detail. He replied to GOODCHILD that stereo-pairs probably would not overcome the difficulty. Since good longitudinal views cannot be obtained, both the lumen of the desmotubule and the central rod remain of questionable reality. There was a pause while GIBBS, who had suggested that the central rod should be re-named a 'desmoquark', was ejected, whereupon GOODCHILD announced his desire to play devil's advocate. He argued that glutaraldehyde, when applied to a streaming cell, can bring about very dramatic changes in the cytoplasm and organelles as seen in the light microscope: hence what reason is there to be sure that the fixative, which might well move through the plasmodesmata as it penetrates pieces of tissue, does not modify or create the central rod or even the desmotubule? ROBARDS reiterated that the desmotubule appears after various fixation procedures - osmium tetroxide, aldehyde-osmium, and even permanganate - and best of all, it appears in unfixed cryosectioned material. It is not present in all plasmodesmata (see text), but all in all there is no more reason to suppose it to be an artifact than in the case of the endoplasmic reticulum in general. ROBARDS pointed to the widespread occurrence of images illustrating a central rod, but agreed that very little is known about it, or whether it is a real structure at all. A case could reasonably be made that it is an artifact of staining (Robards, 1971), but there is at present little evidence to support such an idea. A detailed study of this feature is needed.

VAN STEVENINCK pointed out that several French botanists had tried the technique of Golgi, impregnating tissues for long periods with osmium tetroxide or permanganate solutions at elevated temperatures. Cisternae of endoplasmic reticulum and dictyosomes seem to fill up with electron-opaque deposits, and claims of continuity from cell to cell had been made. The claims were perhaps somewhat premature, for example Benbadis, Lasselain and Deysson (1973) include one micrograph showing deposits in cisternae on either side of a cell plate, but not traversing it, and Chardard (1973) and Poux (1973) show impregnated cisternae on either side of cell walls, but the plasmodesmata between them are very much less electron-opaque. The technique had not, however, been applied specifically to reveal plasmodesmatal ultrastructure, and

should be tried with this in mind. GUNNING added that the impregnation method, particularly in conjunction with high voltage electron microscopy, had shown that networks of extremely fine tubes are common between the endoplasmic reticulum and the forming faces of dictyosomes, the point being that the desmotubule is no longer an isolated, and hence especially suspect, example of a narrow tube in connection with conventional membranes.

Two experimental approaches to the controversy emerged. Measurements of the electrical resistance of cell junctions were, said GUNNING, acceptable to all as a means of assaying symplastic continuity. Would it not be possible to monitor the conductivity while glutaraldehyde was applied, thereby checking on whether the fixative introduces any marked changes as it cross-links the constituents to produce the structures that the electron microscopist sees. It was agreed that this experiment should be attempted. The other approach stemmed from the data on plasmodesmatal frequency that had been cited by ROBARDS. GRESSEL asked what happened to the vast numbers of plasmodesmata when cells are broken, as during biochemical isolation procedures? JONES thought that the plasmodesmata would pinch off and stay within the wall, quoting the work of Burgess (1971) on plasmolysed cells in support. GUNNING too had seen isolated plasmodesmata, complete with desmotubules, in walls between severely plasmolysed protoplasts. There was general optimism that it should be possible to prepare cell walls and to isolate plasmodesmata from them; further details of ultrastructure might then be open to study.