

## **Stomata and Plasmodesmata**

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### **Summary**

In developing epidermal tissue of *Phaseolus vulgare* L. complete plasmodesmatal connections occurred between guard cells and epidermal cells and between sister guard cells of a stoma but they were not seen in fully differentiated tissue. However, incomplete, aborted plasmodesmata were occasionally seen in the common guard/epidermal cell wall, usually connected to the epidermal cell protoplast, in mature tissue. Plasmodesmatal connections between neighbouring epidermal cells were commonly observed in tissue at all stages of development. In all locations, the plasmodesmata were usually unbranched occurring singly or in small pit fields; very rarely branched, incomplete plasmodesmata were also seen in the wall between mature guard and epidermal cells. The significance of these findings were related to stomatal functioning and to the development of plasmodesmata in general.

*Keywords:* *Phaseolus vulgare*; Plasmodesmata; Stomata.

### **1. Introduction**

The presence or absence of plasmodesmata in the wall between the guard cells and the neighbouring epidermal cells remains a controversial subject. The majority of light microscope studies indicate that such connections exist (KOHL 1897, KUHLA 1900, GARDINER and HILL 1901, KIENITZ-GERLOFF 1902, ESAU 1941, SIEVERS 1959, FRANKE 1962 a, LITZ and KIMMINS 1968, INAMDAR 1973) though a few reports indicate they are absent (KIENITZ-GERLOFF 1891, SHEFFIELD 1936, SCHUMACHER 1942). At the electron microscope level, however, where the identification of plasmodesmata is less equivocal, there is ample evidence of plasmodesmatal connections between immature guard cells and neighbouring cells (KAUFMAN *et al.* 1970, SINGH and SRIVASTAVA 1973, PALEVITZ and HEPLER 1974, 1976, RUTTER and WILLMER 1979). In fully developed epidermal tissue, however, virtually all studies have been unable

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to detect plasmodesmata between guard cells and neighbouring cells (BROWN and JOHNSTON 1962, MIROSLAVOV 1966, THOMSON and DE JOURNETT 1970, SRIVASTAVA and SINGH 1972, SINGH and SRIVASTAVA 1973, ZIEGLER *et al.* 1974, ALLAWAY and SETTERFIELD 1972, GALATIS 1977, SANCHEZ 1977) even though, in some cases, deliberate efforts were made to find them. In contrast to these negative findings, PALLAS and MOLLENHAUER (1972 a, b) have reported seeing large numbers (15–20 pores  $\mu\text{m}^{-2}$ ) of plasmodesmata in the end walls of the guard cells of both *Vicia faba* and tobacco and FUJINO and JINNO (1972) also report of many plasmodesmatal connections between the inner lateral subsidiary cells and guard cells of *Commelina communis*. The observations on *V. faba* and tobacco have been described as unconvincing in the review by CARR (1976) and, unfortunately, the quality of the electron micrographs in the *Commelina* study does not allow critical evaluation.

If plasmodesmata are so rare or absent in mature guard cells, as most electron microscope studies would suggest, but relatively frequent in developing guard cells, it may be presumed that these connections break-down during maturation of the guard cells. VELA and LEE (1975) claim to have seen such incomplete plasmodesmata in the common guard/subsidiary cell wall in wheat.

In view of the importance of knowing whether intact plasmodesmata exist or not between guard cells and neighbouring cells to our understanding of how stomata function, particularly regarding solute transport between the guard cells and neighbouring cells, we have undertaken a further search for such structures in these locations. We have particularly searched for aborted and incomplete plasmodesmata in the common guard/epidermal cell wall and we have serially sectioned parts of stomata in efforts to establish such facts.

## 2. Materials and Methods

Electron microscope studies were made on leaf material from *Phaseolus vulgare* L. plants grown from seed in a John Innes potting compost No. 2. The plants were grown in a heated greenhouse so that temperatures never dropped below 12 °C in the winter months and supplementary lighting was given between October and March.

Plant material was taken from the central leaflet of the trifoliate leaves. To obtain the different stages of stomatal development leaflets of different lengths (8 mm, 15 mm, and 150 mm) were cut into 1 mm squares for fixation. The material was fixed for 2 hours at 18 °C in 3% glutaraldehyde in sodium cacodylate buffer (pH 7.2). After washing for 3 hours in several changes of the above buffer the tissue was placed in 2% osmium tetroxide and left overnight. Dehydration was carried out using an ethanol-acetone-propylene oxide series and the material was then embedded in an epon/araldite mixture. Silver sections were viewed with a JEM 100 C microscope after staining (REYNOLDS 1963).

## 3. Results

During differentiation of the protodermal tissue plasmodesmata were frequently observed linking guard mother cells with neighbouring epidermal cells and also linking epidermal cell with epidermal cell (Fig. 1). At a later stage

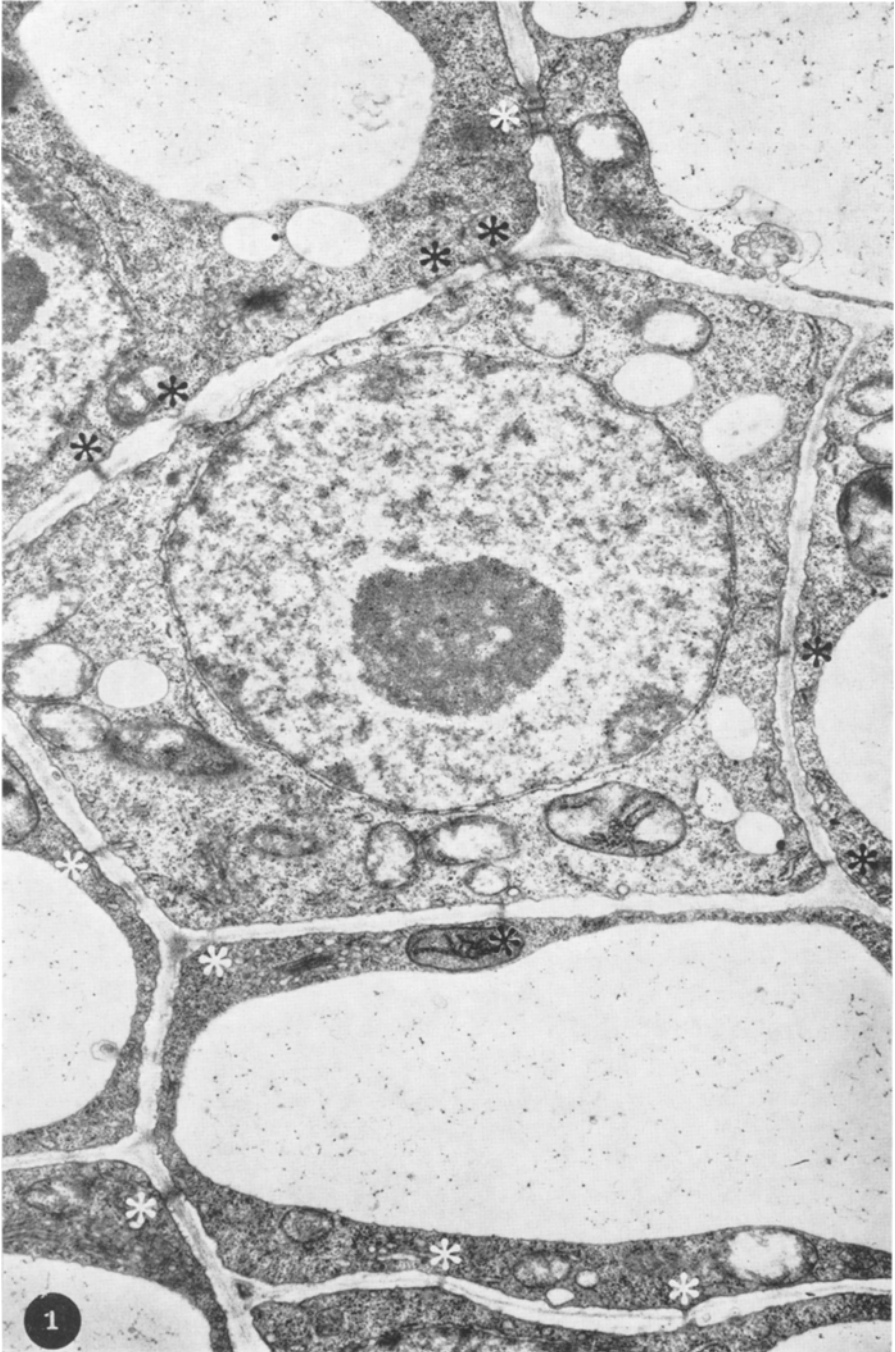


Fig. 1. A guard mother cell surrounded by vacuolated epidermal cells. Plasmodesmata (\*) occur between the guard mother cell and neighbouring epidermal cells and also between epidermal cell and epidermal cell (mag.  $\times 15,000$ )

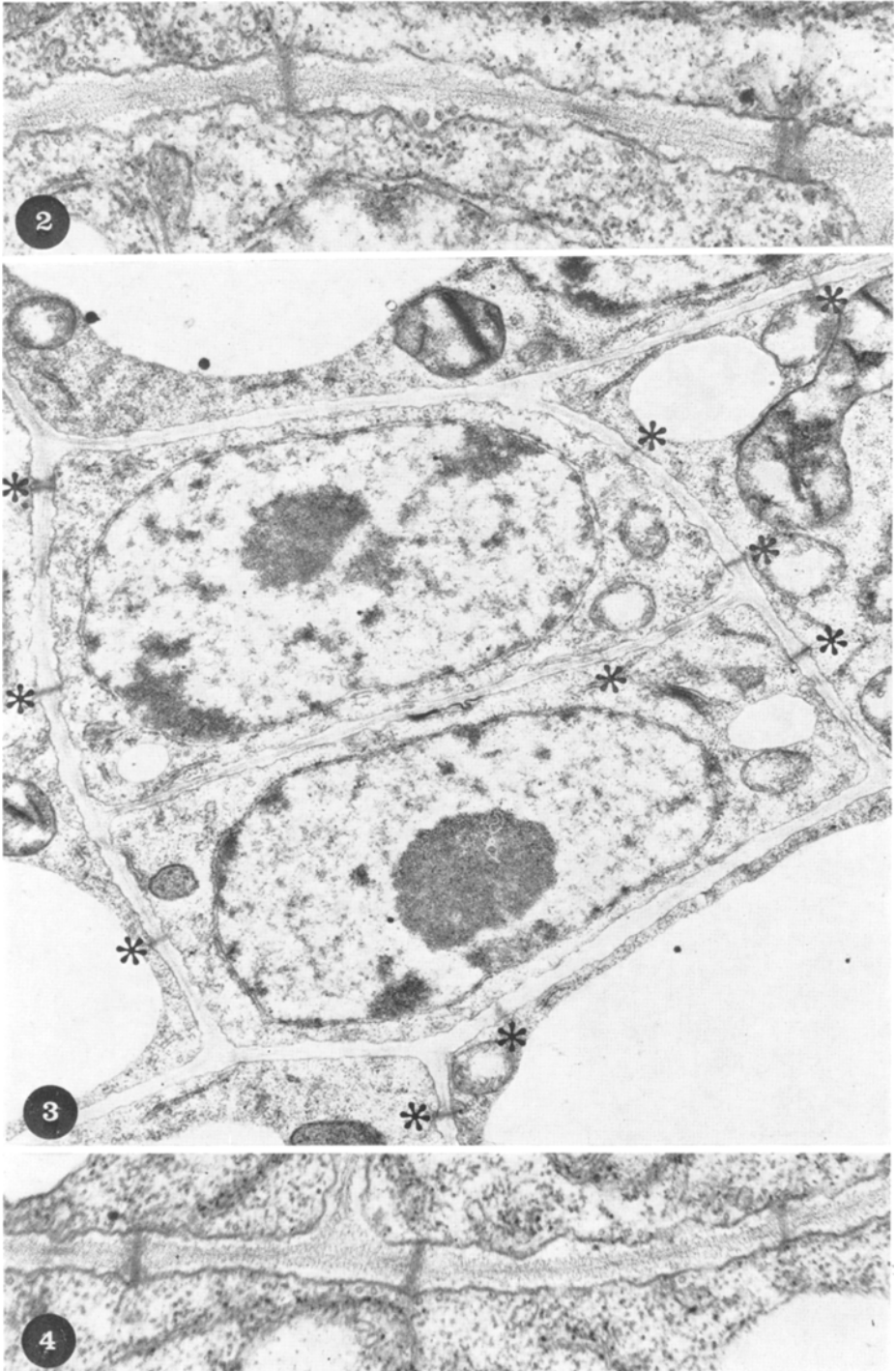
of development of the stomata plasmodesmata were also frequently seen connecting sister guard cells along their ventral walls, connecting neighbouring epidermal cells with guard cells, and connecting epidermal cell with epidermal cell (Fig. 2-4). A common location of the plasmodesmata at this stage of development of the guard cells appeared to be in their end walls (Figs. 2-4). However, this impression may result from a "dilution" effect of the plasmodesmata numbers due to the unequal expansion of the guard cell walls, the end walls expanding much less than the dorsal or ventral walls during growth. At a later stage of development still, when the pore between sister guard cells had partly formed, plasmodesmata were seen between sister guard cells and between guard cells and neighbouring epidermal cells (Figs. 5 and 6). Usually, the plasmodesmata in all the locations occurred as single, unbranched connections but on occasions sparsely populated pit-fields were seen (Figs. 1 and 2).

In mature tissue complete plasmodesmata connections between guard cells and epidermal cells were *never* seen although much effort was expended in trying to find them. However, occasionally, incomplete plasmodesmata were seen, either buried in the common guard/epidermal cell wall (Fig. 11), or penetrating a short distance from the epidermal cell side into the common wall (Figs. 7-10). The view that these plasmodesmata were incomplete connections was substantiated by serially sectioning guard cells with such areas. No convincing evidence of their continuity through the wall on the guard cell side was found though, occasionally, small inconspicuous localized disruptions of the wall microfibrils were found which might conceivably represent the remains of a disrupted canal (Fig. 7).

The incomplete plasmodesmata seen in the common guard/epidermal cell wall usually appeared to be located in a more electron-dense area of the wall and were, in several examples, connected to the epidermal cell protoplast (Figs. 7 to 10). This darker area of the wall was considered to be the remains of the initial primary wall of the developing guard and epidermal cells. The layer of wall which did not contain plasmodesmata represents the thickening applied to the inside of the guard cell wall as it reaches maturity. The branched, incomplete plasmodesmata in Fig. 11 also occur in the darker region of the guard cell wall. The wall in this region has separated along the line of the middle lamella between a guard cell and an epidermal cell apparently breaking the plasmodesmata; the gap between the two walls, therefore, would be

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Figs. 2-4. Fig. 3 (mag.  $\times 15,500$ ) is a developing stoma showing a pair of guard cells before the central pore has formed. Plasmodesmata (\*) occur between sister guard cells along their ventral walls, between guard cells and neighbouring epidermal cells, and between epidermal cell and epidermal cell. Fig. 2 (mag.  $\times 39,000$ ) and Fig. 4 (mag.  $\times 39,000$ ) are enlargements of the plasmodesmata seen in Fig. 3 which occur in the end walls of the guard cells connecting with neighbouring epidermal cells



Figs. 2-4

sub-stomatal cavity space. This process occurs during separation of young angular cells as they expand resulting in the formation of intercellular spaces.

Plasmodesmata were also observed between neighbouring epidermal cells at all stages of the development of the epidermal tissue.

#### 4. Discussion

This study supports earlier reports that complete plasmodesmatal connections occur between guard and neighbouring epidermal or subsidiary cells and between sister guard cells during the early stages of the differentiation of the epidermal tissue. However, contrary to other studies, with the exception of the unconvincing study by VELA and LEE (1975), incomplete, non-functional plasmodesmata were occasionally seen in the common guard/epidermal cell walls.

These incomplete plasmodesmata may have arisen due to deposition of wall material on the guard cell side occluding the plasmodesmata while, on the epidermal cell side, deposition being very much less significant, they remained open. The occlusion of plasmodesmata at both ends has been reported before in cells of the pigment strand in the developing wheat seed (ZEE and O'BRIEN 1970) but we are unaware of reports of plasmodesmata being occluded at one end only between living cells.

During stomatal opening, guard cells of some species bulge into the neighbouring cells, the dorsal cell wall stretching considerably (increases in the length of the dorsal wall of the guard cell of 10% or greater have been recorded (MEIDNER and WILLMER 1975), under the increasing turgor pressure. Also, in some species the guard cells change their alignment in the epidermis moving slightly above or below the surface of the leaf during opening and closing movements (SCHWENDENER 1881). Such guard cell movements with accompanying wall stretching are likely to damage such delicate structures as plasmodesmata and we consider this another reason for believing that cytoplasmic connections break down between the guard cells and neighbouring cells.

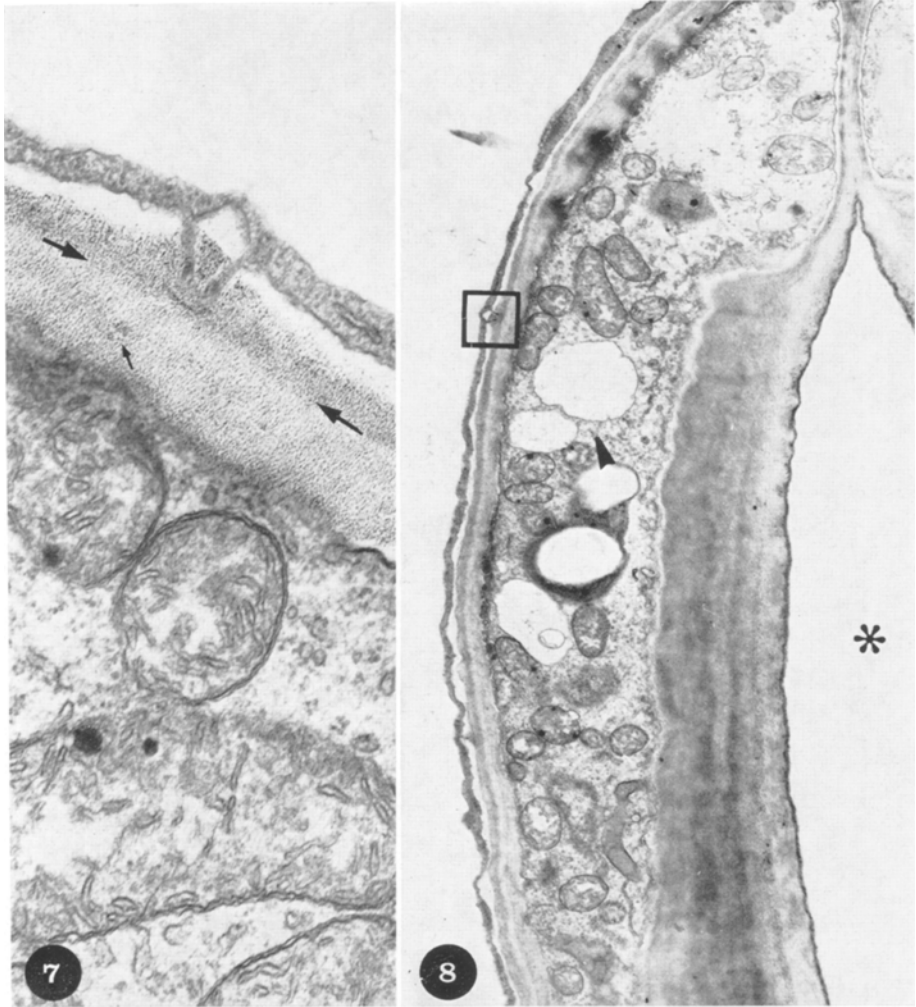
Curiously, we have found no intact, branched plasmodesmata at any stage in the development of the epidermal tissue although incomplete ones were found in mature guard/epidermal cell walls. It is interesting to note, therefore, that

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Figs. 5 and 6. Fig. 5 (mag.  $\times 12,500$ ) shows a developing stoma in which the central pore (large arrow) is beginning to form. Plasmodesmata (\*) occur between sister guard cells in their ventral walls and between guard cells and epidermal cells. Dictyosomes (*d*) are still abundant within the guard cells at this stage of development and numerous vesicles (small arrows) are located around the periphery of the developing cell wall suggesting an active synthesis and deposition of wall material. Fig. 6 (mag.  $\times 35,500$ ) is an enlargement of the plasmodesmata seen in Fig. 5 which connect the guard cell with an epidermal cell



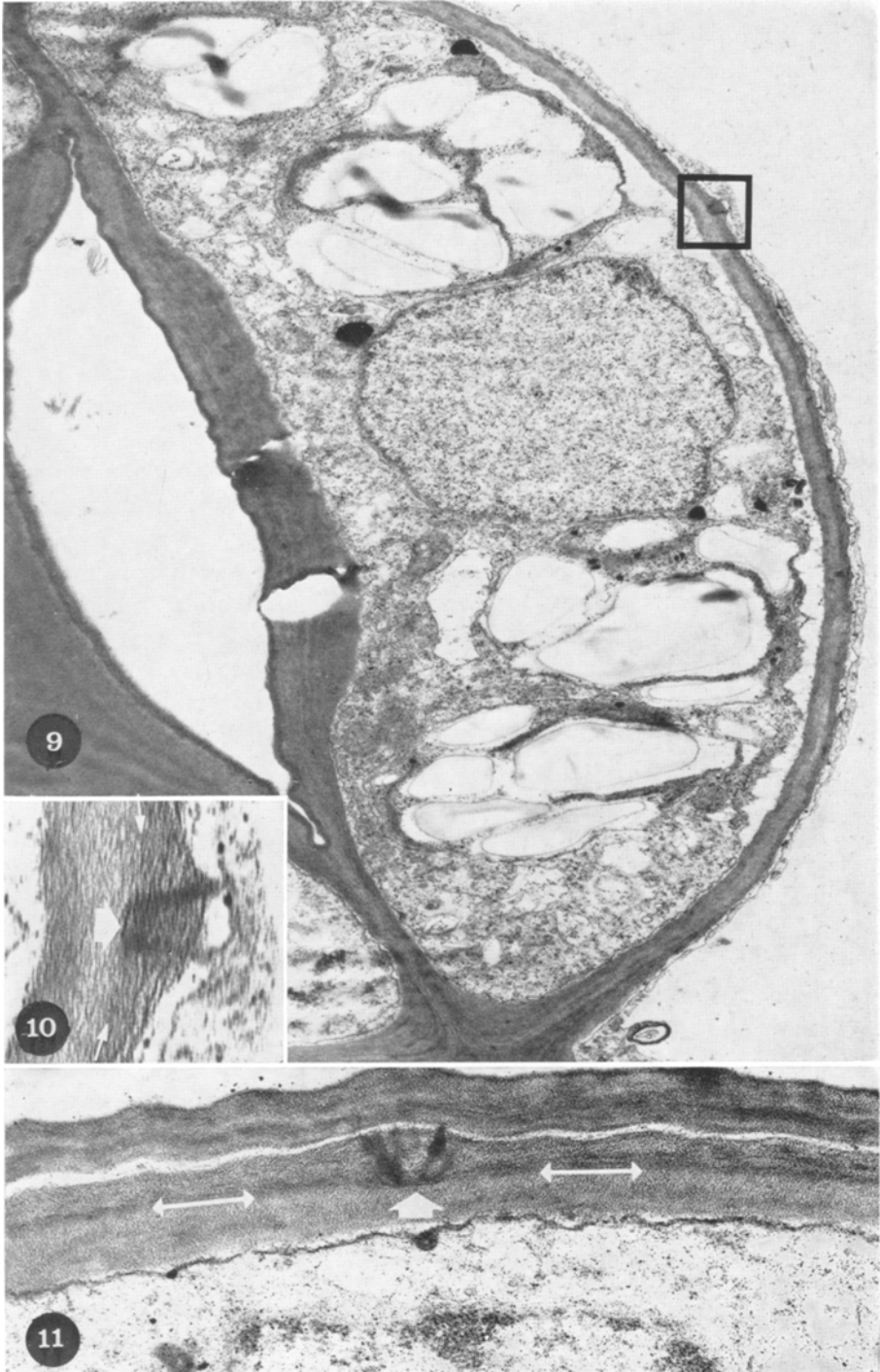
Figs. 5 and 6



Figs. 7 and 8. Fig. 8 (mag.  $\times 8,000$ ) shows part of a fully developed stoma with an open pore (\*). The incomplete plasmodesmata in the enclosed area in Fig. 8 are enlarged and shown in Fig. 7 (mag.  $\times 45,000$ ). The large arrows indicate what is considered to be the border between regions of initially deposited wall material (darker area) and of wall material deposited later, on the inside of the guard cells. The small arrow shows a small disruption of the wall material which is thought to be the remains of an aborted plasmodesmatal canal

Figs. 9–11. Fig. 9 (mag.  $\times 10,000$ ) shows a fully developed stoma with incomplete plasmodesmatal connections between a guard cell and a neighbouring epidermal cell. The enclosed area in Fig. 9 is enlarged in Fig. 10 (mag.  $\times 50,000$ ) to show the incomplete plasmodesmata (large arrow) in more detail. Fig. 11 (mag.  $\times 24,000$ ) shows incomplete plasmodesmata embedded in the common wall between a guard cell and an epidermal cell. The small arrows in Fig. 10 and 11 indicate what is considered to be the border between regions of initially deposited wall material (darker area) and of wall material deposited later





Figs. 9-11

PALLAS and MOLLENHAUER 1972 a, b) found branched plasmodesmata in pit fields with no restriction of wall thickening in these regions. We wonder if their observations represent this intermediate stage of plasmodesmata development.

Light microscope studies have frequently concluded that plasmodesmata exist between mature guard and epidermal or subsidiary cells while electron microscope studies are almost unanimous in concluding that they are absent. One possible explanation for this divergence of opinion is that the histochemical tests used to detect plasmodesmata at the light microscope level may give positive reactions to aborted plasmodesmata and even to weakened or structurally different areas of wall material. Indeed, using light microscopy and histochemical tests it was believed at one time that ectodesmata existed as fingers of cytoplasm extending through the epidermal, subsidiary and guard cell walls penetrating to the outer cuticular layer of the leaf (FRANKE 1962 b). Many now consider that such observations were artifacts and that the ectodesmata really consisted of regions of differently structured, or more permeable wall material.

If functional plasmodesmatal connections are absent between guard and epidermal cells important questions concerning the metabolism of guard cells and the normal functioning of stomata are raised. It is considered that metabolites must be transported from the mesophyll to the guard cells to maintain a carbon balance since photosynthesis appears to be absent from the guard cells (RASCHKE and DITTRICH 1977). Also, it has been suggested that malic acid leaves the guard cells upon stomatal closure (BOWLING 1976, DITTRICH and RASCHKE 1977). However, in the absence of plasmodesmata between guard cells and neighbouring cells and in the absence of features such as transfer cells or extensive membrane convolutions which would increase the speed and efficiency of metabolite transport, it must be assumed that such transport to and from the guard cells is not essential for stomatal functioning. Possibly a carbon supply from the abundant source of guard cell starch (except in the *Allium* genus) can buffer any short-term demand for carbon by the guard cells.

The absence of plasmodesmata connecting guard cells with neighbouring cells would place the guard cells in a special category; few types of mature, living cells do not possess plasmodesmatal connections and those cells which are reported not to have them (see ROBARDS 1976, p. 18) appear to be related to reproductive processes in which, in many cases, a separation of genetic material is necessary. The need for the "isolation" of the guard cells from the main body of the plant is more difficult to envisage particularly in view of the need for the transport of metabolites into and out of the guard cells. One possibility is that their "isolation" may be necessary for stomata to respond rapidly to changing environmental factors such as light intensity, humidity or CO<sub>2</sub> concentrations, though it is difficult to envisage exactly

why this should be so. It is also interesting to note that guard cells are the only cells we know of which can sense changes in the concentrations of  $\text{CO}_2$  within their immediate environment and react rapidly to these changes with a resulting opening or closing of stomata. Moreover, guard cells can sense very small changes in  $\text{CO}_2$  concentrations around atmospheric levels (approximately  $320 \mu\text{l l}^{-1} \text{CO}_2$ ). Thus, the functional significance of the lack of plasmodesmatal connections to guard cells remains to be established.

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