PREPROPHASE MICROTUBULES AND STOMATAL DIFFERENTIATION IN *COMMELINA CYANEA*

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Summary

The relationship between preprophase microtubules and asymmetrical cell division in the formation of the stomatal complex of *C. cyanea* was investigated. Polarization of nuclei and other cell organelles adjacent to the guard mother cell occurred in most cases without a preprophase band of microtubules being present; the grouping of preprophase microtubules appeared immediately prior to cell division, and its situation, even during abnormal stomatal development, predicted the plane of future division. The results show that preprophase microtubules cannot be the cytoplasmic agents involved in orienting and positioning the nucleus prior to division. Clear evidence was obtained indicating that preprophase microtubules move intact into the spindle. Some aspects of abnormal stomatal development are discussed, and the results are related to some other work on stomatal differentiation.

I. INTRODUCTION

During investigations into the fine structure of dividing cells of wheat, Pickett-Heaps and Northcote (1966*a*, 1966*b*) discovered that a grouping or band of microtubules appeared near the cell wall at preprophase. This band accurately predicted the position of the future cell plate, being most conspicuous during highly asymmetrical cell divisions that formed the stomatal complex in the epidermis of the young leaf. Burgess and Northcote (1967) confirmed the existence of this preprophase band of microtubules in *Phleum* (and in pea) root tips, but they found that in the former asymmetrical division could follow a symmetrical disposition of the band in the cell. They proposed that the band was functional in orienting or positioning the uucleus prior to mitosis. More recently, Cronshaw and Esau (1968) described a similar band in premitotic cells of *Nicotiana* leaves.

Pickett-Heaps (1969*a*) demonstrated by use of caffeine treatment on wheat seedlings that the number and position of microtubule band(s) in binucleate or polyploid cells was not related to the number, size, and disposition of nuclei; the band continued to predict the asymmetrical plane of division during stomatal differentiation even if a previous attempt at that particular division had been unsuccessful. Later, Pickett-Heaps (1969*b*) described the effects of centrifugation on differentiating stomatal cell complexes in wheat. In particular, preprophase nuclei at the second asymmetrical division were found to be roughly equivalent to other phases of mitosis in their resistance to centrifugal sedimentation. It was concluded that the preprophase band was not specifically involved in orienting and holding the nucleus against the guard mother cell. Furthermore, evidence was obtained which confirmed a strong

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impression that these preprophase microtubules moved *intact* into the prophase spindle.

In order to extend these observations on the relationship between preprophase microtubules and asymmetrical cell division, a fine structural investigation into formation of stomata in *Commelina cyanea* has been undertaken. These stomata are very complex, since the mature guard cells are flanked by a total of six subsidiary cells, four of these being lateral subsidiary cells and the other two terminal subsidiary cells (Fig. 13). The formation of this complex has been described with the light microscope by Stebbins and Jain (1960).

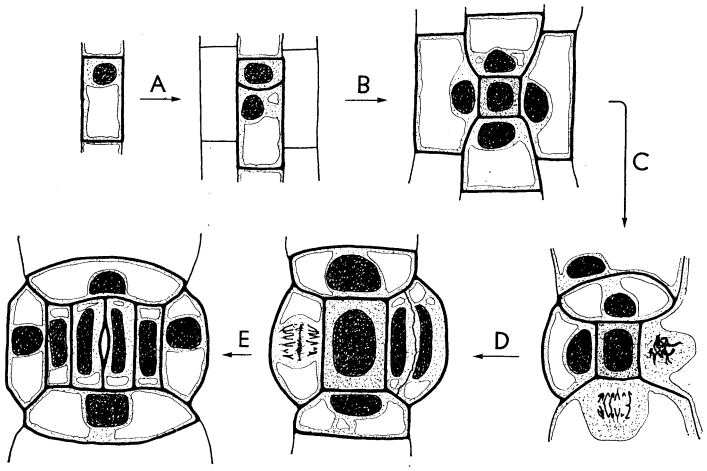

Fig. 1.—Diagrammatic representation of formation of stomatal complex in *C. cyanea*. Polarized epidermal cell divides asymmetrically (*A*) to give small guard mother cell. Nuclei of adjacent epidermal cells become polarized adjacent to guard mother cell (*B*); they then divide asymmetrically (*C*), forming both lateral and terminal subsidiary cells. The lateral subsidiary cells then divide again asymmetrically (*D*). At some stage during these events, the central guard mother cell also divides symmetrically (*E*), forming the guard cells. The wall between the guard cells splits to give the stomatal pore.

II. Materials and Methods

Specimens of *C. cyanea* were originally collected from near Grafton, N.S.W., by Mr. R. R. Willing of the Botany School, Australian National University, who very kindly made available some cuttings. These were placed in damp earth adjacent to a north-facing window, and soon exhibited vigorous growth.

Young leaves were cut into 1-mm segments and placed into 6% glutaraldehyde buffered to pH 7 with 0·025M phosphate buffer containing 0·002M calcium chloride, at room temperature. After 1–24 hr, the segments were well washed with buffer, and postfixed in 1% osmium tetroxide

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Fig. 4.—Light micrograph; lateral divisions. Note orientation of cell plate (top arrows) and metaphase plate of chromosomes (bottom arrows), in association with skew end walls of the cells —cf. Figures 14(*a*), 15, and 16. ×1225. *g*, guard mother cell; *pt*, polarized terminal epidermal cell.

Fig. 5.—Light micrograph; paired chromosomes of metaphase plate (terminal division) visible; first lateral divisions completed. Round, staining objects (top corner) are due to a characteristic exudate on the leaf surface. ×960. *l*, lateral subsidiary cell; *g*, guard mother cell.

Fig. 6.—Light micrograph; guard mother cell (*g*) on right has two terminal subsidiary cells (*t*) and each of its lateral subsidiary cells has also divided (l_1, l_2). The guard mother cell on the left has the lateral subsidiary cell (*l*) undivided at this stage; epidermal cell nucleus (arrow) is at prophase, having already supplied one terminal subsidiary cell to the other guard mother cell. ×1190.

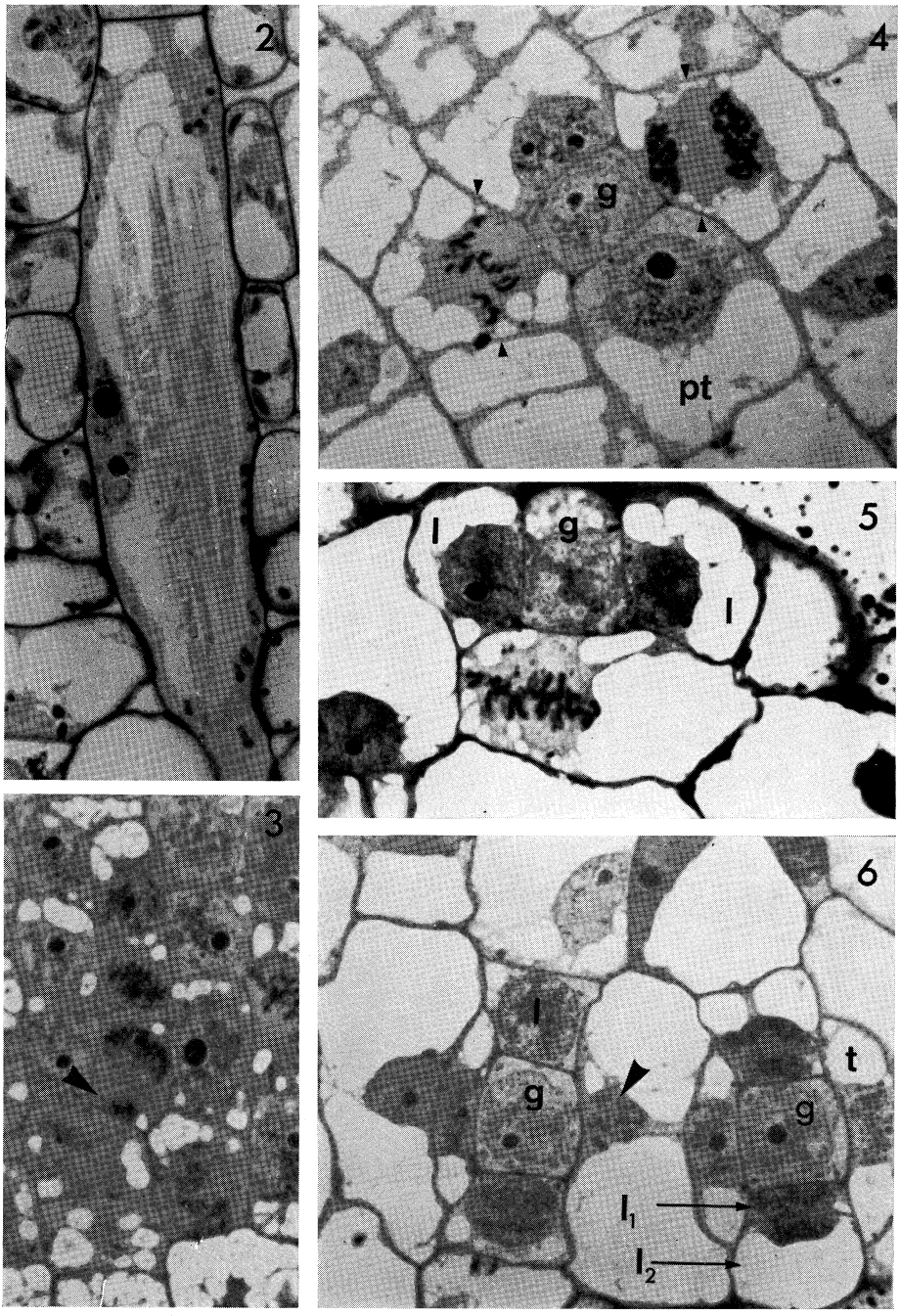

Fig. 2.—Light micrograph; elongated, crystal-containing cell lying under the epidermis. These crystals were very hard, invariably damaging the knife edge, which resulted in severe knife marks on the sections (e.g. Figs. 15 and 16). ×960. Note.—All micrographs are in longitudinal section, in the plane of the leaf surface.

Fig. 3.—Light micrograph; first asymmetrical division (Fig. 1, step *A*); the small cell (arrow) was a guard mother cell, or possibly an epidermal hair cell. ×960.

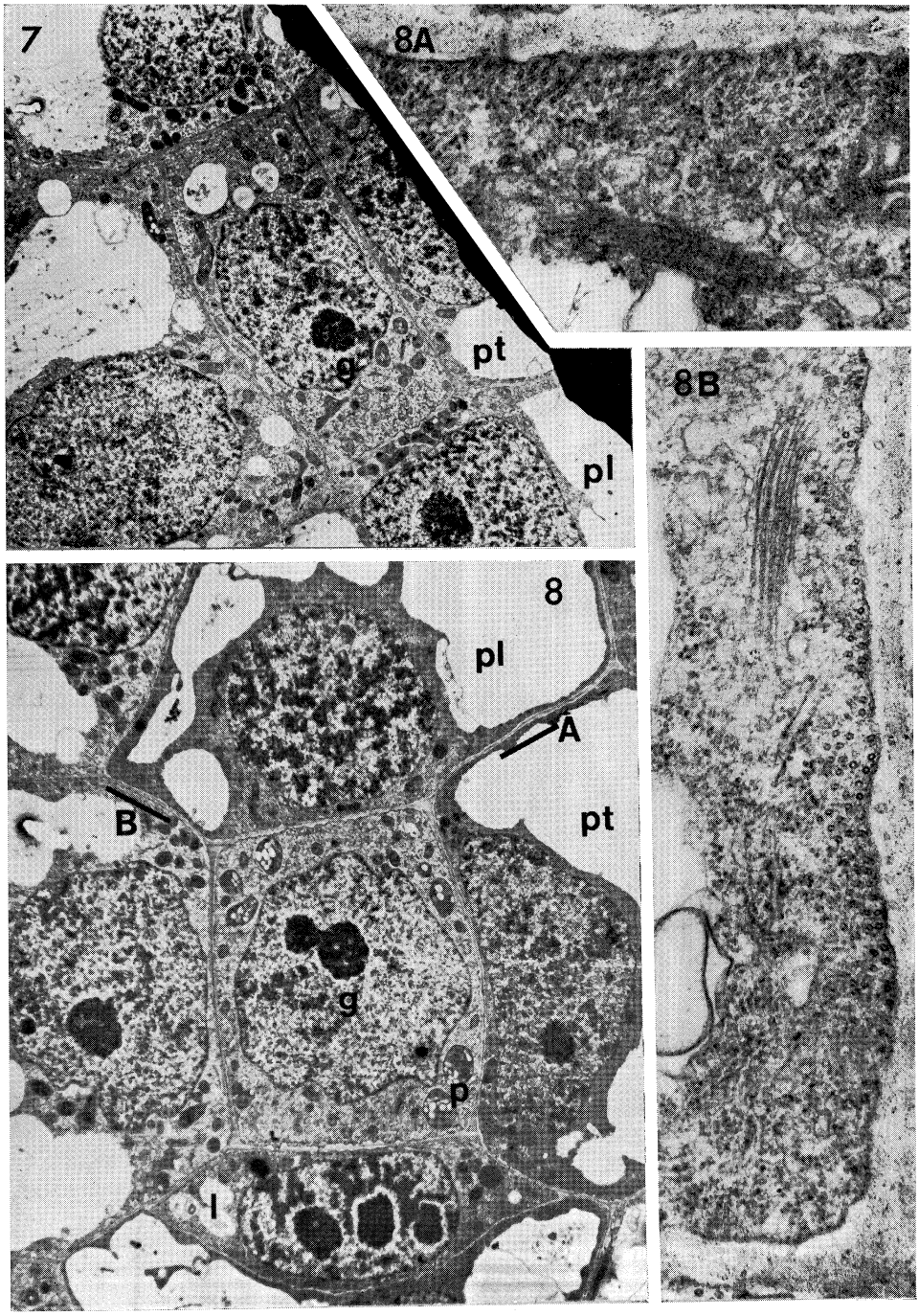

Fig. 7.—Typical group of polarized epidermal nuclei and cell organelles around the guard mother cell *g* (cf. Fig. 1, step *B*). *No* preprophase microtubules were present in these or most other such epidermal cells. Polarized terminal (*pt*) and lateral (*pl*) epidermal cells are indicated. ×3400.
Fig. 8.—Lateral subsidiary cell formation; top polarized epidermal cell (*pl*) is entering prophase. Preprophase band of microtubules at *A*, *B*. [In this and subsequent figures, and in accordance

buffered with veronal acetate for 1 hr, also at room temperature. Washing, dehydration, and embedding in Araldite followed as usual. Sections were cut on glass knives, stained with uranyl acetate and lead tartrate, and examined in a Phillips EM 200 or an Hitachi HU 11E electron microscope.

For light microscopy, thick ("blue-green") sections, cut adjacent to thin sections, were transferred to a drop of water on a clean slide. The drop was evaporated by gentle heating, and the sections stained for a few seconds with hot 1% toluidine blue in 1% borax solution, then washed, dried, mounted, and photographed as required.

III. Observations

The progression of events that are observed in the formation of the stomatal complex in *C. cyanea* are illustrated diagrammatically in Figure 1. Polarization of an epidermal cell nucleus results in asymmetrical cell division (Fig. 1, step *A*), the smaller cell becoming the guard mother cell. During further growth, the nuclei in the cells on all four sides adjacent to the guard mother cell then become polarized (Fig. 1, step *B*), a situation which persists for some time judging from the large number of complexes seen at this stage [see Section III(*b*)]. All four surrounding epidermal nuclei divide highly asymmetrically at various times (Fig. 1, step *C*); then each lateral subsidiary cell divides again (Fig. 1, step *D*); this division is also asymmetrical. The guard mother cell also divides at about this stage (Fig. 1, step *E*) to give the two future guard cells. The wall between the guard cells later splits to give the stomatal pore.

Some variability was encountered in the proportion of dividing cells in different blocks of tissues. In differentiating stomatal complexes, for instance, some sections revealed very few dividing epidermal cells, whilst in sections from other blocks many stomatal complexes contained dividing cells. This was probably related to the times of fixation (i.e. diurnal variation in mitotic rhythms).

One serious problem was invariably encountered when sectioning the material of *C. cyanea*, due to the presence of very hard crystals (oxalate?) in the vacuoles of most cells (e.g. Figs. 10, 13, 16, 17); these were generally small, but certain clearly defined cells also contained much larger crystals (Fig. 2). The crystals, often apparent as holes in the section (e.g. Figs. 13, 16), always caused damage of the knife edge, leading to knife marks on the sections which was often serious (e.g. Figs. 15, 16). Drastic measures (including surgery of the block face to remove the big crystal-containing cells) alleviated the situation to some extent.

(*a*) *Formation of the Guard Mother Cell* (*Fig. 3*)

Few stages of the first asymmetrical division (Fig. 1, step *A*) have been found. This cell division took place in a very limited region of tissue in the author's material (Fig. 3) and so observations on mitosis were few. However, one preprophase nucleus

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with a convention previously adopted (Pickett-Heaps 1969*a*, 1969*b*), black bars are placed on the opposite side of the wall to the region occupied by the preprophase band of microtubules; when the bars are labelled ("*A*" etc.), the region is shown in more detail in the following micrograph(s).] Other lateral subsidiary cell (*l*) already formed. Differentiating plastids (*p*) in guard mother cell. ×3950.

Figs. 8*A*, 8*B*.—Preprophase microtubules along wall at *A*, *B* in Figure 8. These micrographs were actually taken from serial sections of the cell in Figure 8. ×52,500.

which was eventually found did have preprophase microtubules where expected (i.e. encircling the polarized nucleus, predicting the future plane of division). The general pattern of epidermal differentiation at this stage was much less clearly defined (Fig. 3) than is seen, for example, in wheat, where files of cells contain a fairly regular alternation of guard mother cells (or future epidermal hair cells) and epidermal cells. In *C. cyanea* these cells, easily distinguishable from epidermal cells by their much smaller size and lack of vacuoles, appeared in a somewhat random distribution along cell rows.

(*b*) *Terminal and First Lateral Division* (*Figs. 4, 5, and 6*)

Many cell complexes were found with all four epidermal cell nuclei closely appressed to the guard mother cell (Fig. 7), the frequency indicating that this configuration is maintained for some time. Many plastids and some mitochondria were also generally gathered adjacent to the guard mother cell (Fig. 7 and others).

These polarized cells were closely examined for preprophase microtubules. At first sight, microtubule bands were clearly *not* present since the microtubules were distributed along the cell wall in the normal fashion. Indeed, first impressions suggested that no preprophase band of microtubules preceded these asymmetrical divisions. However, an obvious early prophase nucleus was then discovered with the band of microtubules as expected (Figs. 8, 8*A*, 8*B*). Subsequent work soon revealed many such bands and confirmed that only in a small but variable proportion of both lateral and terminal epidermal cells were they present (Figs. 8, 8*A*, 8*B*, 9, 9*A*, 9*B*). The band of microtubules predicted with considerable accuracy the plane of division; these bands were not close to the guard mother cell (as is found in wheat epidermis), and neither does the cell plate meet the older cell wall close to the guard mother cell (see Figs. 6, 8, 11, and others). The relationship between the position of preprophase microtubules and the future plane of division was also clearly seen in several epidermal cells whose end wall was diagonally oriented [Fig. 14(*a*)], where the anticipated intersecting plane of division [Figs. 14(*a*), step *B*, 16, 16*A*, 16*B*] matched that of the preprophase microtubules. This is described later.

The general appearance of the polarized epidermal cell nuclei and the absence of the microtubule band confirmed the impression that most of these should be more correctly regarded as interphase nuclei (Fig. 7 and others). The evidence strongly suggests that preprophase grouping occurs only just before mitosis, and is therefore *not* related to nuclear polarization.

In a previous paper (Pickett-Heaps 1969*b*) evidence was presented suggesting that (some) microtubules move intact from the preprophase band into the spindle. In most preprophase *C. cyanea* cells, a few profiles and groups of microtubules could be found between the preprophase band and the nucleus, suggesting a similar conclusion (as in Fig. 11*B*). Particularly in terminal divisions, a cytoplasmic strand containing these microtubules was often present between the nucleus and region near the cell wall occupied by the preprophase tubules (Figs. 9, 9*A*, 9*B*). Serial sectioning (Fig. 9*C*) confirmed the widespread distribution of microtubules around such strands.

Fig. 9*C*.—Preprophase microtubules and cytoplasmic strand of Figure 9*B*, sectioned at a different level; many microtubules obvious in the strand (arrows). ×46,500.

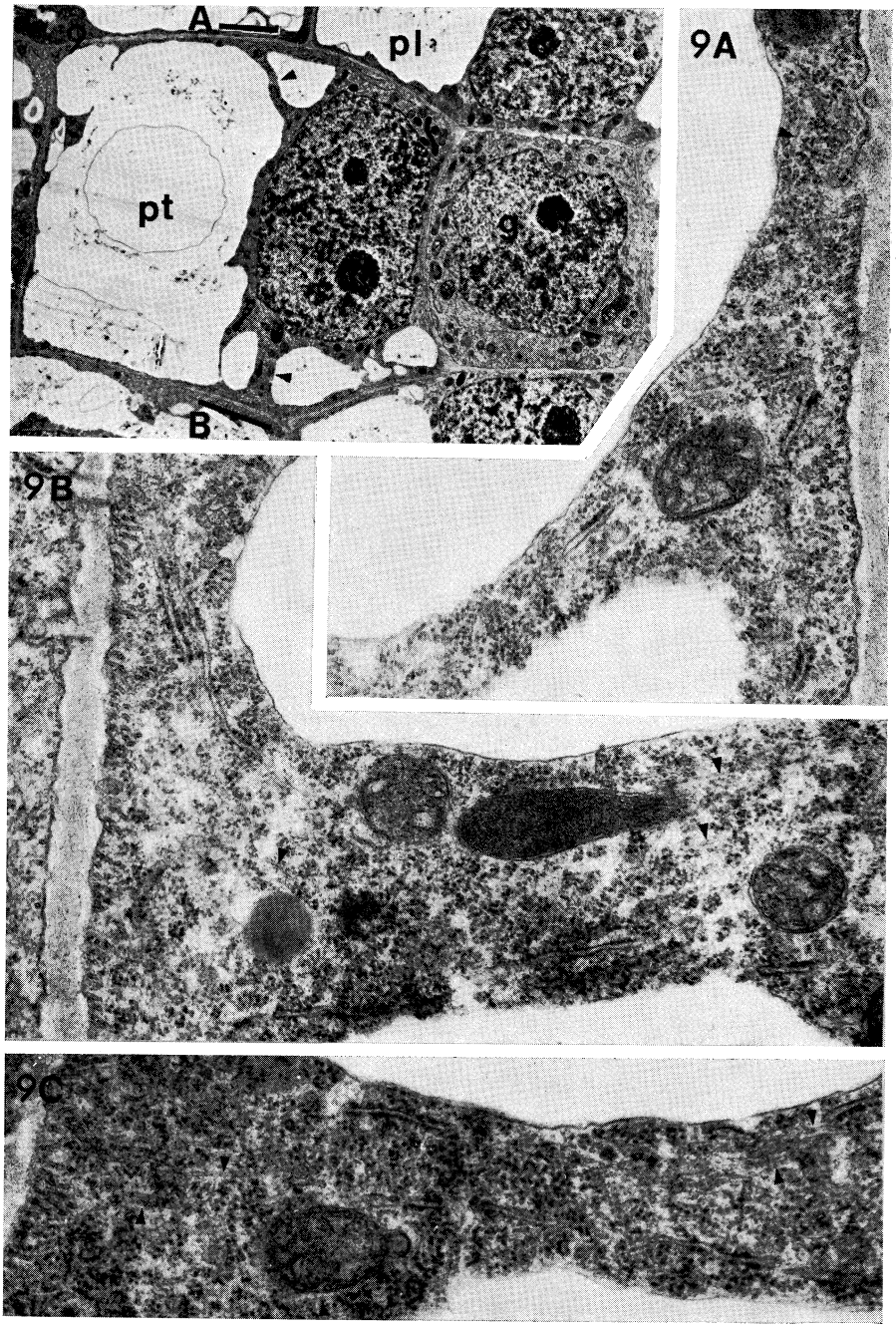

Fig. 9.—Terminal subsidiary cell formation; preprophase microtubule bands at *A*, *B*. Note cytoplasmic strands (arrows) between preprophase bands and nucleus. These frequently contained some microtubules (see Figs. 9*A*, 9*B*, 9*C*). ×2800.

Figs. 9*A*, 9*B*.—Preprophase bands of tubules from regions *A*, *B* in Figure 9. Some segments of microtubules (arrows) were almost always found away from the band, or in the cytoplasmic strand in these cases. ×39,500.

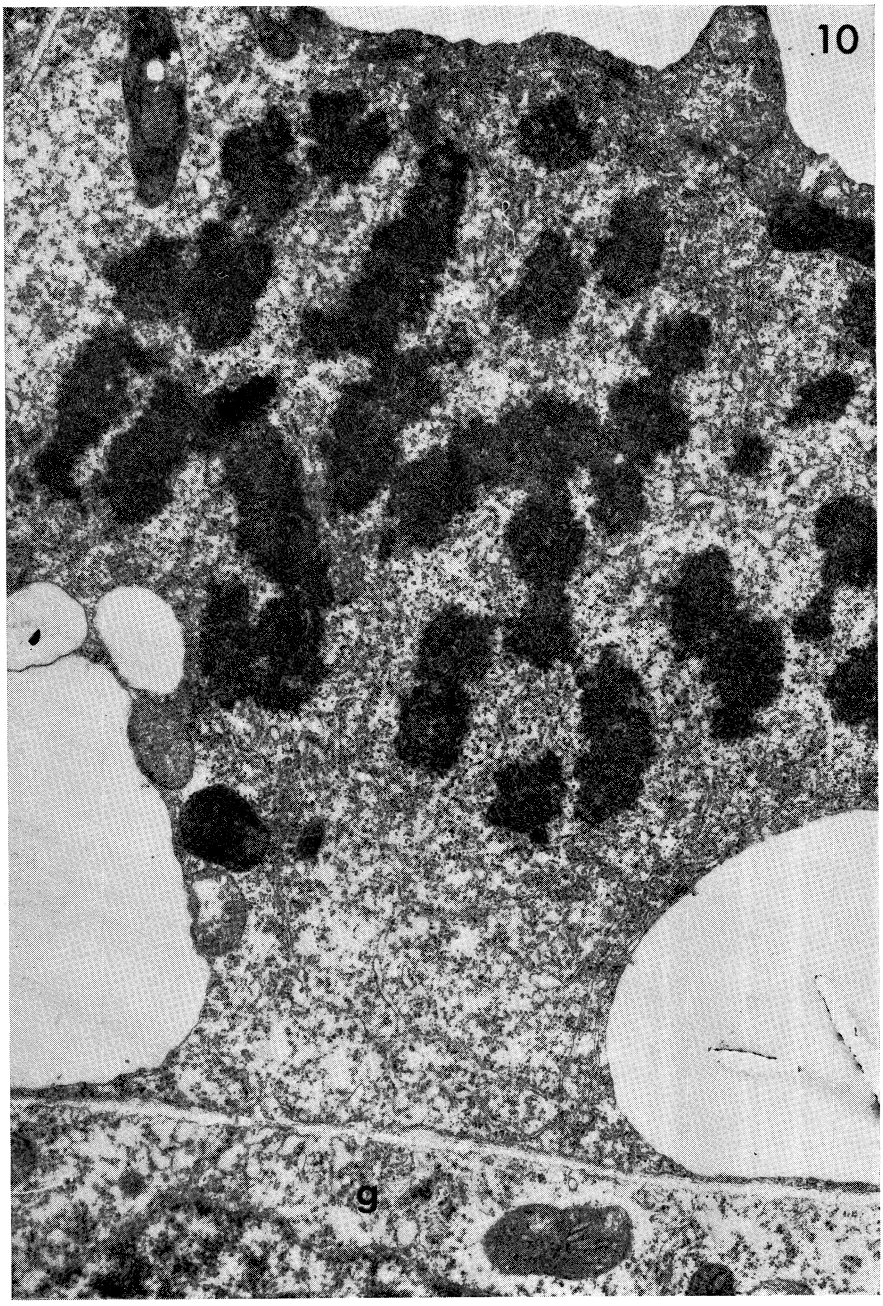

Fig. 10.—Pole, prometaphase spindle adjacent to guard mother cell *g* (terminal division). The poles of dividing cells are very firmly attached to the guard mother cell during stomatal differentiation (Pickett-Heaps 1969*b*) but there is no characteristic ultrastructural features associated with the attachment. ×14,000 approx.

Fig. 11*B*.—Preprophase microtubule band, region *B* in Figure 11. Some microtubules are also seen close to the nuclear envelope (arrows). ×37,000.

Fig. 12.—Division of guard mother cell (*g*); lateral subsidiary cells (*l*) are already formed, but no terminal subsidiary cells are yet present. ×2800.

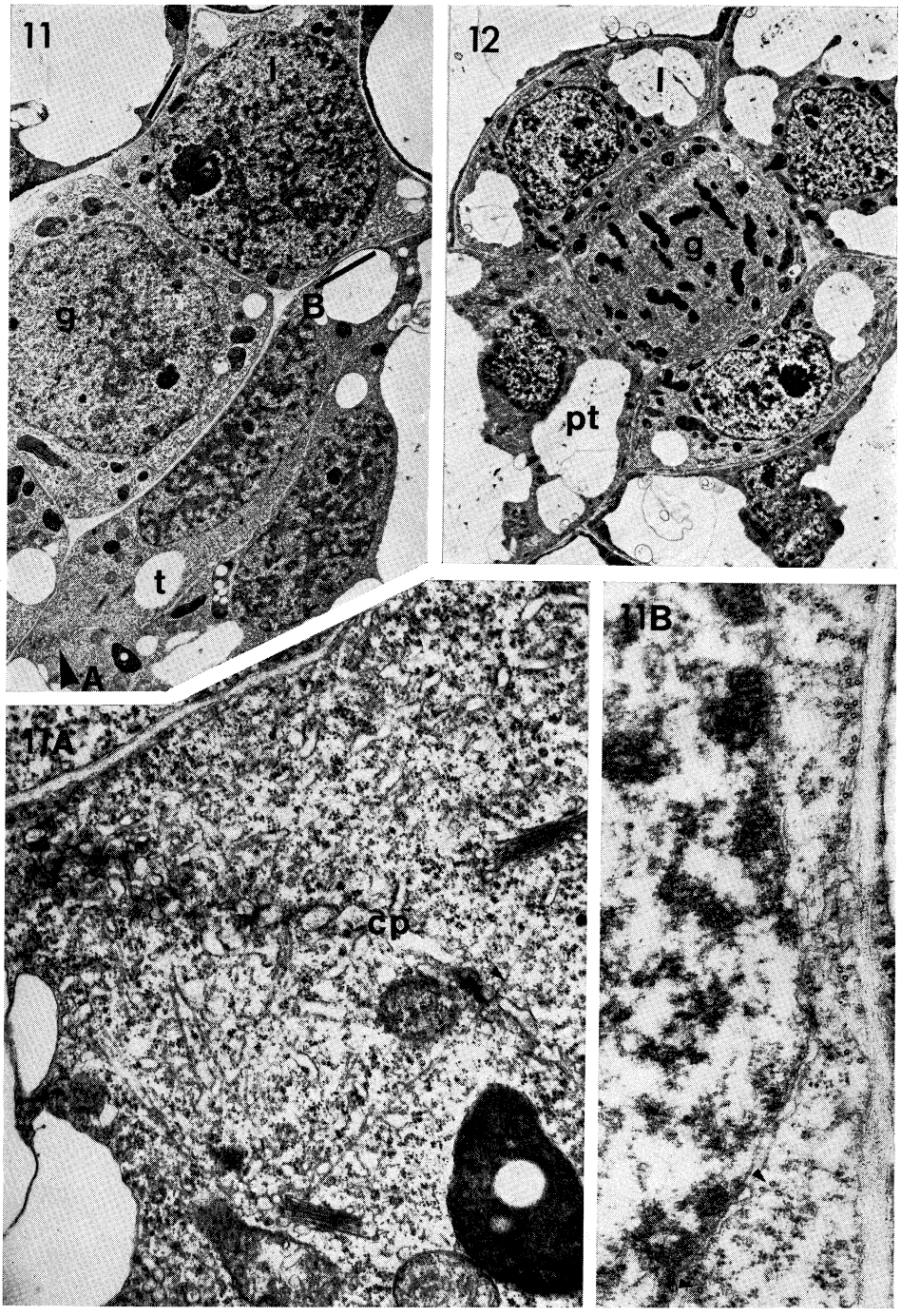

Fig. 11.—Preprophase, lateral subsidiary cell division; microtubule band found at region shown with black bars. Cell plate formation (arrow) following terminal subsidiary cell formation (*t*); phragmoplast is far removed from daughter nuclei. ×3600.

Fig. 11*A*.—Phragmoplast (region *A*, Fig. 11). Considerable degree of spatial orientation evident, although this part of the cell plate (*cp*) is far removed from the daughter nuclei. A continuous microtubule (arrow) passes through the cell plate. ×18,500.

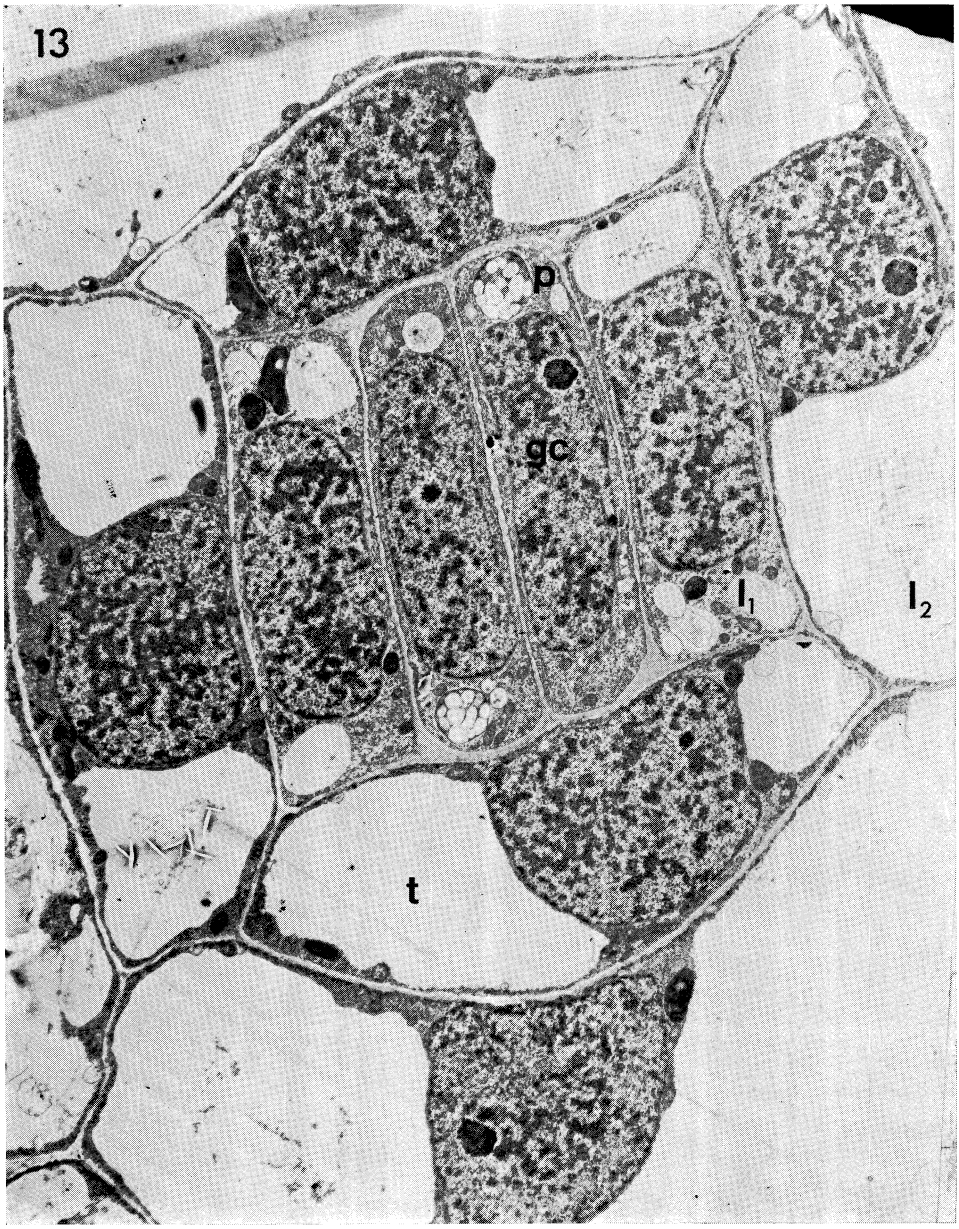

Fig. 13.—Fully formed complex of eight cells. Differentiated plastids (p) in guard cells (gc). Wall between these guard cells later splits to form stomatal pore. Compare with Figure 1. l_1, l_2, pair of lateral subsidiary cells following division; t, terminal subsidiary cell. $\times 4150$.

As is the case with many other highly polarized divisions, the spindle pole near the guard mother cell lacks any obvious ultrastructural component (Fig. 10—cf. Fig. 5) that could be related to the strength of attachment that exists between the guard mother cell and pole, and that is evident for example in centrifugation experiments (Pickett-Heaps 1969*b*).

Cell plate formation is interesting in that the edges of the new wall meet the older walls often at considerable distance from the daughter cell nuclei (Fig. 11). This region of phragmoplast, typical in all respects (Fig. 11*A*), must extend its periphery in a highly controlled manner, without necessarily being associated with the nuclear spindle apparatus.

(*c*) *The Second Lateral Division*

The lateral subsidiary cells divided a second time, generally following the formation of the young five-cell complex (Fig. 1, step *D*). Prior to division the lateral subsidiary cells are vacuolated to varying extents, but they are fairly compact in size (Figs. 6, 11). Nuclei were always polarized adjacent to the guard mother cell, even when these cells were appreciably vacuolated. As before, only a few of such polarized cells contained preprophase microtubules. However, the band of microtubules was unmistakable when present (Figs. 11, 11*B*); it predicted as usual the expected plane of division. This division is also asymmetrical, as the cell formed next to the guard mother cell is slightly smaller (Fig. 1, step *D*—cf. Figs. 6, 13).

When the cells were more vacuolated, a cytoplasmic strand was frequently observed to lie between the nucleus and wall region occupied by the preprophase microtubules. As before, the strand generally contained a few short segments of microtubules (as in Figs. 9*B*, 9*C*). Other microtubules could also be found near the nucleus (Fig. 11*B*).

(*d*) *Division of the Guard Mother Cell*

A few preprophase guard mother cells were found and these had the microtubule band oriented as expected (i.e. longitudinally); this division is symmetrical (Figs. 12, 13). Plastids in the guard mother cell differentiated early in development (e.g. Fig. 8 and others).

(*e*) *Division in Other Cells of the Leaf*

Many preprophase and mitotic cells were encountered in both the leaf epidermis and young mesophyll tissue. Typical microtubule bands were present in preprophase cells, and the position of these always coincided with the expected plane of division.

IV. Some Variations and Abnormalities in Stomatal Differentiation

(*a*) *Effect of Young Guard Mother Cell on Normal Adjacent Mitosis*

Examination of young epidermal cell complexes in some blocks of tissue revealed that an appreciable proportion of lateral epidermal cells surrounding the complex were separated by a diagonal end cell wall, which was close to the guard mother cell (Figs. 4, 16). This seemed to occur as a result of an ordinary division occurring in a cell row alongside a guard mother cell when the latter had not yet begun to exert its full influence on adjacent epidermal cells. The inferred sequence

of events is shown diagrammatically in Figure 14(*a*); an epidermal cell division produces a slightly skewed cell plate [Fig. 14(*a*), step *A*—see Fig. 15], apparently a result of spindle reorientation at telophase (Fig. 15). Subsequent asymmetrical cell division in the epidermal cell [Fig. 14(*a*), step *B*] then forms a characteristically shaped lateral subsidiary cell, triangular in profile (Figs. 4, 18).

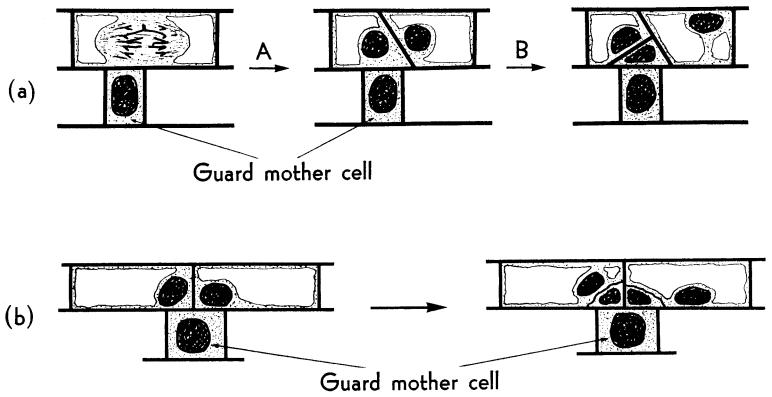

Fig. 14.—(*a*) Diagrammatic representation of effect apparently induced by the young guard mother cell on adjacent dividing epidermal cells. It appears that the cell plate in the epidermal cell is formed skew (cf. Fig. 15) because of the proximity of the guard mother cell (step *A*). Subsequent lateral subsidiary cell formation in the epidermal cell (step *B*) generally results in a cell with a triangular profile [cf. Figs. 4, 15, 16, 18 (top cell)]. (*b*) Diagrammatic representation of abnormality encountered if the wall between epidermal cells happened to lie adjacent to the guard mother cell. Both epidermal cells are then induced to divide.

Only one epidermal cell has been found at preprophase whose subsequent division might have been expected to be thus affected by the young guard mother cell. The band of microtubules in this cell did not seem to be appreciably displaced away from the usual transverse orientation. Later in the development of such a complex, the spindle is normally angled between the skew end wall and the guard mother cell wall. This is apparent, for example, from the orientation of both the metaphase chromosome plate and cell plate at telophase (Fig. 4). It is significant that in such cases, the preprophase band of microtubules also predicts such an orientation of the spindle and cell wall (Figs. 16, 16*A*, 16*B*—compare with the top lateral subsidiary cell in Fig. 18). Figure 8 seems to represent a departure from this general rule; the reason for this is not obvious, but it can be seen that the lower lateral subsidiary cell in Figure 8 has also *not* produced the triangular-shaped cell that might be expected. The departure from the general trend is at least consistent in this stomatal complex.

(*b*) *Formation of Abnormal Complexes*

On rare occasions, the wall separating epidermal cells was situated alongside the guard mother cell; both epidermal cells are then apparently induced to divide [Fig. 14(*b*)], forming a stomatal complex with a greater number of subsidiary cells than normal (i.e. six—Figs. 1–13). Two such partially formed, abnormal complexes were found in *C. cyanea* during this work. Both in terminal (Fig. 17) and lateral

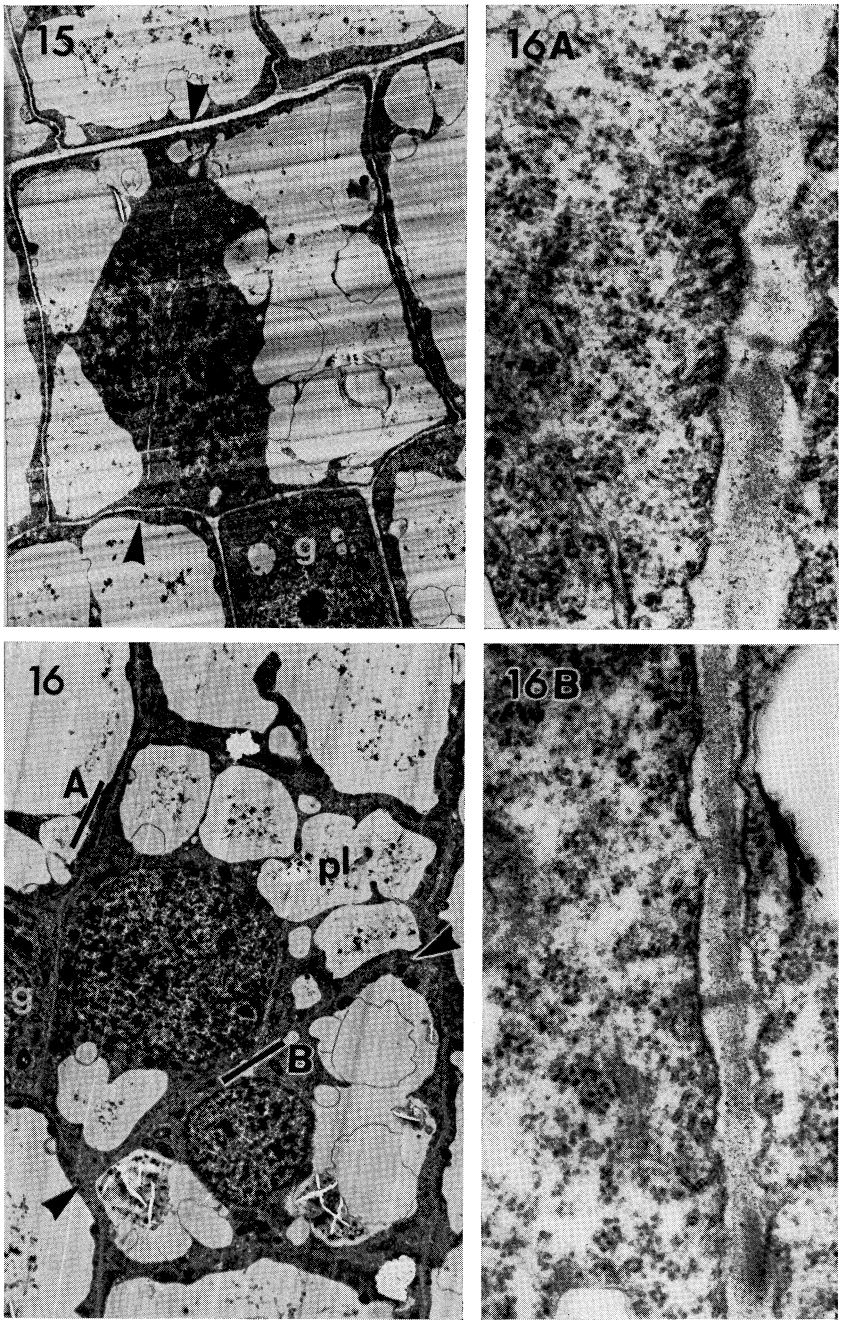

Fig. 15.—Telophase in epidermal cell adjacent to a young guard mother cell (*g*). Cell plate formation is skew (arrows). Knife marks due to crystals (e.g. as in Fig. 2) in cells. ×2950.
Fig. 16.—Preprophase, lateral subsidiary cell formation. Preprophase band of microtubules was found at *A*, *B* which predicted the normally observed cell plate orientation when the end wall of the epidermal cell is skewed (arrows)—compare with Figs. 4, 14, 15, and 18 (top cell). ×2950.
Figs. 16*A*, 16*B*.—Preprophase microtubules at *A*, *B* in Figure 16. ×65,000.

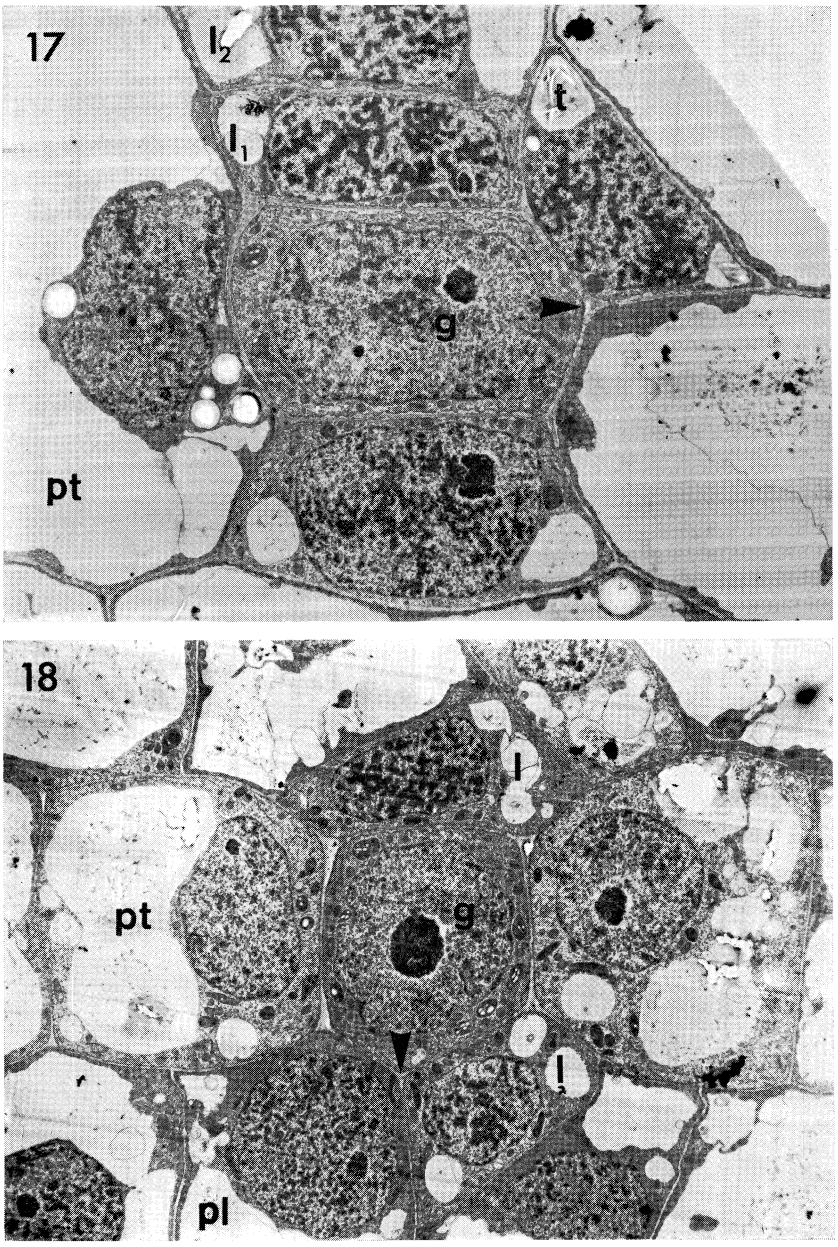

Fig. 17.—Abnormality, due to wall between epidermal cells (arrow) lying alongside guard mother cell—as in Figure 14(*b*). In this case, one of the abnormal pair of terminal subsidiary cells (*t*) has already been formed. One lateral subsidiary cell has divided (l_1, l_2). ×3600.

Fig. 18.—Top lateral subsidiary cell (*l*) shows typical triangular profile, due to skew end wall of epidermal cell (as in Fig. 14 and others). Abnormality present due to end wall between epidermal cells (arrow) coinciding with guard mother cell wall—as in Figure 17. However, in this case, it is on the lateral (not terminal) side. ×2500.

(Fig. 1) cell formation, development of such an abnormal complex was under way. Neither of these complexes contained preprophase cells with microtubule bands, but the bands would probably have predicted the plane of division (see Pickett-Heaps 1969*a* for the similar situation in wheat leaves).

V. Discussion

The observations described above are considered interesting for the following reasons:

(1) The existence of a preprophase grouping of microtubules in another higher plant is confirmed, and the general relationship of this band with the plane of future cell division, symmetrical or asymmetrical, is reasserted.

(2) Polarization of nuclei (and other organelles) is clearly *not* dependent on the existence of the microtubule band.

(3) Some additional evidence supports the suggestion that microtubules might move intact from the microtubule band into the prophase spindle.

The existence of preprophase microtubule bands is becoming more widely confirmed (Pickett-Heaps and Northcote 1966*a*, 1966*b*; Burgess and Northcote 1967, 1968; Cronshaw and Esau 1968). Recently Pickett-Heaps (1969*a*) has shown that the number of microtubule band(s) in caffeine-treated wheat tissues is apparently unrelated to the number and size of the nuclei; furthermore the microtubule band will continue to predict another asymmetrical cell division even following previous abortive attempts at that division. Burgess and Northcote (1967) have evidence that the plane of cell division is not always the same as the orientation of the microtubule band. This aspect is discussed in previous papers (Pickett-Heaps 1969*a*, 1969*b*).

The fact that many polarized nuclei in *C. cyanea* are found without a corresponding microtubule band indicates that the latter cannot be instrumental in polarizing the nuclei. A similar conclusion was reached after centrifugation of wheat stomatal complexes, when all stages of mitosis in the second asymmetrical division are highly polarized (i.e. with or without the preprophase microtubule band) and the spindle always resists sedimentation away from the guard mother cell (Pickett-Heaps 1969*b*). The grouping of microtubules is now seen as a *result* and not a *cause* of polarization (Pickett-Heaps 1969*b*). This conflicts with the views of Burgess and Northcote (1967) who suggest that the band orients the nucleus prior to mitosis, i.e. it "is associated with the prior positioning of the nucleus . . ." (Burgess and Northcote 1967, p. 325).

There is now clear evidence that the microtubules can move intact into the spindle region, since in both *Triticum* and *Commelina* some or quite a few segments of the tubules are generally visible on close inspection of the cytoplasm between the nucleus and preprophase band. The presence of a cytoplasmic strand lying between the nucleus and preprophase band of microtubules was common; very often segments and small groups of microtubules were also to be found in this strand. This confirms similar results discussed in more detail in a previous paper (Pickett-Heaps 1969*b*), where it was tentatively concluded that the preprophase grouping of microtubules represented a forgathering of future spindle material (the microtubules) before these are moved into the forming spindle. It appears that the microtubules are depolymerized once they have reached the prophase spindle, although some "open" microtubules can be found at the wall region which suggests that depolymerization was also

occurring there (Burgess and Northcote 1967). In some other types of plant cells at present under investigation, further evidence will soon be presented that a massive movement of intact microtubules from the wall to the spindle region occurs (Fowke and Pickett-Heaps, unpublished results).

Little comment can be made at this stage regarding the effect that a young guard mother cell seems to have on an adjacent spindle structure [Fig. 14(*a*)]. (The guard mother cell might also be able to affect the pattern of growth of the cells surrounding it.) An ability to deflect the spindle structure (Fig. 15) is hardly surprising in view of the extraordinary control the guard mother cell extends later over its neighbouring epidermal cells, inducing them to divide in such a very precisely controlled manner. The deflection of the cell plate is probably effected by the same means that later achieves the polarized divisions. The effect may become progressively greater during the symmetrical mitosis as the guard mother cell develops its inductive potentials; in the only case examined, preprophase microtubules in such an epidermal cell were not detectably altered from the transverse orientation. During the next asymmetrical division of this epidermal cell, to form the initial lateral subsidiary cell, the plane of the preprophase microtubule band (Fig. 16) is oriented skew as is the spindle and cell plate (Fig. 4), the latter being angled to form a lateral subsidiary cell with a triangular profile (top cell, Fig. 18). Figure 8 represents an exception to this general observation—but it will be noted that the exceptional behaviour is at least consistent. In Figure 8, the lower lateral subsidiary cell is not triangular in profile either, as was expected since one wall is clearly angled as described above.

The abnormalities occasionally encountered when the wall separating epidermal cells lies alongside a guard mother cell have been described in the Gramineae by Stebbins and Shah (1960). Pickett-Heaps (1969*a*) has confirmed that this can happen in wheat leaves (where stomatal complexes have normally one lateral subsidiary cell on each side of the guard mother cell); one is rarely lucky enough to find nuclei at preprophase in such a situation but in two cases found in wheat, the band predicted the position of the future cell plate. This type of abnormality can occur in both lateral and terminal epidermal cells in *C. cyanea* (Figs. 17, 18).

The way the phragmoplast maintains its ordered cell plate formation is obscure. In many small dividing plant cells, phragmoplast microtubules could obviously be related to the interzonal microtubules at telophase (e.g. as in Fig. 4); the mechanism by which the wall is oriented between such nuclei is relatively easy to visualize in simple terms. However, in some divisions in *C. cyanea* (as in many other divisions in vacuolated plant cells), the phragmoplast is able to extend itself far beyond the interzonal region between the nuclei (Fig. 11). In *C. cyanea*, this extension is highly organized spatially, and forms a cell wall of quite predictable geometry related to the overall pattern of other cells. The means by which the cytoplasmic organization of the phragmoplast is controlled spatially is not evident from micrographs (Figs. 11, 11*A*). It is an interesting subject for speculation and further experimental investigation.

VI. References

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