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CELL DIVISION IN THE FORMATION OF THE STOMATAL COMPLEX OF THE YOUNG LEAVES OF WHEAT

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SUMMARY

During the formation of stomata in the young leaves of wheat the cells divide in a characteristic manner; two of the cell divisions are asymmetrical and produce cells of unequal sizes. A study of the fine structure of the cells during mitosis has shown that a band of microtubules appears at each preprophase stage. This band, although it is not present in the subsequent stages of mitosis, indicates the location on the wall of the mother cell where the cell plate will join it at the final division of the cytoplasm at telophase. Thus the future plane of cell division is indicated by these microtubules at preprophase.

Microtubules are also found at the growing edge of the cell plate and appear to function in directing the vesicles which are brought up to extend the plate. The cell plate which is formed to cut off the subsidiary cells on either side of the guard mother cell is curved, and the microtubules present in conjunction with this plate during its formation could function to align and hold it on the required position.

The relationship of the guard mother cell to the divisions of the adjacent epidermal cells which form the subsidiary cells is discussed, and related to general problems of growth and differentiation.

INTRODUCTION

The development of stomatal complexes, as seen in the light microscope, has been described by Stebbins & Shah (1960), who examined the stomata from several types of plants. The number of cells that finally form the functional complex in these varied from two to eight.

The present work has attempted to study with the electron microscope the development of the four cells (two of these being incompletely separated) which are organized into the young stomatal complex on the leaf surface of the wheat seedling. The first two mitoses that occur result in very asymmetric division of the epidermal cells, but the resultant position of the cell plate in the cytoplasm is predictable, because the final distribution of the cells in the complex is known. In the last division, which forms the two guard cells, the cell plate develops normally, except that it does not extend completely to separate the daughter nuclei from one another. Subsequently the dividing wall thickens considerably in the central region, where it splits to form the stomatal pore between the young guard cells.

It was considered that a detailed study of the fine structure of these dividing cells, in which the future plane of division can be predicted at an early stage, might reveal a prior organization of cytoplasmic organelles determining the subsequent plane of

division. Such a correlation would be important for any theory of plant growth and differentiation.

MATERIALS AND METHODS

The wheat seedlings used and the methods of fixation and microscopy were the same as those previously described (Pickett-Heaps & Northcote, 1966).

RESULTS

In order to distinguish the three cell divisions that are an integral part of the differentiation process, these will be called the asymmetrical first and second divisions and the symmetrical third division respectively. These events are shown in Fig. 1 *a–g*.

Asymmetrical first division

During the growth of the young leaf inside the coleoptile, the lines of cells of the epidermis differentiate into rows of cells which in some cases give rise to stomatal complexes, or in others, to rows of epidermal hairs. The distribution of these rows has been indicated by Stebbins & Shah (1960). The first sign of the differentiation process is the asymmetric division which results in two different cells (Fig. 1 *a*).

Before the cells divided, the nucleus in all cells became displaced in one direction, the cytoplasmic vacuoles occupying the other end of the cell (Fig. 2). It has not been possible to determine, with the small sections of tissue used, whether this polarization can be correlated in any way with gravity, or with the direction of the tip of the cotyledon. Stebbins & Jain (1960) say that in these cells, the polarization is normally little, if at all, influenced by the external environment.

Cell organelles other than the microtubules were distributed evenly in the cell around the nucleus and peripheral cytoplasm. The microtubules were concentrated at that end of the cell where the 'polarized' nucleus had come to rest prior to division; they formed an 'equatorial' band against the wall, around this nucleus. When the cell was cut in longitudinal sections they were clearly seen in transverse section near the wall (Figs. 3–5). This band was generally quite clear, particularly since very few microtubules were distributed along the wall of the rest of the cell. Quite frequently, they were found two or three deep in some regions of the band, which was most noticeable just prior to prophase. Once the chromosomes started to condense, the band became less obvious.

Prophase was accompanied by a progressive condensation of the nuclear chromatin, and disintegration of the nucleolus (Fig. 3). Two polar zones, from which elements of endoplasmic reticulum appeared to proliferate, became apparent at each end of the spindle, and frequently short elements of microtubules were found in these zones as well as occasional longer elements in close proximity to the nuclear envelope (Pickett-Heaps & Northcote, 1966).

The sequence of events leading to telophase was very much the same as that observed in other cells of the wheat seedling, except that the spindle was confined to one

end of the cell. Microtubules were found in between the chromosomes, lying along the spindle axis, and some were apparently connected to the chromosomes themselves (Pickett-Heaps & Northcote, 1966). At telophase, cell-plate formation was accompanied by the characteristic proliferation of microtubules, and as the coalescing plate extended it apparently contracted around the nucleus of the smaller cell (Fig. 6). Thus

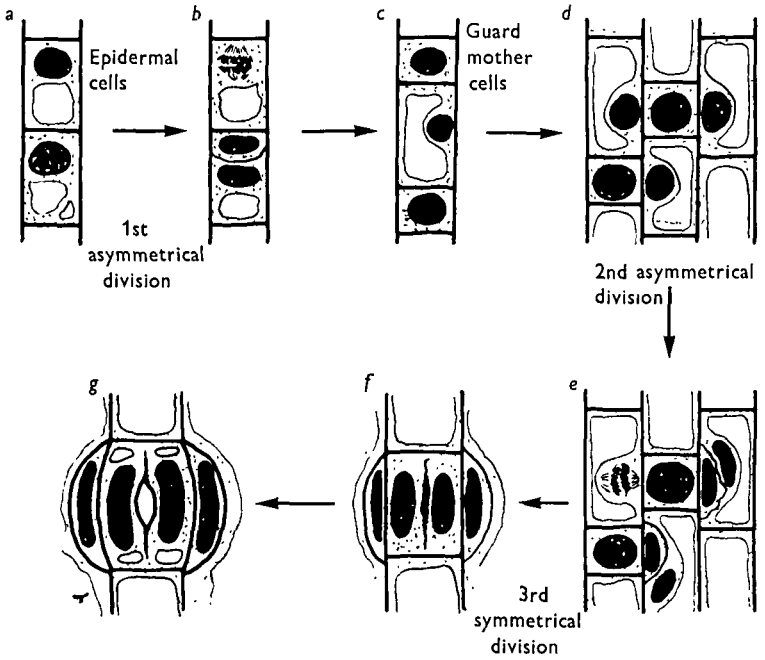

Fig. 1 *a–g*. Formation of the stomatal complex. This diagram shows the three divisions by which the stomatal complex is formed. The cells of epidermal rows become polarized so that each nucleus of each cell is situated at the same end (*a*). The cells divide asymmetrically (*b*) and a small compact daughter cell (the new guard mother cell) and a larger vacuolated daughter cell are formed; each of these cells elongates slightly (*c*). During the next preprophase stage the nuclei in each epidermal cell adjacent to the guard mother cell take up a position at the common wall between it and the guard mother cell (*d*). These epidermal cells then divide independently giving a second asymmetrical division (*e*) which results in the formation of two small subsidiary cells one on each side of the guard mother cell; the latter then divides (the symmetrical third division) to give two equal guard cells (*f*). Later the guard cells grow and the wall between them splits to form the pore (*g*).

this cell, which was now the guard mother cell, had a very characteristic shape at this stage. Many small vesicular components were seen between the microtubules at the edges of the plate (Fig. 7). The microtubules in the cytoplasm of the guard mother cell were often angled quite sharply away from the longitudinal wall (Fig. 7).

After this division, both cells grew in length, the guard mother cell becoming square, and the young curved wall which was formed from the cell plate came to resemble the other lateral wall of the guard mother cell. By the time that the next stage of the differentiation started, the rows of cells of the epidermis were comprised of alternating

small square cells—the guard mother cells—and the much larger oblong vacuolated cells (Fig. 8).

Asymmetrical second division

The second asymmetrical division takes place in the large vacuolated cells alongside the guard mother cell. The nuclei of these cells came to rest against the wall which was common to them and the guard mother cell, and this disposition indicated that the cells were about to divide, even though no other nuclear changes were visible at this stage.

It was noticeable that a very large number of the cells observed at this stage in the formation of the stomata were in the preprophase configuration (Fig. 8). It would appear therefore that once division started, it proceeded quite quickly.

The alignment of this pre-mitotic nucleus was accompanied by a very characteristic and invariable distribution of microtubules (Figs. 9–12). This was seen in the sections as two groups of microtubules. Each group was situated in the cytoplasm of the preprophase cell, near that part of the wall close to, but not shared with, the guard mother cell (Figs. 9–12). Sections of microtubules seen in the cytoplasm near the wall shared with the guard mother cell were extremely rare. However, at a little distance past the limits of the common wall, this preprophase concentration of microtubules was very marked, and was further enhanced by the almost complete absence of microtubules around the remainder of the wall. Some microtubules were seen often in cross-section, at the usual position, near the plasmalemma of the guard mother cell. It should be emphasized that this grouping of the microtubules is consistent and invariably present in all cells at this stage of pre-mitotic activity. From a study of the sections it can be deduced that a band of microtubules runs in a circular manner around the cytoplasm near the wall adjacent to the guard mother cell; microtubules were never seen grouped in this configuration against the wall distal to the guard mother cell.

The number of these microtubular elements appeared to reach a maximum before the obvious onset of prophase. As in the asymmetrical first division, the number decreased as prophase became more evident.

The cells on either side of the guard mother cell divided independently (e.g. Fig. 10). Prophase, metaphase and anaphase followed the familiar pattern. Sometimes the guard mother cell divided without one of the subsidiary cells being even partially formed.

One of the pole areas of the mitotic spindle was situated very close to the guard mother cell. Nothing characteristic could be found in this region; the endoplasmic reticulum was present as a network and the microtubules of the spindle appeared to penetrate this area without terminating in any obvious structure.

At telophase the wall was formed by the coalescence of vesicles which were organized into a hemispherical cell plate (Fig. 13). Microtubules appeared in the usual position at the edge of the plate. Since the cell plate formed in such a characteristic manner, the distribution of the cell plate microtubules is worthy of note. As the line of vesicles formed the semicircular pattern around the nucleus of the subsidiary cell,

microtubules were most obvious in large numbers radially aligned from this nucleus (Fig. 14). It would appear that these tubules were actively engaged in aligning the plate as it formed, by both determining its shape and guiding the vesicles into it.

Symmetrical third division and further growth

To complete the formation of the stomatal complex, the symmetrical last division occurred in the guard mother cell. This generally took place after one or both of the subsidiary cells had been formed. Somewhat surprisingly, preprophase cells were comparatively rare. However, when they were found (Fig. 15), they all showed evidence of the usual banding of microtubules in the 'equatorial' position (Fig. 16). This grouping was not so marked as in the previous two asymmetric divisions. Again, the microtubules disappeared before the onset of later prophase.

The cell plate formed in the centre of the cell as usual. The wall was not completely formed, there being one or several holes apparent at either or both ends of the wall (Fig. 17), often opposite a stub projecting from the older transverse wall. All the older stomata examined had gaps in the wall; however, it was necessary to take serial sections to show this, as sometimes one end of the wall would appear to be continuous with the lateral wall, the gap only being revealed in sections at a different level. In view of this difficulty it was impossible to decide whether the wall was formed and broken down at a later stage or never completely developed. The latter explanation is considered more likely. No particular distribution or occurrence of any cell organelle was seen which would explain this incomplete development. Elements of endoplasmic reticulum occasionally went through the gap, but this always occurs during the formation of the cell plate and there is no reason to think that this organelle was in any way connected with the maintenance of the wall-free area.

DISCUSSION

It has been noted previously that in highly organized meristematic regions of the wheat seedling, both in root and coleoptile, cell division is usually preceded by a grouping of microtubules in a characteristic position in the cytoplasm. It is likely that this grouping might prove to be an almost invariable sign of the preprophase condition. To describe their position, they were initially termed the 'equatorial' band of microtubules, since they ran around the middle of the nucleus near the wall, and this position usually corresponded to the central region of the cell. However, the terminology appears incorrect when applied to the asymmetrical second division of the epidermal cells, described above, where the band is most marked. In these cells although it has not as yet been possible to show the microtubules in longitudinal sections, as has been done with the root and the coleoptile cells (Pickett-Heaps & Northcote, 1966), there is every reason to believe that they run as a circular band in the cytoplasm of the epidermal cell, adjacent to the wall which separates it from the guard mother cell. They are never found at the other walls of the epidermal cell. They therefore take up that position in the cell where the future cell plate is going to meet the older walls. In the epidermal cells, this position is not equatorial with respect

to the nucleus, but is confined to one side of the cell, at its common wall with the guard mother cell. The preprophase band of microtubules therefore indicates the future plane of division of the cells. This was also inferred from studies on the preprophase band of microtubules which occurs in the dividing isodiametric cells of root and coleoptile meristems (Pickett-Heaps & Northcote, 1966).

Stebbins & Shah (1960) found an 'inequality' in the cytoplasm of the preprophase epidermal cells. The cytoplasm in these cells when plasmolysed was shown to remain appressed to the guard mother cell area of the wall, while it had come away elsewhere. This might have been due to the presence of the preprophase band of microtubules.

It appears likely that microtubules also play a large part in the orientation of the cell plate during its formation. This was indicated by the studies on cytokinesis in root and coleoptile meristems (Pickett-Heaps & Northcote, 1966). It is particularly evident from the observations made on the cell plate as it is being formed in the first and, more particularly, in the second asymmetrical division. There must be some very positive cytoplasmic force acting on the vesicular components which are lined up in the hemispherical position to form the plate, and it is difficult to escape the conclusion that the microtubules are involved (Figs. 7, 14).

It is interesting to note that the young cell wall between the immature guard cells is incomplete. Brown & Johnson (1962) report this 'entirely unexpected' finding in their description of the fully developed stomata in sixteen species of grass. This could be of functional significance and may be necessary to keep the internal osmotic pressure of the guard cells identical.

Stebbins & Jain (1960) and Stebbins & Shah (1960) discuss the influence which apparently emanates from the guard mother cell, and which induces the epidermal cells on either side to divide to give the subsidiary cells. They show that this influence varies between guard mother cells which are competing for the nucleus of one epidermal cell. This is particularly noticeable in the results described for *Commelina*, a plant whose stomata normally have a total of six subsidiary cells; two of these are situated at each end of the guard cell pair, and are thus derived from an epidermal cell which also provides the lateral subsidiaries to other guard mother cells. Our results indicate that one observable effect of the influence of the guard mother cell is the occurrence of the preprophase banding of microtubules in the adjacent epidermal cell. Stebbins and his co-workers suggest that the organization of the subsidiary cells is a property of the individual cells, and that it depends not only on a 'hormone', passed from the guard mother cell, but also on some gene-cytoplasmic interaction within the epidermal cell. Our results would suggest that this can be interpreted as a specific synthesis or perhaps organization of the microtubules at a position dictated by the adjacent guard mother cell. Stebbins & Jain also suggest that the emanating substance is produced by the guard mother cell at varying rates, and diffuses directionally, to cause (in the case of wheat stomata) the lateral epidermal cells to divide, without affecting those epidermal cells in the same row, although these latter cells can again divide laterally forming subsidiary cells to guard mother cells in other rows. It seems very likely that during this diffusion, the substance or substances passes from the guard mother cell to the epidermal cells through the plasmadesmata rather than

by general diffusion through the wall and plasmalemma. If these pores contain endoplasmic reticulum, then the diffusible materials could be passed directionally by the endoplasmic reticulum from cell to cell, and in such highly polarized cells the endoplasmic reticulum might exist as distinct systems, one of which could lie along the axis of the cell. Such a concept could explain other forms of cell differentiation, particularly that which occurs in those rows of cells in meristems which differentiate in columns to form phloem and xylem elements. Stebbins & Jain (1960), and Stebbins & Shah (1960), describe the persistent occurrence of an abnormal type of stomatal complex which has two subsidiary cells on one side. This occurs when the wall between the epidermal cells on that side coincides with the middle of the guard mother cell. This can be explained if the substances causing division diffuse laterally into both epidermal cells, causing them both to divide asymmetrically.

Observations which show the influence of one cell upon the plane of division of another are of extreme importance to the understanding of the general problem of organized growth and differentiation. In the work described above the influence of the guard mother cell on the organization and eventual plane of division of the adjacent epidermal cell has been shown. When the fine structure of the cells is investigated a result of this influence can be seen in the formation of a preprophase band of microtubules. It is possible that a similar effect of one cell upon another occurs during the organized divisions of cambial cells and the other meristematic cells of growing roots and stems (Pickett-Heaps & Northcote, 1966). In these tissues the phenomenon could be part of a general influence of the micro-environment (the surrounding cells) on the actively dividing cell.

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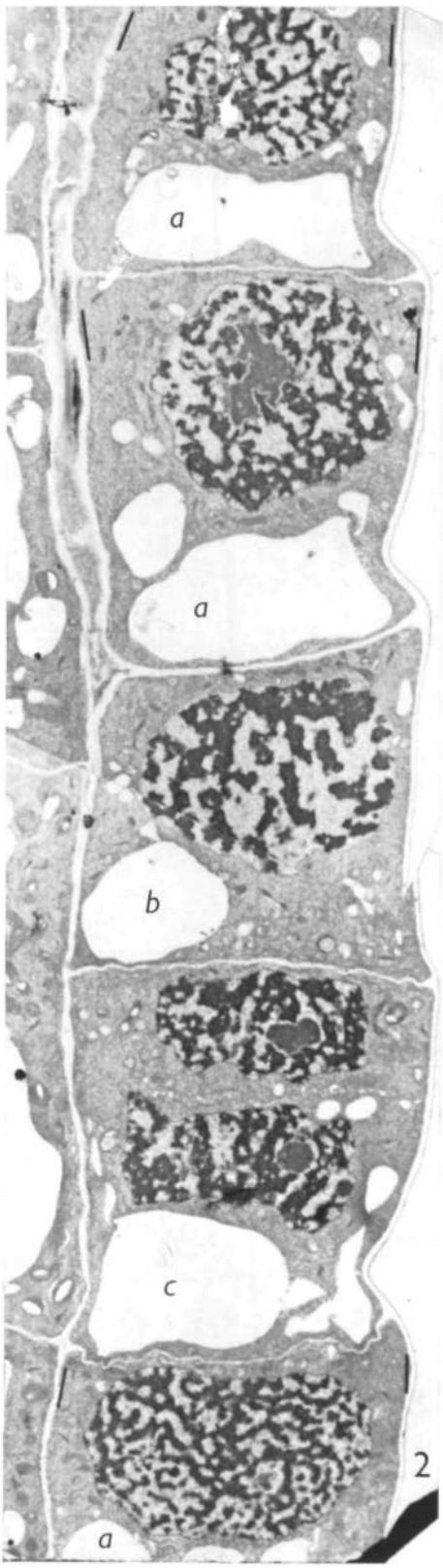
ABBREVIATIONS

cp	cell plate	*nc*	nucleolus
ec	epidermal cell	*sc*	subsidiary cell
gc	guard cell	*t*	microtubules
gmc	guard mother cell	*w*	cell wall
m	mitochondrion		

Fig. 2. Longitudinal section at right angles to the leaf surface of epidermal cells during the asymmetrical first division. A row of five polarized differentiating cells is shown; the nuclei are positioned at the same end of each cell. Cells marked '*a*' are at preprophase and the short black lines indicate the position in the cytoplasm of the preprophase band of microtubules. Cell '*b*' is at prophase and cell '*c*' at telophase (compare Fig. 6). The appearance of the nucleoli is characteristic of these mitotic stages. × 2400 approx.

Fig. 3. Longitudinal section in the plane of the leaf surface of one of a row of differentiating epidermal cells at preprophase of the asymmetrical first division. Positions '*a*' and '*b*' indicate the location of the preprophase band of microtubules (shown at higher magnification in Figs. 4 and 5, respectively). The same end wall of the epidermal cell in Figs. 3–5 is designated w_1. × 5400.

Figs. 4, 5. Details of preprophase band of microtubules shown at '*a*' and '*b*' in Fig. 3. Both × 33000.



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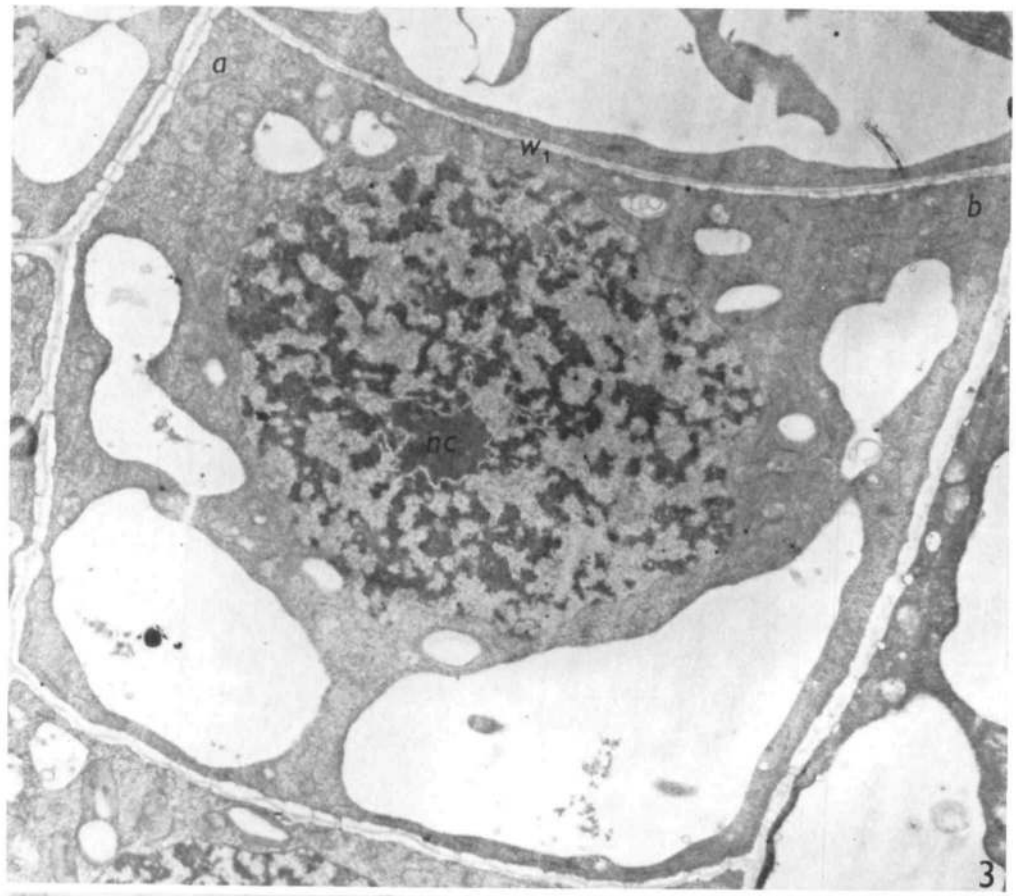
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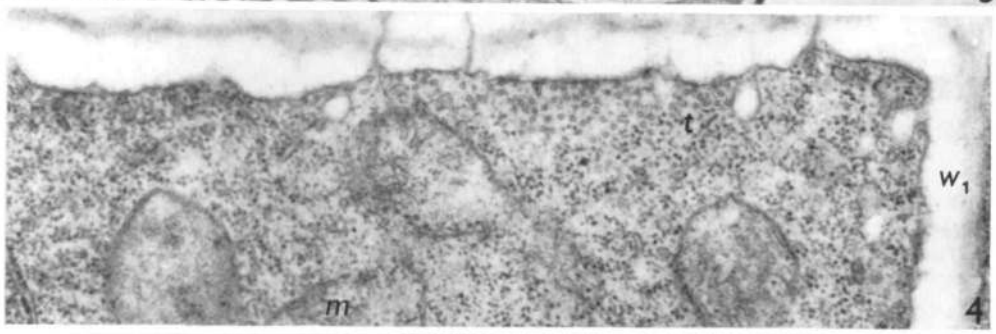
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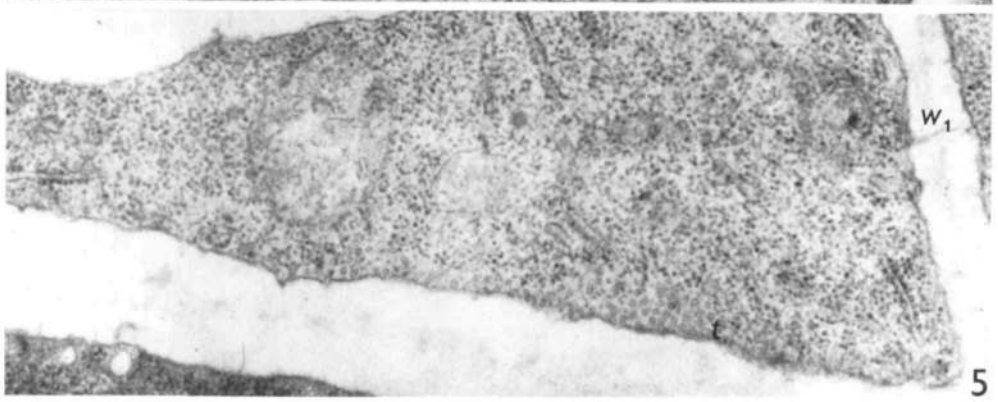
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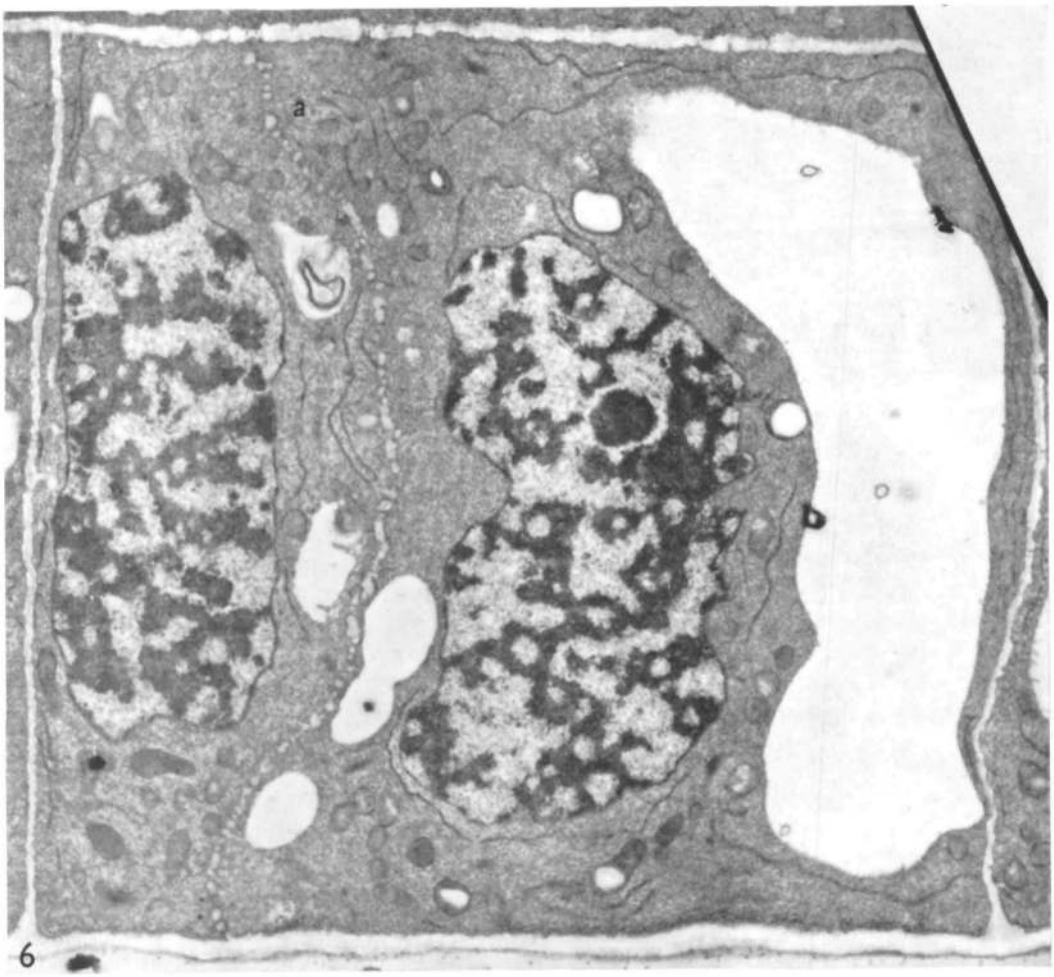
Fig. 6. Similar cell to that shown in Fig. 3 but at telophase. The curved cell plate has divided the cell into a small compact daughter cell (the guard mother cell) and a larger vacuolated epidermal cell (compare Fig. 1*b*). The region indicated by '*a*' is shown at higher magnification in Fig. 7. ×6000.

Fig. 7. Higher magnification of region '*a*' in Fig. 6. Microtubules are seen at the edge of the plate; the vesicles which are collected at this region were apparently being aligned by the microtubules (arrow). ×30000.



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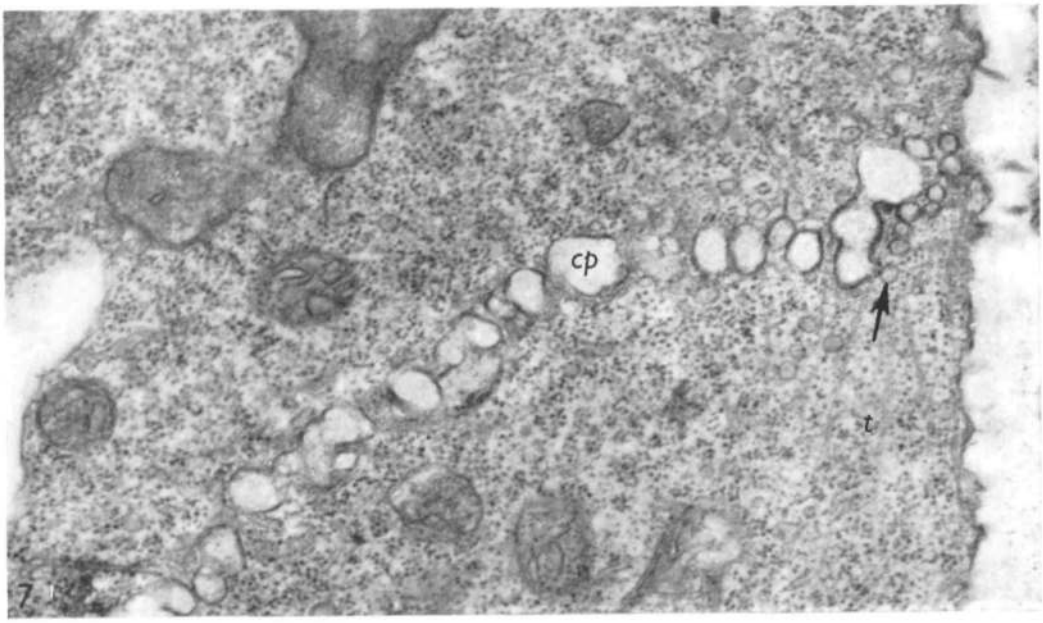

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Fig. 8. Longitudinal section in the plane of the leaf surface of rows of differentiating epidermal cells prior to the asymmetrical second division (compare Fig. 1 *d*). The central column of cells consists of an alternating sequence of small compact guard mother cells and larger vacuolated epidermal cells. The nuclei of the epidermal cells on either side of the guard mother cells are positioned adjacent to it. In each of these preprophase epidermal cells, the preprophase band of microtubules was found at the region indicated by the short black lines (compare Figs. 9–12). × 2600 approx.

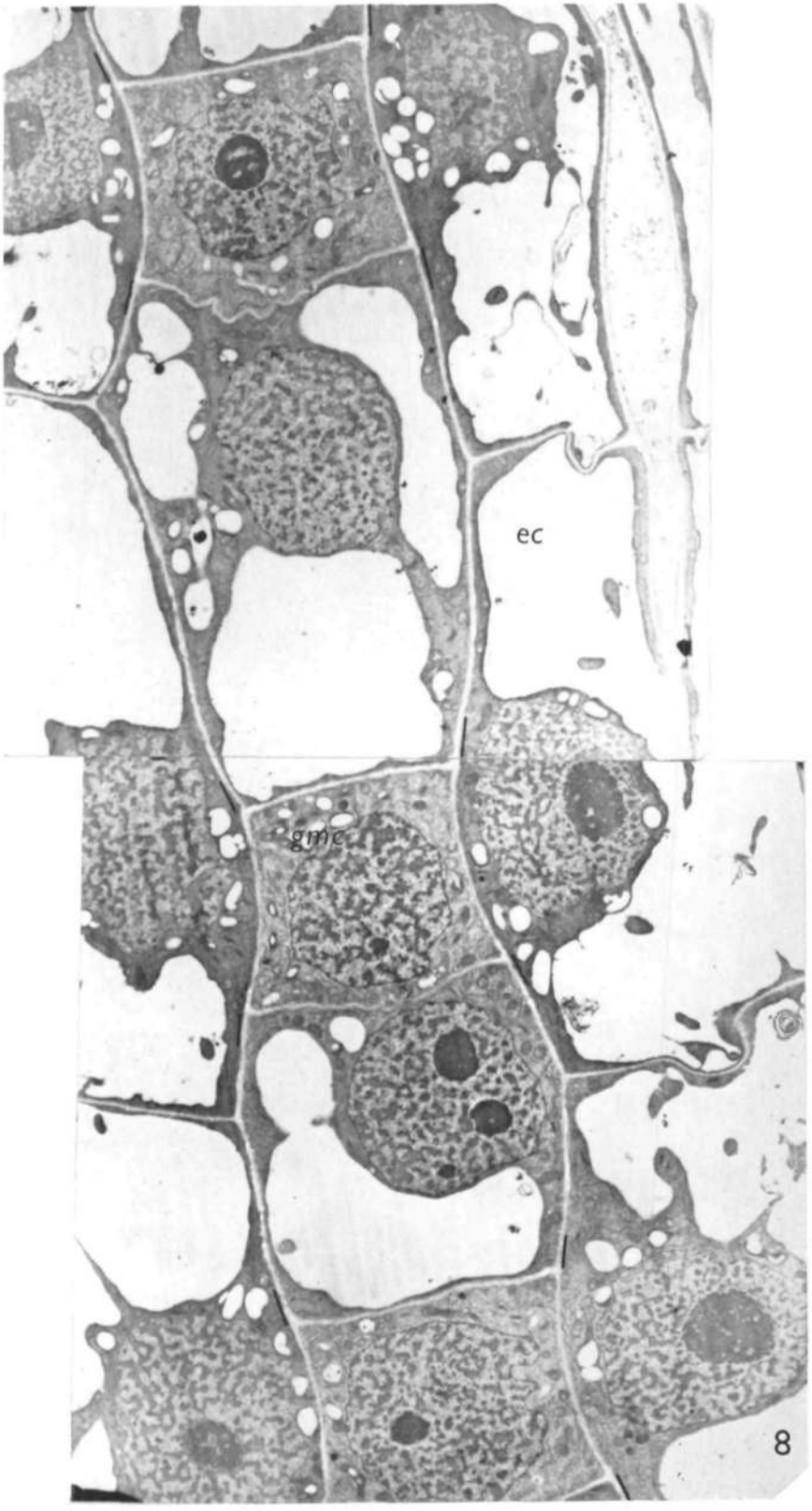
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Fig. 9. Higher magnification of region '*a*' in Fig. 10. The position of the guard mother cell is indicated by the arrow. The preprophase band of microtubules in the epidermal cell can be seen at the position indicated in Fig. 8. × 37000.

Fig. 10. Longitudinal section in the plane of the leaf surface of differentiating epidermal cells. On one side of the guard mother cell the asymmetrical second division has been completed to form a small subsidiary cell. On the other side a preprophase nucleus is seen (compare Fig. 1*e*). The preprophase band of microtubules seen at regions '*a*' and '*b*' are shown at higher magnification in Figs. 9 and 11 respectively. × 3400.

Fig. 11. Higher magnification of region '*b*' in Fig. 10 (compare Fig. 9). × 36000.

Fig. 12. The preprophase band of microtubules in an epidermal cell equivalent to that shown in Figs. 8 and 10. The microtubules are shown in transverse section. They are at the same position relative to the guard mother cell as that shown in Figs. 8–11. This phenomenon is an invariable characteristic of these preprophase cells. × 34000.

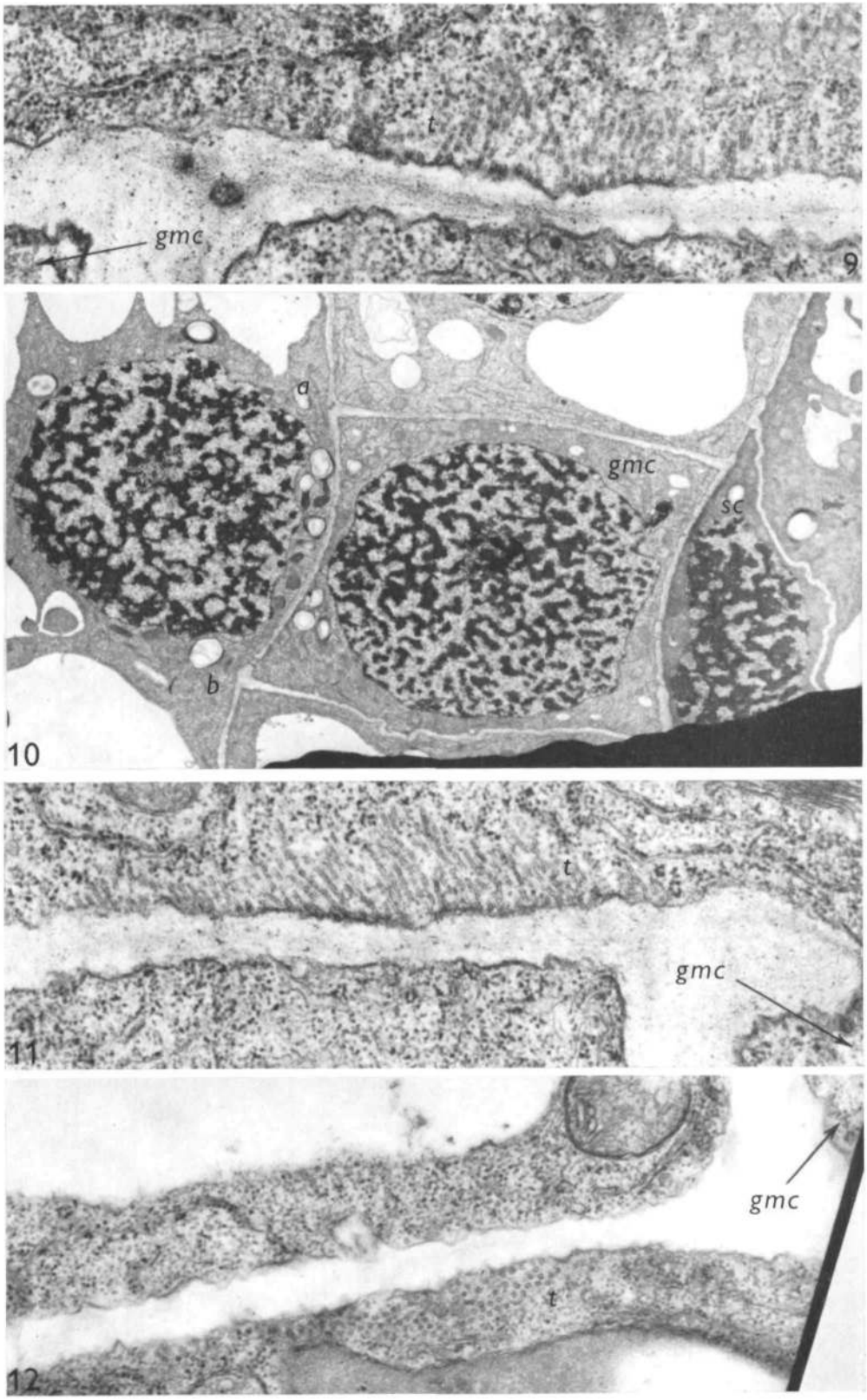
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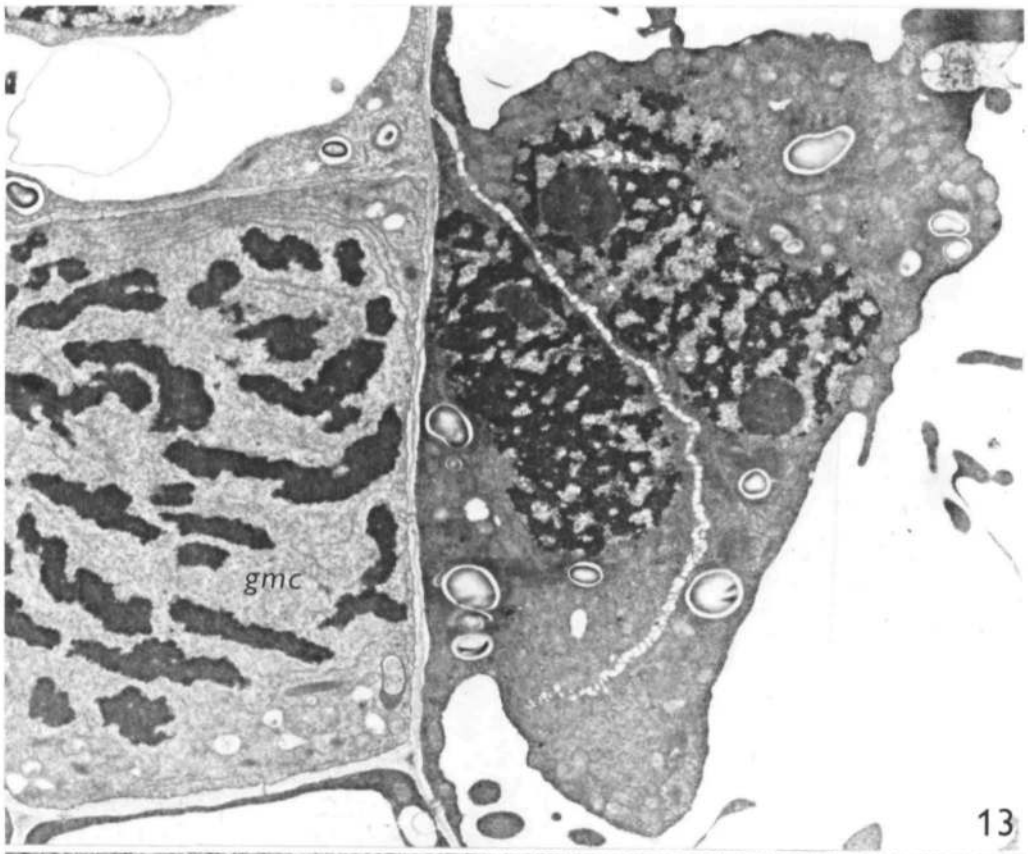
Fig. 13. Longitudinal section in the plane of the leaf surface of differentiating epidermal cells. The guard mother cell is at about late metaphase of the symmetrical third division (compare Fig. 1*f*). The epidermal cell adjacent to it is at telophase of the asymmetrical second division (compare Fig. 1*e*). The curved cell plate can be clearly seen and numerous microtubules were found at the edge of the plate (compare Fig. 14). ×4800.

Fig. 14. Higher magnification of the cell plate being formed at telophase in an epidermal cell at the asymmetrical second division similar to that shown in Fig. 13. Numerous microtubules are present at the growing edge of the plate where vesicles were being directed and incorporated into it. ×23000.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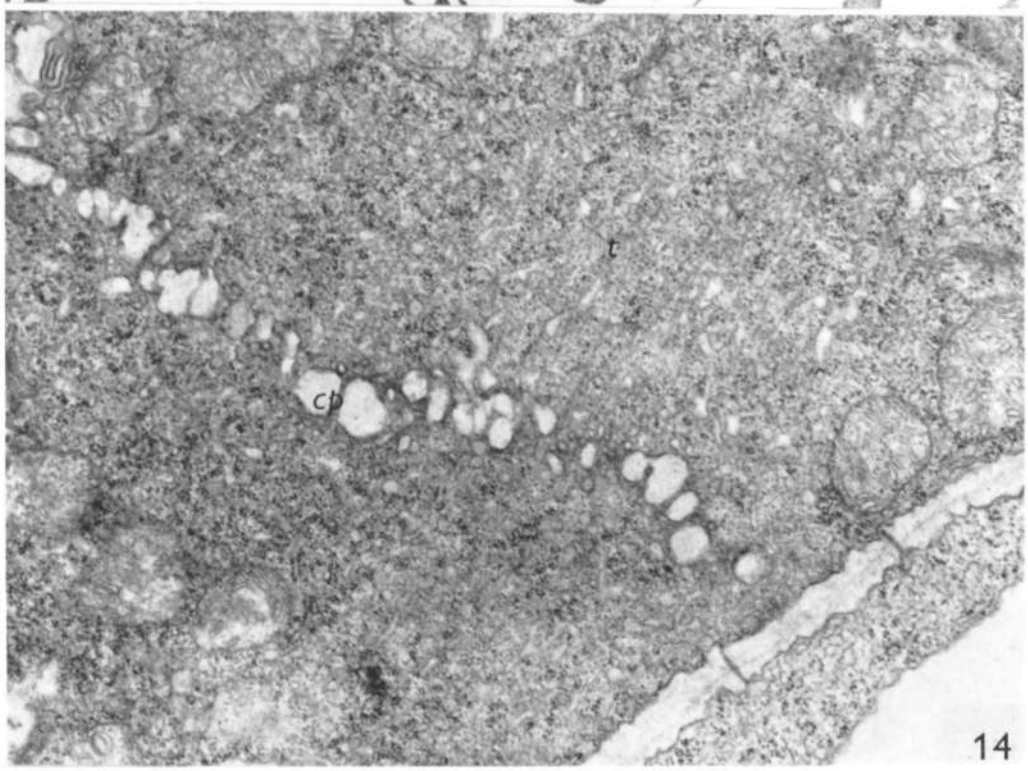
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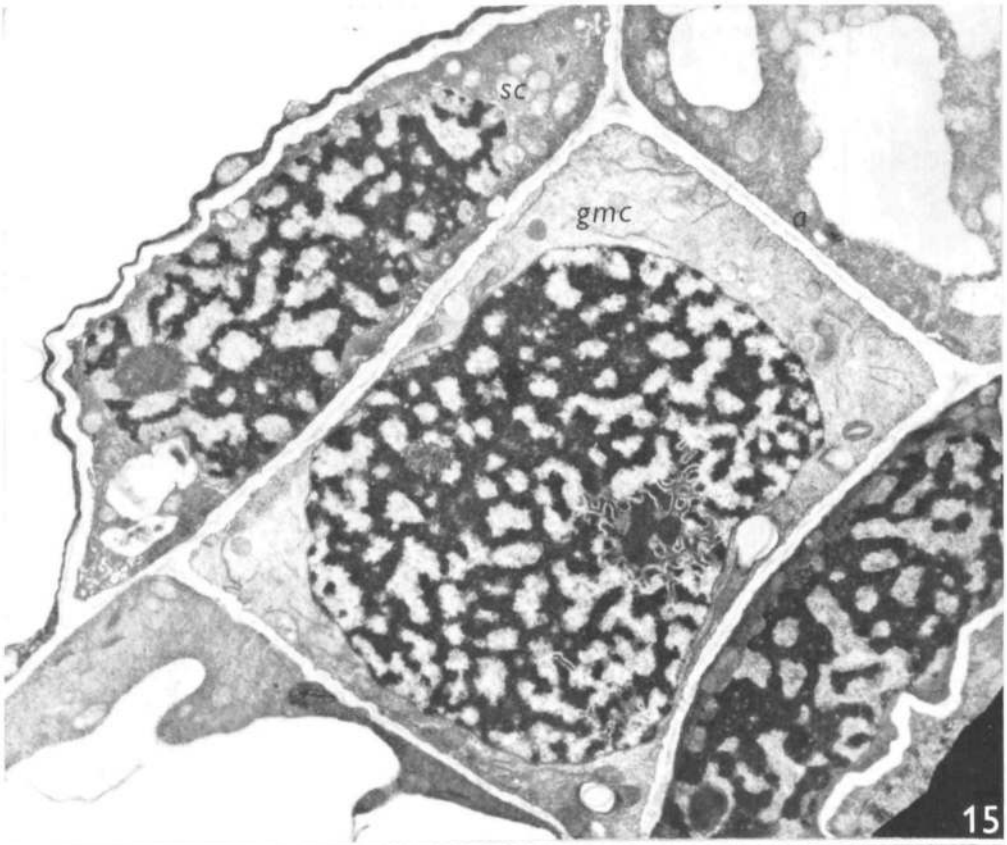
Fig. 15. Longitudinal section in the plane of the leaf surface of a nearly fully formed stomatal complex. The guard mother cell is at preprophase of the symmetrical third division; the characteristic form of the nucleolus at this stage of mitosis can be clearly seen. A preprophase band of microtubules was found running in an equatorial position around the nucleus; one section of this at region '*a*' is shown at higher magnification in Fig. 16. ×5000.

Fig. 16. Higher magnification of preprophase band of microtubules found at region '*a*' in Fig. 15. ×35000.

Fig. 17. Longitudinal section in the plane of the leaf surface of a fully formed stomatal complex. Two guard cells have been formed from the guard mother cell. These are not entirely separated during this and later stages of development because of incomplete formation of the dividing wall (arrowed). ×4000.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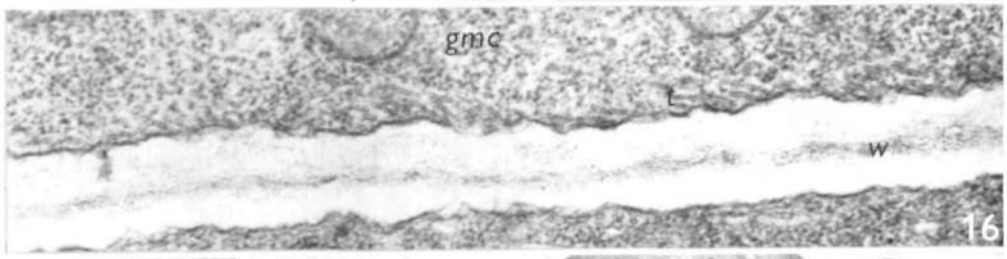
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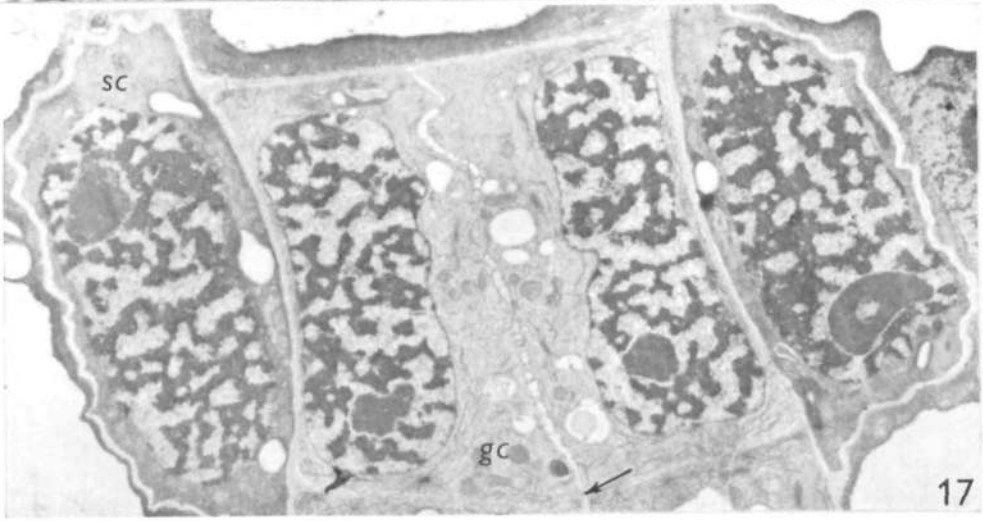
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