

## ORGANIZATION OF MICROTUBULES AND ENDOPLASMIC RETICULUM DURING MITOSIS AND CYTOKINESIS IN WHEAT MERISTEMS

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### SUMMARY

The fine-structural changes accompanying mitosis in meristematic cells of the roots and coleoptile tissue of wheat have been studied. A band of microtubules encircling the nucleus appeared in the cytoplasm before the cells entered prophase. These microtubules were oriented at right angles to the direction of the mitotic spindle and were located at the position on the mother cell wall where the future cell plate dividing the daughter cells would have joined it. During prophase the number of microtubules in this preprophase band decreased and eventually disappeared, while microtubules were found to be aligned along the spindle axis. These spindle microtubules appeared as a cone-shaped array of units radiating from the polar zones of the spindle and passing very close tangentially to the nucleus. At late prophase they penetrated the disintegrating nuclear envelope and were seen between the chromosomes. During metaphase and anaphase many microtubules were present running throughout the length of the spindle, and others were found to be attached to chromosomes. Paired sister chromosomes were found joined to microtubules from opposite poles of the spindle. The position and orientation of the lamellae of the endoplasmic reticulum which invaded the spindle from the two poles was closely related to the position and alignment of the microtubules. During the formation of the cell plate vesicles were seen to be collected between the microtubules. As the vesicles fused to form the plate the microtubules were found only at its growing edge, where the vesicles were still being aligned. At the initial stage of its formation the microtubules passed right through the plate, but as it extended they appeared to end at the plate region.

The results of the investigation are discussed in relation to the descriptions of mitosis and cytokinesis based on optical microscopy of living cells.

### INTRODUCTION

The work of Porter & Machado (1960) showed very clearly the distribution of the elements of the endoplasmic reticulum in the mitotic spindle of meristematic cells of onion root. These authors noted in particular the proliferation of the lamellae of the endoplasmic reticulum from two poles in the cell and the invasion of the mitotic spindle by these lamellae at a very early stage of mitosis. During telophase the endoplasmic reticulum formed the new nuclear membranes of the daughter cells and became at the cell plate region a close lattice of tubular profiles which was associated with the vesicles that gave rise to the cell plate.

Observations with the light microscope have shown the presence of birefringent fibrils in the mitotic spindle of animal and plant cells (Bajer & Molé-Bajer, 1963; Mazia, 1961), and these spindle fibres are connected directly with the kinetochore during the chromosomal movement that occurs at metaphase and anaphase. Early

investigations with the electron microscope showed that the spindle contained oriented fibrillar material (Sato, 1958, 1959; Leak & Wilson, 1962). More recently, Harris & Bajer (1965) have shown that the birefringent fibres seen with the optical microscope are equivalent to an organized distribution of microtubules seen with the electron microscope. Manton (1964 *a, b*) has also shown the existence of spindle microtubules in plant cells, and Ledbetter & Porter (1963) have shown the presence of these tubules at telophase in meristematic root cells.

In this paper a detailed account is presented of the organization and distribution of the microtubules and endoplasmic reticulum at the various stages of mitosis and cytokinesis in wheat meristem cells. A new distribution of microtubular elements in dividing cells is described.

#### MATERIALS AND METHODS

Wheat seeds (*Triticum vulgare*) were washed and germinated on damp filter paper at 25 °C. Sections (1 mm) from the central portion of coleoptiles grown to about 0.7 cm in length and from terminal root tips (1 to 2 mm) were taken and immediately placed in the fixative.

Glutaraldehyde (6%), buffered to pH 7.0 with 0.02 M phosphate buffer, with CaCl<sub>2</sub> added, was used to fix tissue for 1–2 h at room temperature. It was found empirically that the amount of calcium added was not critical; 4–6 small drops of 0.1 M CaCl<sub>2</sub> were generally used for 25 ml of fixative (the pH having been adjusted to 7.0). The insolubility of calcium phosphate effectively limits the amount of calcium present in solution. The material was post-fixed in veronal-buffered 1% OsO<sub>4</sub> for 1 h (Ledbetter & Porter, 1963). The washed tissues were dehydrated in an ethanol series and finally soaked in propylene oxide and embedded in Araldite. Sections were cut using glass knives in a mechanical-advance microtome, collected on carbon-coated grids and stained with lead (Millonig, 1961) for between 5 and 20 min. It was found advantageous to stain with a saturated solution of uranyl acetate (in 50% ethanol) for 0.5–1 h before the lead staining. Sections were examined with a Philips electron microscope (model EM 100) at 80 kV.

#### RESULTS

The mitotic cycle is normally divided into four convenient and recognizable stages: prophase, metaphase, anaphase and telophase. Although the time spent in each of these phases varies considerably, the cycle is continuous and it would be difficult to state exactly when one of the phases ended and the next began. In this account a rigorous separation between descriptions of metaphase and anaphase cells has not been attempted, since it was generally difficult to distinguish between these two phases of division by examination of thin sections of the cell with the electron microscope. By mid and late anaphase, however, the stage reached became obvious. It appeared from the micrographs that the chromosomes did not take up a clearly recognizable metaphase position. In the coleoptile cambial cell, particularly, there seemed very little

room to enable the chromosomes ( $n = 42$ ; Darlington & Wylie, 1945) to achieve such a recognizable distribution, and whether these cells divided longitudinally or transversely, no particular metaphase configuration could be seen. In more isodiametric cells, such as those of the root meristem, there often appeared in the sections of metaphase cells small sections of chromosomes some distance from the equatorial position, and it is difficult to tell whether these represented moving chromosomes at early anaphase, sections of the trailing limbs of true metaphase chromosomes, or perhaps neocentric activity (Bajer & Molé-Bajer, 1963).

The description of mitosis which follows applies generally to all the meristematic cells of the root and coleoptile which have been examined.

### *Preprophase*

It seemed a reasonable possibility that, prior to the normally recognized onset of mitosis, there would be visible in the cytoplasm of the cell some activity or organization related to the subsequent division, and this was therefore looked for. In particular, some evidence was sought at the ultrastructural level of a 'decision' having been made by the cell, determining its future plane of division.

One of the problems was the identification of such 'preprophase' mitotic stages. The 'preprophase' cell is one whose nucleus has not undergone the obvious changes that produce the typical prophase image, and obviously there comes a stage when such a nucleus is indistinguishable from that of a resting or interphase cell. Careful study of electron-microscope sections resolved this difficulty. All the cells that were adjacent to others undergoing division were examined; since localized regions in the tissue containing several mitotic figures next to one another were always obvious, it was reasonable to assume that some of the cells in close proximity to a dividing cell would be in the preprophase condition. It became clear that in preprophase nuclei, early condensation of chromatin was occurring (Fig. 2). The appearance of the nucleoli in preprophase cells was also often characteristic. During interphase periods, these organelles were circular in cross-section, with a somewhat diffuse edge. In preprophase cells, a radical change could often be seen; the nucleolus lost its compact form, and long twisted 'arms' of its material could be seen radiating from it and apparently penetrating into the nucleoplasm. A typical late stage of this change is seen in Fig. 2. While these signs were not infallible guides such changes frequently indicated preprophase activity and with practice many such nuclei were found.

It seemed most likely that spindle organization, and in particular microtubule synthesis, might be observable in what was going to be the future polar zone of these cells, but long and careful scrutiny of these regions failed to reveal any changes or structures that could be implicated in mitosis. However, a band consisting of a large number of microtubules was found near the wall of the cell, far removed from the polar zone. In a typical longitudinal section, many profiles of these organelles could be seen packed together, often three or four (sometimes more) units deep along a restricted area of the wall (Figs. 4, 5). They appeared in this position on each side of the nucleus (Figs. 1, 3), encircling it as what can best be described as a preprophase band in the equatorial position. Appropriate transverse sections of the cells confirmed

the occurrence and extent of this band of microtubules (Figs. 6–8). The microtubules were seen in such transverse sections to run around the wall, several units deep, curving gracefully away from the sharper corners of the cells (Fig. 6); sometimes individual elements appeared to veer away from the wall (Fig. 8). These microtubules were not equivalent in their distribution to those which are characteristically found associated with the young cell wall (see Ledbetter & Porter, 1963). Once compared, the difference between the disposition of the singly occurring, typical ‘wall’ microtubules, and these preprophase microtubules, which sometimes exhibited evidence of hexagonal close-packing in their bunched grouping, can be clearly seen. The actual number of ‘wall’ microtubules along a unit length of wall in a typical meristematic interphase cell, compared with the number found in the preprophase band, was not very large, and they were almost invariably found situated very close to the wall. This can be contrasted with the appearance of the preprophase band, where 150 or more profiles of the tubules occurred, confined to a localized region of the wall. The microtubules shown in Fig. 4, which have an outside diameter of about 200 Å, are spread along the wall for a distance of 2.6  $\mu$ ; the ones farthest from the wall are situated at a distance of 0.24  $\mu$  from the plasmalemma. This photograph is typical of many others taken from cells in the preprophase condition. It was impossible to ascertain how these tubules originated, but in view of their numbers it seems likely that some were synthesized *de novo*. There is, however, no reason to suppose that some of the others were not originally ‘wall’ microtubules.

It is difficult to estimate how the number of these organelles varied over the preprophase period, but it seems unlikely that they would all appear simultaneously in the cytoplasm. As the nucleus went into true prophase, the number decreased and other microtubules appeared in the cytoplasm of the cell, aligned along what was almost certainly going to be the future spindle axis, and consequently perpendicular to the preprophase ‘equatorial band’ (Figs. 3, 10).

When the preprophase cell was cut in transverse section, so that continuous profiles of microtubules of the preprophase band could be seen around the wall, a careful scrutiny of the nuclear envelope occasionally revealed that other microtubules seen in transverse section were closely applied to this membrane, aligned along the spindle axis. It was quite likely that other microtubules running along the spindle axis were situated farther out in the cytoplasm. However, they would be very difficult to see against the dense background of ribosomal particles in the cytoplasm of root tip cells, and they were not found in these sections, although longitudinal sections (Fig. 3) indicated that they were present. They were visible in transverse section in the less dense cytoplasm of the coleoptile cambial cells, resembling those seen in the prophase cell shown in Fig. 12.

### *Prophase*

The prophase condition was clearly recognizable as the chromatin material became progressively more condensed; this often resulted in the nucleus having a banded appearance. As has frequently been described, the nuclear envelope progressively lost its identity, and, by late prophase, the densely stained chromosomal bodies were

present in a nuclear region which was not limited by a membrane (Porter & Machado, 1960); but the ground substance of this region, following glutaraldehyde/osmium fixation, was distinctly less heavily stained than that of the cytoplasm.

Following all methods of fixation there was never any indication that any of the wheat cells examined possessed a structure that might be equivalent to a centriole. The polar zone became delineated by the progressive development of organized, convoluted layers of endoplasmic reticulum; these were not extensively developed in cells of the root meristem (Fig. 9) (compare Porter & Machado, 1960) but were much more evident in coleoptile tissue. Careful observations of prophase cells showed that development of the spindle, which was occurring at this stage, involved the formation and organization of microtubules. Profiles of microtubules were seen at very early prophase running along the spindle axis in the cytoplasm around the nucleus (Fig. 12). Some of these were very closely apposed to the nuclear membrane which had ribosomes on the cytoplasmic membrane surface (Fig. 13). Frequently, long, fairly straight profiles of microtubules were visible nearer the pole region. In the root cells these were distributed as a cone-shaped umbrella of units radiating out from a small region of the polar zone (Fig. 9) and passing very close (tangentially) to the nucleus; at the middle of the cell they were often packed several units deep in the cytoplasm. It became apparent at this stage that penetration of the disintegrating nuclear envelope by the microtubules had started to occur (Fig. 9), although this was not extensive. The number of microtubules in the spindle region appeared to increase as prophase continued. Once the membrane had entirely dispersed, profiles of microtubules were visible between the rows of the chromosomal bodies.

The disposition of the microtubules in relation to the endoplasmic reticulum is interesting. In early prophase they were found mainly outside the nuclear envelope and were not always applied closely to it (Fig. 12). After the nuclear membrane had broken down, a number of the spindle microtubules could be seen in transverse section, distributed mainly among the elements of the endoplasmic reticulum which had penetrated the spindle region, around the condensed chromosomes.

#### *Metaphase, early anaphase*

In a typical metaphase cell of the root tip it was found that the distribution of the chromosomes in a metaphase plate was not always clear, and in the coleoptile cambial cells it was impossible to distinguish between metaphase and early anaphase in thin sections. The work of Bajer & Molé-Bajer (1963) showed that the active part of the chromosomes (that is, the kinetochores) moved into this position after prophase, and the mitotically-inert arms of the chromosomes trailed into the spindle region. This position was held until all the chromosomes were correctly aligned, whereupon the cell proceeded into anaphase.

The chromosome masses appeared to some extent to be aggregated or stuck together; microtubules penetrated these masses (Fig. 11), although an actual connexion to them, shown later in anaphase (Fig. 20), was more difficult to observe. By the time the metaphase configuration had been established in the root-tip cells the spindle region was almost entirely cleared of cellular organelles. Microtubules were

present, running throughout the length of the spindle, often passing between the chromosomes. The polar zones were still marked by the proliferation of elements of endoplasmic reticulum, a number of which penetrated downwards into the spindle region. It is interesting that almost always one of the two polar zones was seen to be far less developed than the other. This may have been due to the plane of sectioning which did not cut both zones equally. Microtubules penetrated into the polar zones and were often very intimately associated with the endoplasmic reticulum. This was most noticeable in the coleoptile cambial cells (Figs. 14, 15).

The cisternae of the endoplasmic reticulum became bloated in appearance and the contents of the lumen were electron-transparent (Fig. 18). Groups of microtubules were seen to be attached to chromosomes but no particular kinetochore structure was seen (Figs. 17, 20). An attachment of microtubules on two sides of a chromosomal mass was sometimes seen. The axis of the spindle in the long thin cells often lay diagonally across the cell; the resultant position of the chromosomes at late anaphase or early telophase can be seen in Fig. 21.

At higher magnification the orientation of the components of the spindle was very obvious, particularly in coleoptile cambial cells. In the nucleoplasm around the chromosomes, many vesicular bodies were visible, some being apparently small bloated segments of endoplasmic reticulum. These were interspersed and aligned with many profiles of spindle tubules in close proximity to them (Fig. 18). Farther away, towards the poles, it could be seen that several of the microtubules seen in the section were in close contact with, or sandwiched for part of their length between, the invading elements of endoplasmic reticulum (Figs. 14, 15). In a very few cases it appeared that there existed some sort of physical continuity between the walls of the microtubules and the membranous components of the spindle region (Fig. 17).

Study of transverse sections of the cell during this period of mitotic activity (when the spindle region was being invaded and apparently organized by both the endoplasmic reticulum and the microtubules) made the association between these two organelles more apparent. It was, however, difficult to ascertain the exact stage of the mitotic cycle by an examination of transverse sections. During 'presumptive metaphase' many transverse sections of microtubules could be seen and these were generally in close association with endoplasmic reticulum occurring in the peripheral regions around the chromosomes. Others were present in the nucleoplasm between the chromosomes, and again these were often in close proximity to the shorter, more bloated, cisternae of the endoplasmic reticulum found in this region. Occasionally, near the outer edges of the spindle, there appeared to be connexions between the microtubules and endoplasmic reticulum in the form of fine radiating filaments (Fig. 16).

At anaphase the organization of the microtubules in the spindle became more apparent. They appeared in large numbers running through the whole region, apparently unconnected to chromosomes. Some outer microtubules were curved to follow the shape of the spindle.

*Late anaphase and telophase*

Later stages of anaphase in the cells were clearly recognizable by the progressive migration of the chromosomes to each end or side of the cell. The trailing arms of the chromosomes were seen to lie along the contours of the spindle. The region between the chromosomes and the interzonal region between the two migrating groups contained many microtubules and some elements of the endoplasmic reticulum; most other cellular organelles were excluded from these regions. The chromosomal movement and the orientation of the spindle structure generally (comprising microtubules, endoplasmic reticulum, etc.) were directed towards a small area in the polar zone. During late anaphase elements of endoplasmic reticulum became more evident in the interzone region, where they sometimes became applied to the trailing arms of the chromosomes.

It was often apparent that the two groups of chromosomes had moved towards polar zones located at the sides of the cambial cells (Fig. 21). In the cell shown in Fig. 21 many spindle microtubules were present, and some of these can be seen in the interzone region between the chromosomes. At the polar zone itself, the chromosomal and interzonal microtubules all converged on a small focal area, but as usual they were never seen to terminate at any organelle. There was always a 'cap' of layered, convoluted endoplasmic reticulum in or over this zone.

At this stage the first signs of cell-plate formation became visible. In localized areas of the interzone region, the microtubules appeared to aggregate slightly and the vesicular components began to collect between the tubules; two distinct components were generally visible in the root-tip cells, the larger of them probably being phragmosomes (Porter & Machado, 1960). The vesicles appeared to fuse extending the young wall outwards at its edge. As this occurred it was noted that the microtubules were present only at the edge of the extending plate, where the vesicles were still being aligned; the orientation of the tubules was approximately radial with respect to the daughter nuclei (Fig. 23). This meant that they were often angled quite sharply away from the lateral walls by the time the plate extended to those walls. At the initial stages the microtubules passed right through the young plate area. As it extended, however, they appeared merely to end in the plate region (Fig. 23), which at this stage had many elements of endoplasmic reticulum traversing it. The actual number of microtubules present at a given cell plate must have been great. Fig. 22 shows a transverse section of the edge of a cell plate. These microtubules were found radiating out from the area between the chromosome arms during anaphase, and they increased in number throughout this stage.

By late telophase a new nuclear membrane formed around each daughter nucleus. Nothing has been observed to suggest that the membranes forming the new nuclear envelope at this stage were in any way distinguishable from the ubiquitous, cytoplasmic endoplasmic reticulum. By the time that the young cell wall was completed across the cell, most spindle tubules had disappeared and the other organelles then became redistributed as in the normal interphase cell.

## DISCUSSION

There can be little doubt that the spindle fibres seen in living cells are groups or bundles of microtubules (Bajer, 1965; Harris & Bajer, 1965). Since the movement of the components within the mitotic spindle of endosperm cells has been very completely described by Bajer and his colleagues (see Bajer & Molé-Bajer, 1963) it is important to correlate the fine-structural observations described here with the dynamic sequence of events seen in the living cell. With the exception of the preprophase band, the appearance of spindle microtubules at the ultrastructural level and their organization within typical mitotic cells in wheat follows very closely the pattern of birefringence observed by Bajer & Molé-Bajer. In prophase microtubules appear in the cytoplasm close to the nucleus and radiate out from each future polar zone. Inoué & Bajer (1961) have demonstrated an exactly equivalent birefringent nuclear 'envelope' in the cytoplasm of such cells. During later stages the general distribution of microtubules can be directly compared with the birefringence of the spindle shown by Bajer & Molé-Bajer (1963). Bajer (1965) observed that spindle fibrils became attached to the kinetochore regions of the chromosomes, and that the kinetochores of sister chromosomes became slightly separated at metaphase, so that a distinct clear patch appeared between them, even though the chromosomes were paired at this stage. These observations are directly comparable with the ultrastructural studies in endosperm tissue (Harris & Bajer, 1965) and with the results obtained with the coleoptile tissue described here (Fig. 19). The involvement of microtubules in metakinesis has not been clearly established in the tissues examined in this study, though it seems likely that they are concerned in this process. Recently Harris (1965) has shown that microtubules became attached to chromosomes in prometaphase sea urchin eggs; she says: 'The data presented . . . support the idea that chromosomal fibres are instrumental in moving the chromosomes to the metaphase plate.' From observations made on the movements of the chromosomes during cell division it is evident that the kinetochore is almost always the leading point of the movement, and thus the attachment of the microtubules to this region (Harris, 1961, 1962; Roth & Daniels, 1962) may be very significant in any theory of chromosomal movement. It is probably also important that these microtubules form continuous tracts leading to the mitotic poles to which the chromosomes move at anaphase.

Forer (1965) has shown by ultraviolet microbeam irradiation of the spindle of spermatocytes *in vivo*, that the birefringent spindle fibres are apparently undergoing active movement during mitosis. Using this technique, he destroyed the birefringence in small regions of the spindle; these regions were then seen to move polewards immediately.

Since a large number of the microtubules (seen as spindle fibres with the optical microscope) in the spindle are not attached to chromosomes but run between them, they may also have a cytoskeletal function and serve to direct movement of vesicles, etc., along the channels formed between them. This latter function becomes very apparent at cell-plate formation, when the vesicles giving rise to the plate, although apparently produced in the interzonal region, are seemingly directly lined up and



aggregated at the plane of cell division (Fig. 21). If the microtubules do function in this way it would be expected that large concentrations of these organelles would be found at the edge of the expanding cell plate and this can be clearly seen in Fig. 22. Even when the cell plate is not formed at the mid-plane of an isodiametric cell and becomes curved, as is apparent in the asymmetric cell division which occurs in the formation of leaf stomata, a similar distribution of the microtubules and vesicles is found (Pickett-Heaps & Northcote, 1966).

The appearance of the preprophase band is of interest, and some important premitotic activity must be associated with this structure. The results presented on meristematic division must be compared with a similar grouping of microtubules seen in the divisions of the stomatal complex (Pickett-Heaps & Northcote, 1966). In the latter cells the position of these microtubules coincides with the regions at the mother cell wall where the future cell plate joins it, and consequently this position indicates the plane of division of the cell. The position of the preprophase grouping of microtubules in the two premitotic epidermal cells is very clearly related to the position of the adjacent guard mother cell, and it is not unlikely therefore that such a relationship is also evident in the meristematic cells, where an influence on the plane of division of one cell by neighbouring cells must be maintained for the formation of an organized meristematic cell pattern.

The importance of this phenomenon therefore seems to lie in the possibility that it might be at least one of the initial manifestations at the ultrastructural level of a mechanism whereby such a pattern of cells and cell division in an organized tissue is propagated by the interaction of the young cells.

The origin and fate of these preprophase tubules has not yet been determined. They may well be organized into the developing spindle structure. If the tubules moved from their wall position into the spindle, the overall profile of a tubule during this movement would be a complex curve in three dimensions, and thin sections of the cells would not readily indicate the path taken by such a curving tubule. Portions of microtubules away from the main groupings can often be seen in sections of preprophase cells (Figs. 3, 10).

The functions, synthesis and breakdown of spindle microtubules are very obscure. Some preliminary radio-autographic work has suggested that they are protein in nature (Mangan, Miki-Noumura & Gross, 1965). In many flagellate and ciliate animal and plant cells, basal bodies have been shown to extrude filaments which form the core of the motile organ (e.g. Sorokin, 1962; Szollosi, 1964; Gall & Mizukami, 1963) and flagella and cilia in general seem to be proteinaceous in nature (Jones & Lewin, 1960). The involvement of centrioles in the organization of the spindle and their close relationship to basal bodies is well known.

Plant cells do not have centrioles and if the spindle structure of microtubules are partly protein in nature some other cytoplasmic organelle must be involved in their organized synthesis. The work presented here has shown an association between the endoplasmic reticulum and the microtubules and since the endoplasmic reticulum is concerned with protein synthesis and is characteristically distributed during mitosis in the spindle it might well be important in microtubule synthesis. Another possibility,

however, is that the endoplasmic reticulum is supplying metabolites or ions either for transport inside the tubules or for the control and/or maintenance of the tubules' function.

One of us, J.D.P.-H., gratefully acknowledges the receipt of an Agricultural Research Council Studentship, during the tenure of which this work was done.

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(Received 23 August 1965)

## ABBREVIATIONS

<i>ch</i>	chromosome	<i>n</i>	nucleus
<i>cp</i>	cell plate	<i>nc</i>	nucleolus
<i>er</i>	endoplasmic reticulum	<i>pz</i>	polar zone
<i>m</i>	mitochondrion	<i>w</i>	cell wall

Figs. 1-4. Longitudinal sections of root meristematic cells at preprophase. Figs. 1 and 3 show a higher magnification of regions *a* and *b* respectively of the cell in Fig. 2.

Fig. 1. The microtubules of the preprophase band can be seen along a limited region of the cell wall. They appear in short longitudinal section because of the angle of the plane of the section.  $\times 26000$ .

Fig. 2. The appearance of a typical preprophase nucleus is shown. The chromatin is slightly aggregated and the nucleolus is dispersed with material radiating out into the nucleoplasm. (The black spot in the centre of the nucleus is a staining artifact.)  $\times 7000$ .

Fig. 3. Microtubules of similar appearance to those shown in Fig. 1, but at the opposite lateral wall of the cell, can be seen. A spindle microtubule running perpendicular to those of the preprophase band is indicated by the arrow.  $\times 29000$ .

Fig. 4. The preprophase band of microtubules has been cut transversely. They are spread along the wall for a distance of  $2.6 \mu$ .  $\times 43000$ .

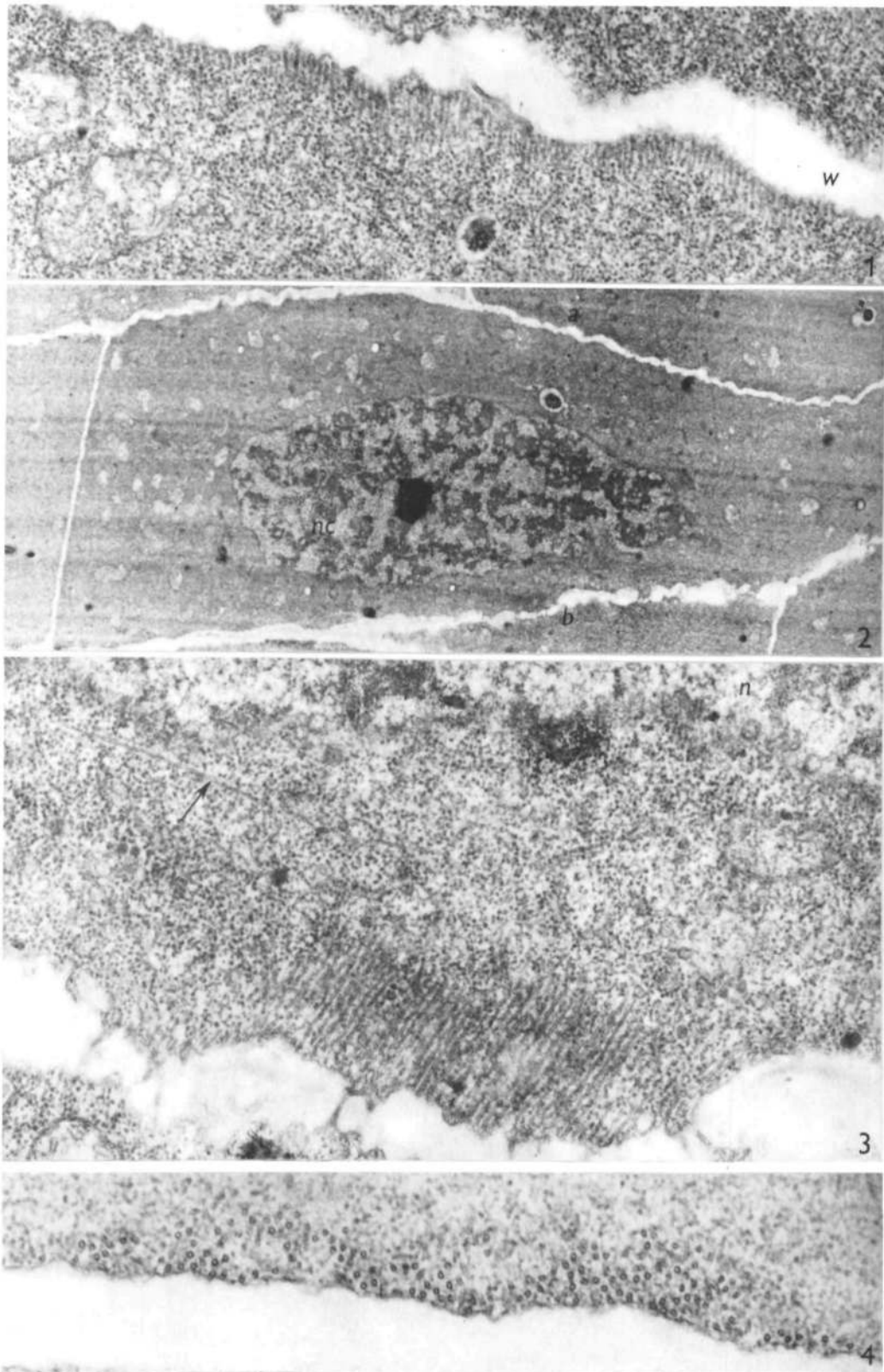


Fig. 5. Longitudinal section of a coleoptile cambial cell. Part of the early preprophase band of microtubules (cut transversely) by the wall can be seen.  $\times 38000$ .

Figs. 6-8. Transverse section of a root meristematic cell cut through the preprophase band.

Fig. 6. The microtubules at the corner of the cell curve away from the wall.  $\times 47000$ .

Fig. 7. The preprophase band of microtubules is shown along the wall about four units deep.  $\times 48000$ .

Fig. 8. Similar to Fig. 7. Some of the microtubules of the preprophase band appear to veer away from the wall (arrow).  $\times 44000$ .

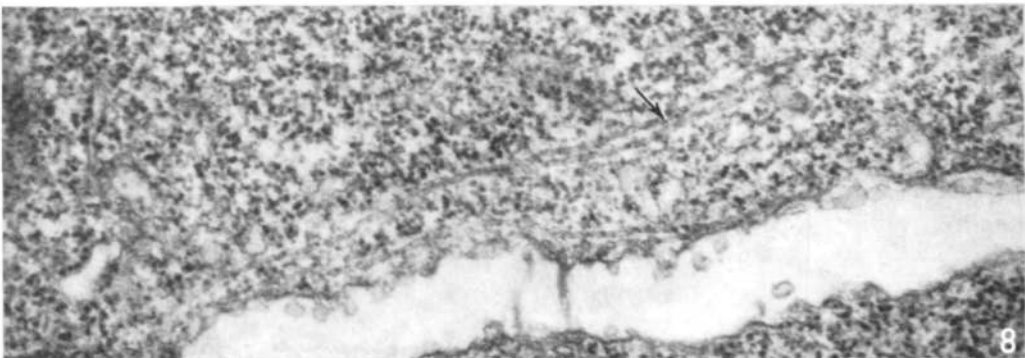
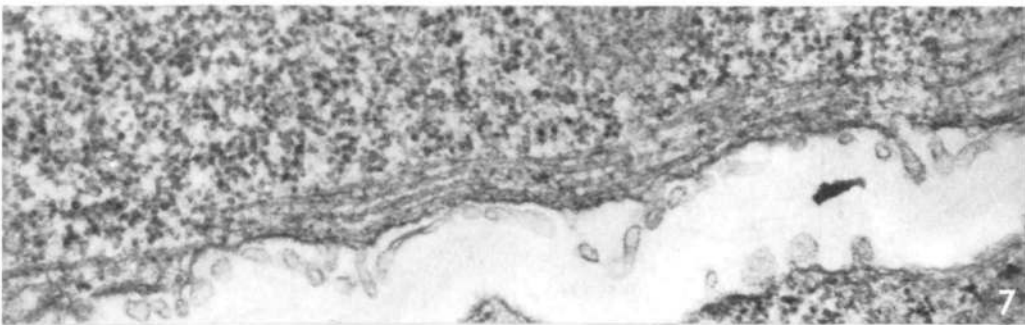
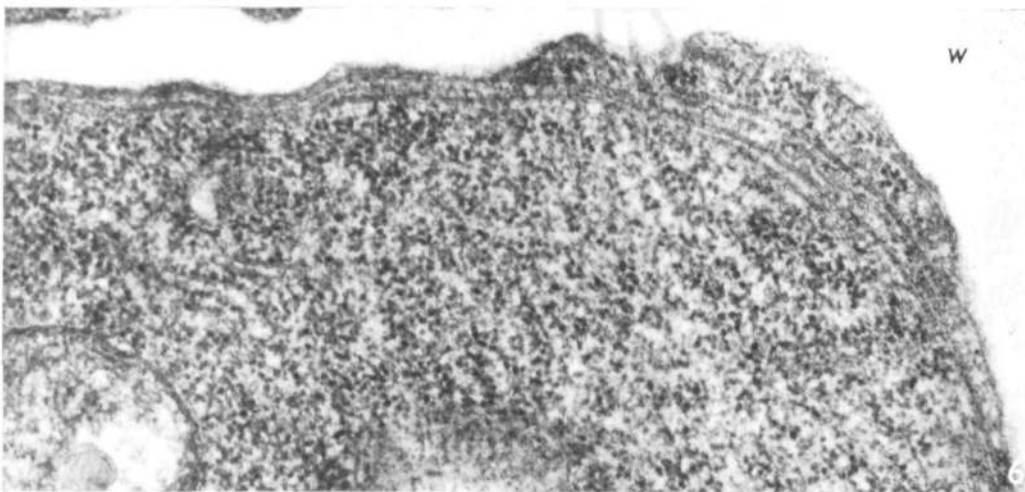
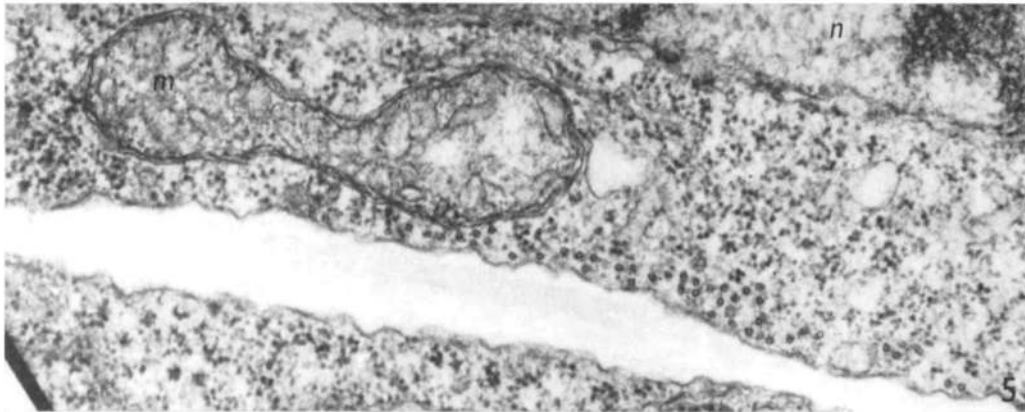
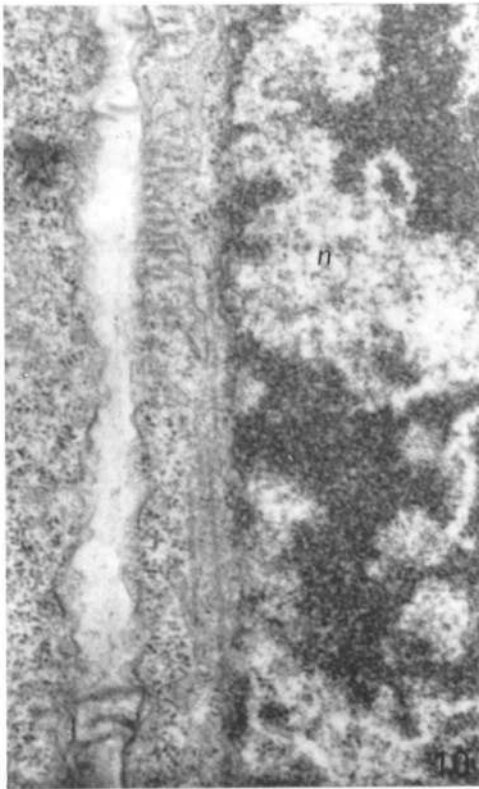
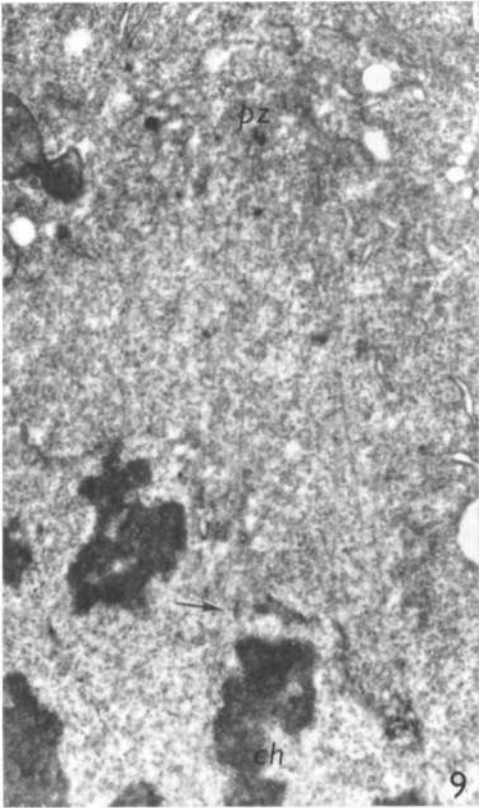


Fig. 9. Longitudinal section of a meristematic root cell at prophase. Microtubules are shown radiating out from a polar region at one end of the cell. Some microtubules (arrow) have penetrated the nucleoplasm.  $\times 18000$ .

Fig. 10. Longitudinal section of a coleoptile cambial cell at early prophase. Several spindle microtubules can be seen adjacent to the nuclear envelope. The preprophase band of microtubules is still visible at this stage near the wall. (Compare with Fig. 3.)  $\times 33000$ .

Fig. 11. Longitudinal section of coleoptile cambial cell at metaphase. Profiles of the endoplasmic reticulum can be seen penetrating into the mitotic spindle and between the chromosomes. Many of the spindle microtubules are applied to the lamellae of the endoplasmic reticulum (see Figs. 14 and 15); a few of these are indicated by the arrows.  $\times 11000$ .





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Fig. 12. Transverse section of coleoptile cambial cell at prophase. Spindle microtubules (arrowed) cut in transverse section can be seen in the cytoplasm close to the nucleus.  $\times 51\,000$ .

Fig. 13. Longitudinal section of root meristematic cell at prophase. Microtubules aligned along the spindle axis can be seen near the intact nuclear membrane. (Compare with Fig. 10.)  $\times 41\,000$ .

Figs. 14, 15. Longitudinal section of coleoptile cambial cell at metaphase.

Fig. 14. The microtubules (arrowed) are each sandwiched between elements of the endoplasmic reticulum which is penetrating the spindle region between the chromosomes.  $\times 56\,000$ .

Fig. 15. The microtubules (arrowed) are closely applied to the endoplasmic reticulum. This photograph is a higher magnification of the cell shown in Fig. 14 and is of a region away from the chromosomes, nearer one of the poles of the spindle.  $\times 41\,000$ .

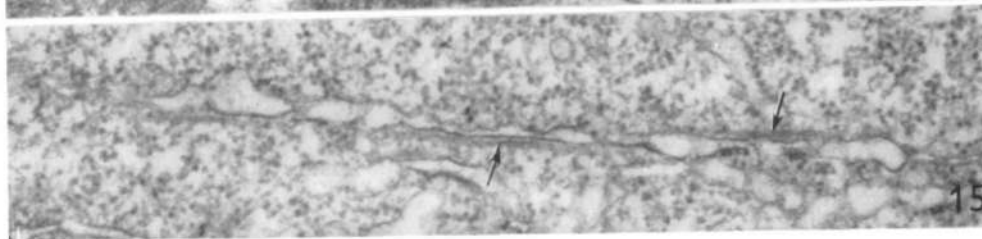
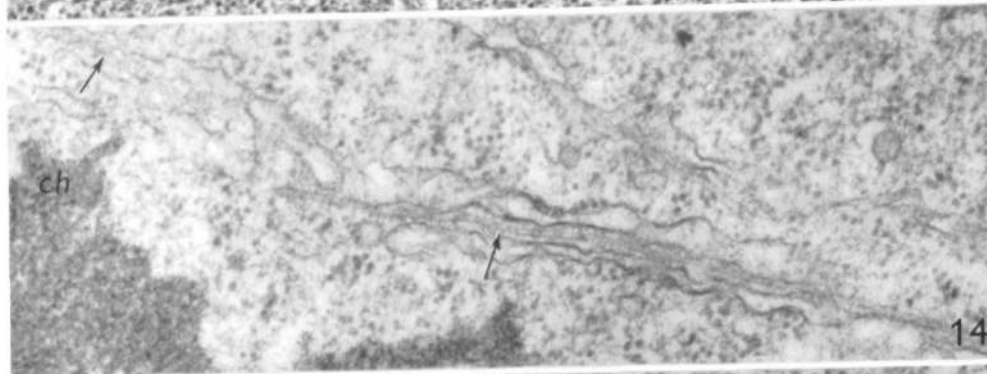
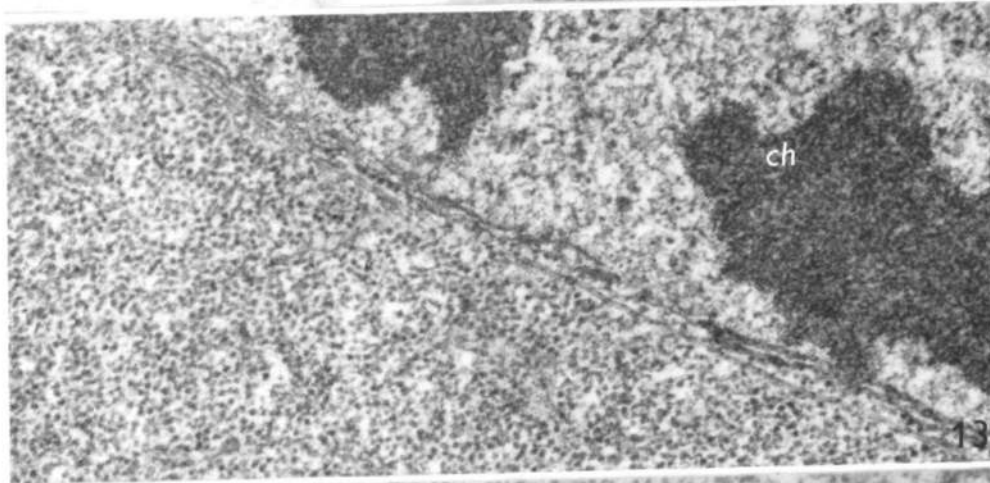
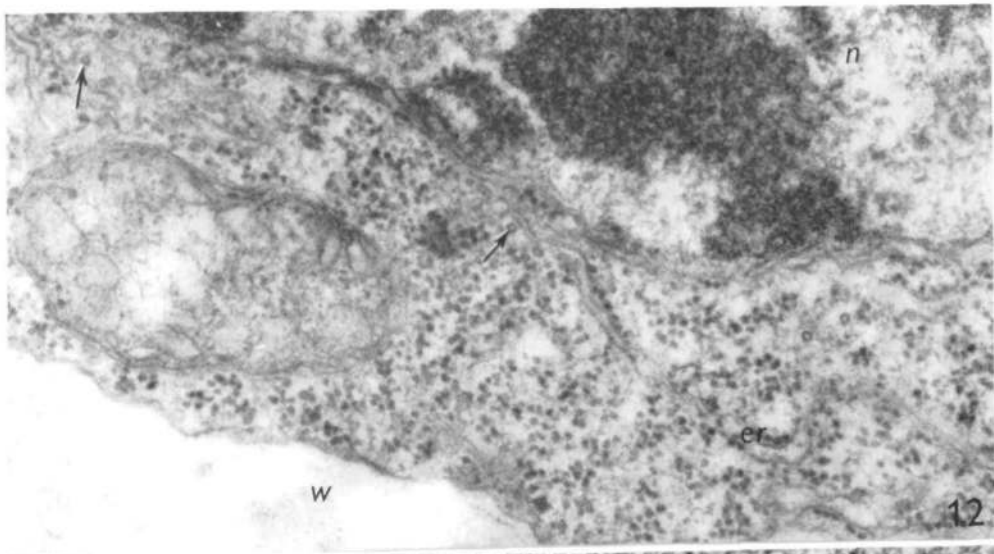


Fig. 16. Transverse section of a coleoptile cambial cell at metaphase. Microtubules in transverse section near the endoplasmic reticulum can be seen. At the arrow, fine strands appear to connect the microtubule to the lamella of the endoplasmic reticulum.  $\times 110000$ .

Figs. 17, 18. Longitudinal sections of coleoptile cambial cells at early anaphase or late metaphase.

Fig. 17. A microtubule can be seen to end at a chromosome (double arrow). At the single arrow the microtubule appears to be continuous with a membranous component of the spindle region.  $\times 38000$ .

Fig. 18. A group of microtubules can be seen aligned with vesicular components of the spindle.  $\times 37000$ .

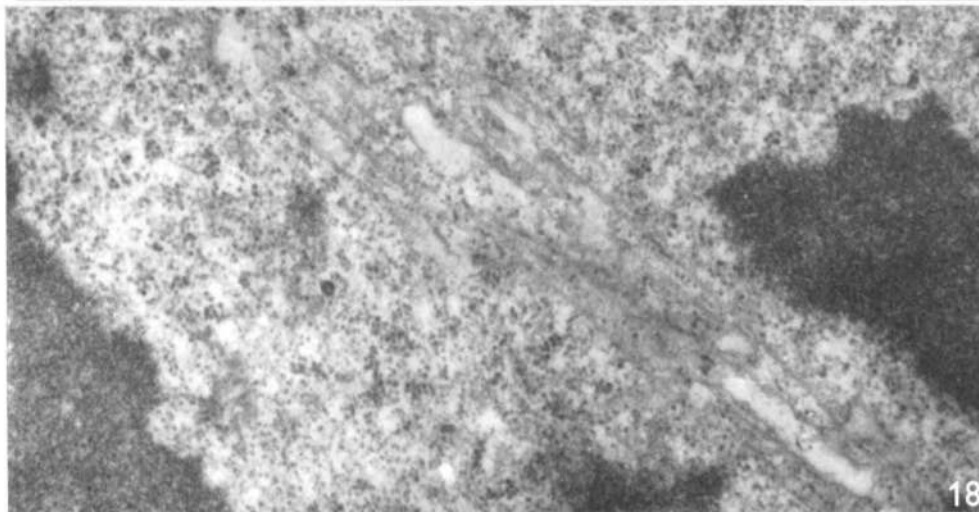
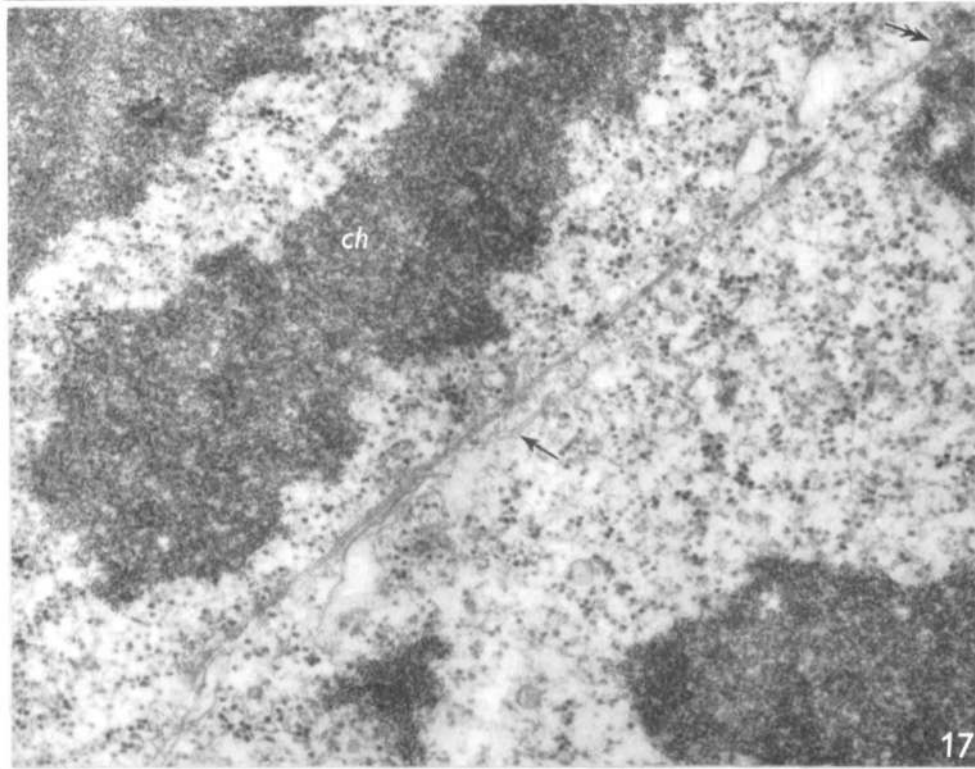
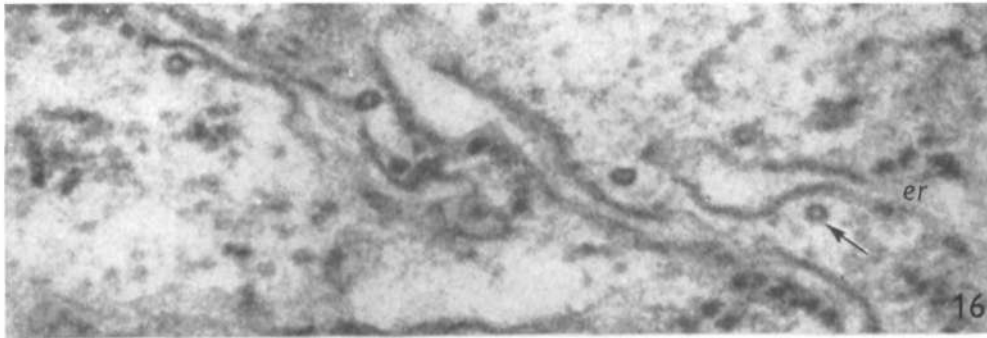
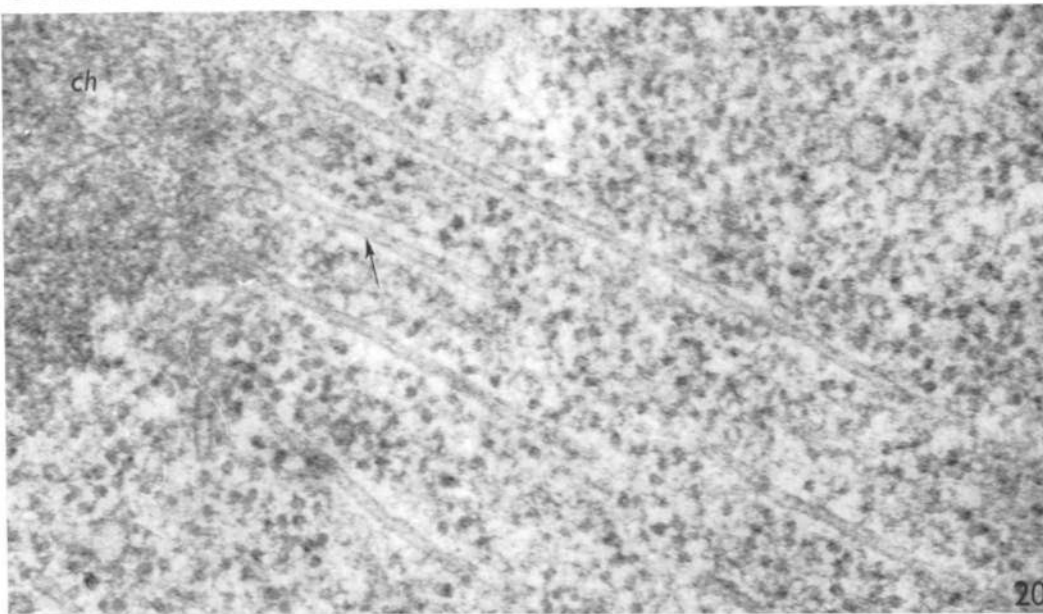
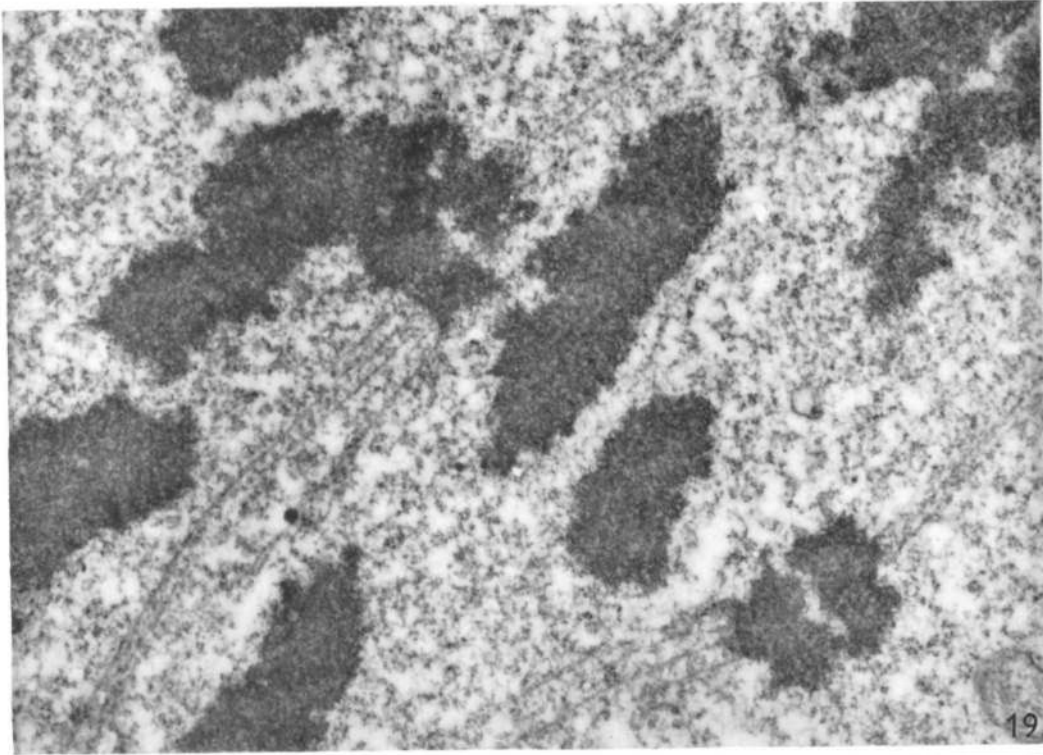


Fig. 19. Longitudinal section of a parenchymatous cell, in the coleoptile cambial region, at metaphase. Groups of microtubules attached to paired chromosomes can be seen.  $\times 19000$ .

Fig. 20. Longitudinal section of a root meristematic cell at anaphase. Several microtubules can be seen to end at a chromosome.  $\times 90000$ .



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Fig. 21. Longitudinal section of a coleoptile cambial cell at late anaphase or early telophase. The daughter nuclei are reforming at each side of the cell adjacent to the polar zones. Interzonal microtubules are present and the cell-plate components, vacuoles and other organelles have been aligned across the cell.  $\times 4200$ .

Fig. 22. Transverse section of a root meristematic cell at telophase. Microtubules in transverse section are seen in large numbers among the vesicular components (arrowed) at the edge of the cell plate and distributed between the edges of the plate and the mother-cell wall.  $\times 43000$ .

Fig. 23. Longitudinal section of a root meristematic cell at telophase. The cell plate has extended completely across the cell; near the mother-cell wall the microtubules still present can be seen to end at the plate.  $\times 42000$ .



