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Longitudinal microtubules and their functions during asexual reproduction in *Paramecium tetraurelia*

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SUMMARY

Longitudinal microtubules appear in *Paramecium tetraurelia* when cells are ready to divide (*ca.* interfission age 0·9). They arise in the longitudinal cortical ridges between kineties and form, in each ridge, an incomplete circlet of 15–18 microtubules, disappearing 10–15 min after separation of the fission products (*ca.* interfission age 0·03–0·04). Vinblastine, colchicine, colcemid, mercaptoethanol, and cold treatments all result in loss of these microtubules, rounding up of the cells, and some suppression of cell division. The tubules are thought to play a role in cellular elongation, morphogenesis, and separation.

1. INTRODUCTION

In the cortex of *Paramecium* the regularly observed microtubules are the post-ciliary and transverse tubular fibrils associated with the kinetosomes. However, Ehret, Albinger & Savage (1964) have reported that supra-kinetodesmal micro-tubules occur in the longitudinal ridges during fission in *P. bursaria*. Jurand & Selman (1969) report seeing similarly placed microtubules only during fission in *P. tetraurelia*. Our present study confirms this. We have found that the appearance, number, and arrangement of the longitudinal microtubules (Allen, 1967; see Discussion) are chronologically related to the course of binary fission. Further, by exposure to chemical inhibitors of microtubules the probable functions of these have been investigated. A preliminary report was published earlier (Sundararaman & Hanson, 1974).

2. MATERIALS AND METHODS

The organism used was *P. tetraurelia* (Sonneborn, 1975), stock 51, mating type VII, sensitive, maintained in Cerophyl medium innoculated with *Aerobacter cloacae* (Hanson, 1962). Cells were maintained and all experiments conducted at room temperature (25 °C). Cells on the verge of fission (predividers *ca.* interfission age 0·90 (Hanson, 1974)) are easily identifiable under the dissection microscope because of a slightly flattened ventral side and their larger size. From this stage they take about 25 min to complete fission, and although the furrow site has been established, it is not distinctly seen until after another 5–10 min. Predividers were isolated from

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a log phase culture in batches of 20–25 and fixed at different times thereafter until 30 min after cell separation. Fixation was done with 6% glutaraldehyde in 0·1 M phosphate buffer at pH 7·2 with postfixation in 1% osmium tetraoxide in the same buffer. Cells were then dehydrated in an ethanol series and embedded in Epon–Araldite. Sections were cut with an LKB ultramicrotome and stained with uranyl acetate and lead citrate. Sections were observed in a Philips 300 electron microscope. For a given stage of fission, grids from 5–10 different cells were observed.

In order to investigate the various functions of the longitudinal microtubules, predividers were treated with vinblastine (100–150 μg/ml), colcemid (1 mg/ml), colchicine (15–20 mg/ml) and β-mercaptoethanol (0·05 M). Also, the cells were kept in the cold (4 °C). For each experiment, predividers were exposed to the appropriate chemical in batches of 10–20 cells and the treatment time was 40–60 min. At the end of each treatment, cells were fixed for electron microscopy. Experiments were repeated three or more times with each chemical. Both treated and control cells were also photographed using phase-contrast microscopy.

3. RESULTS

(i) *Normal cells*

Several electron microscopical studies have been conducted on the cortex of *Paramecium* (Hufnagel, 1969; Jurand & Selman, 1969; Allen, 1971; Ehret & McArdle, 1974). These investigators agree that the cortex of a non-dividing paramecium is devoid of longitudinal microtubules in the longitudinal ridges. Cross-sections of a non-dividing cell show only granulo-fibrillar material in the longitudinal ridges (Allen, 1971) (Fig. 1). Even in cells fixed after 5 h (generation time 6 h), no sign of microtubules is seen. However, when the predividers are observed (*ca.* interfission age 0·90 and later), longitudinal microtubules are seen in the apex of longitudinal ridges (Fig. 2). At an early stage only 3–6 microtubules are present. In cells fixed at a later stage (about 15 min before fission) 8–10 microtubules are seen, arranged in a curve (Fig. 3). Cells in an advanced stage of fission (almost separated) show 15–18 microtubules (Fig. 4); this maximum number of microtubules is also seen in cells fixed immediately after the completion of fission (Fig. 5). At this stage the microtubules are arranged in almost a circle. Within about

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Plate 1

Fig. 1. Transverse section of *P. tetraurelia*, cortex of interphase cell. Kinetodesmata (*k*) are seen stacked one above the other in the longitudinal ridges which are devoid of microtubules. × 40000.

Fig. 2. Cross-section of a longitudinal ridge in a predivider (about 15 min before the completion of fission). × 68000.

Fig. 3. Cross-section through the cortex of a cell which has just begun to divide. Note an increase in number of microtubules as compared to Fig. 2. × 68000.

Fig. 4. Cross-section through the cortex of dividing cell with a well-advanced fission furrow. The microtubules are seen in each ridge; they are arranged in an incomplete circle. × 51000.

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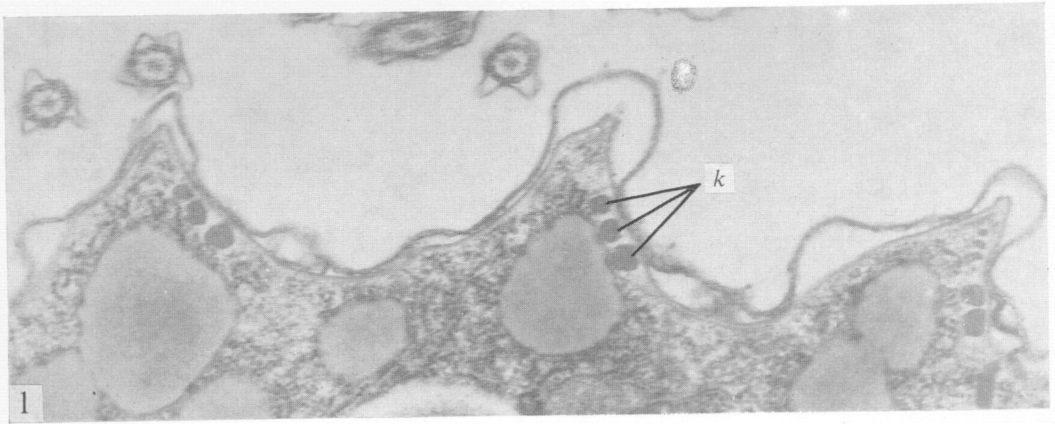

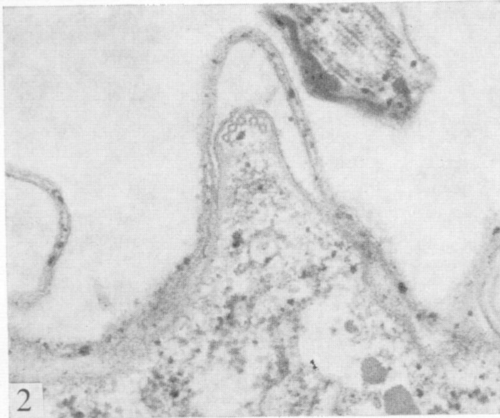

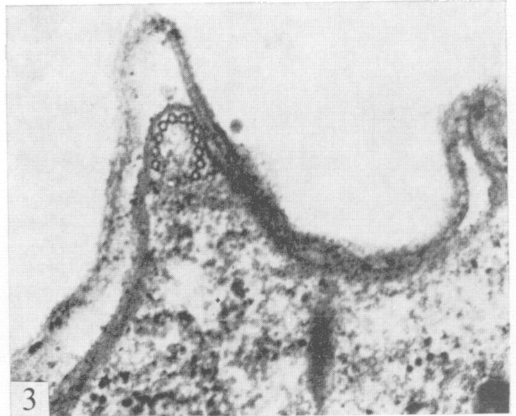

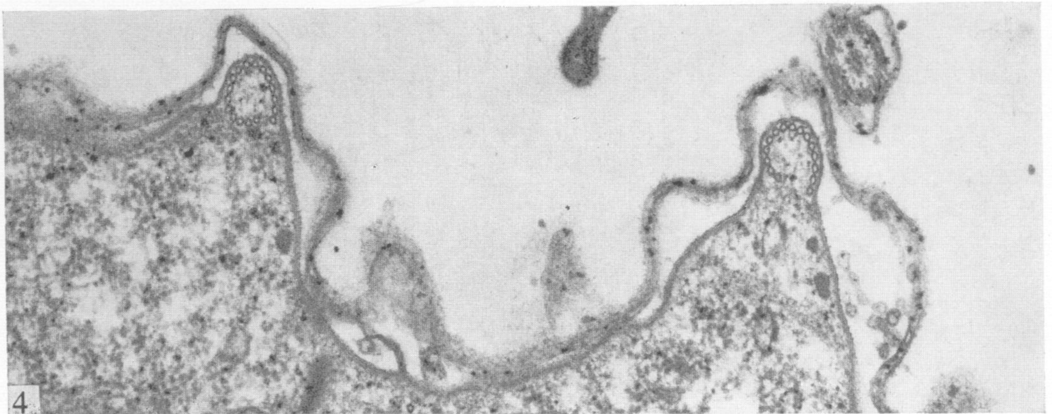

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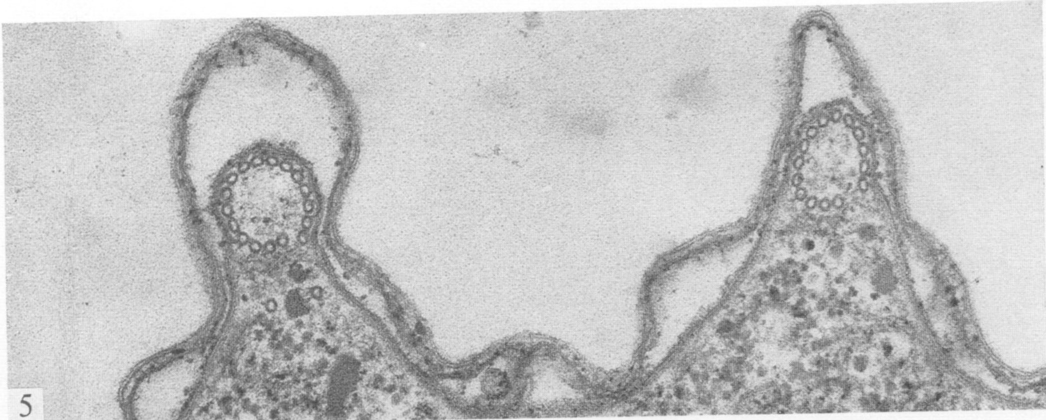

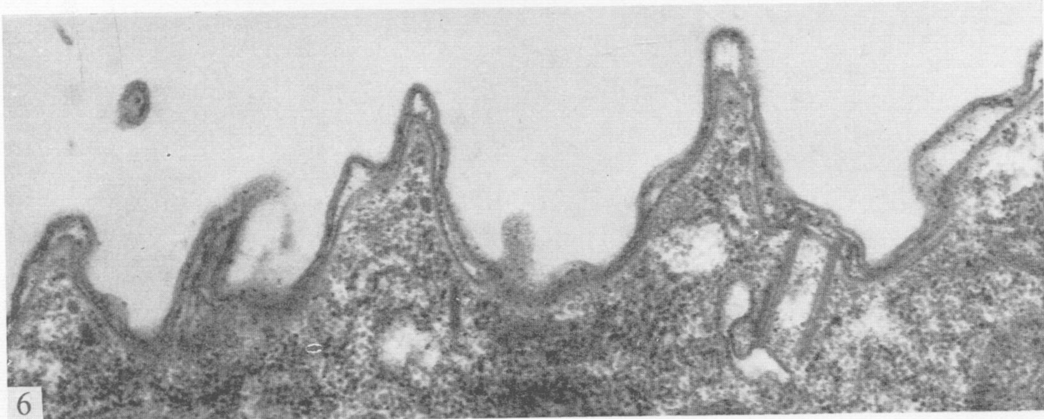

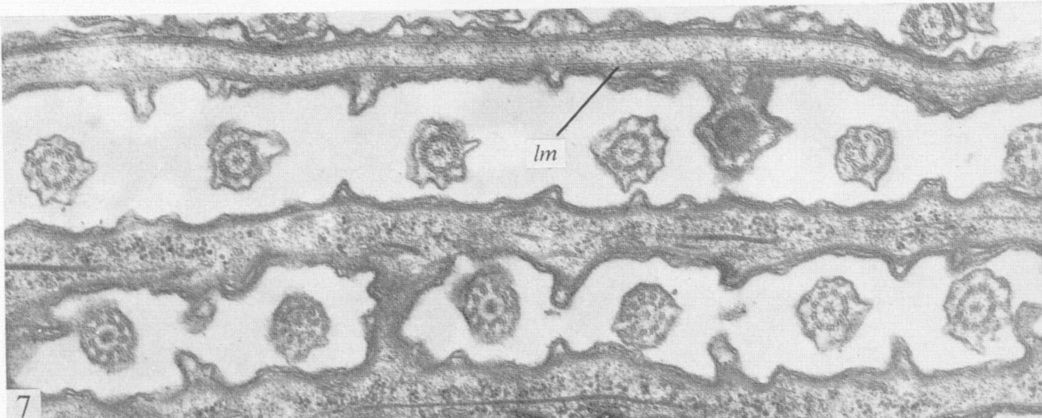

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10–15 min after separation (*ca.* interfission age 0·03–0·04) the microtubules disappear (Fig. 6). The diameter of the longitudinal microtubules is about 25 nm and they are present throughout the length of the dividing paramecia. Although the length of individual microtubules was not determined, some were seen to extend the length of at least a few ciliary units (Fig. 7). Also, these microtubules were seen in all the ridges observed.

The fate of the longitudinal microtubules is not clear during the final constriction of the fission furrow. In very early stages of furrow formation, the microtubules are seen to extend through the furrow region. As the furrow deepens, it seems that the microtubules somehow break at the fission line and curve into the deepening furrow from both sides.

(ii) *Effect of inhibitors*

When predividers are treated with vinblastine the process of binary fission is arrested and the cells become rounded as compared with controls (Figs. 8, 9). In 80% of the division-arrested cells, furrowing progresses but final cell-separation is inhibited as long as the cells are under the influence of the chemical. Those cells which complete fission while being treated with vinblastine do so with a delay of 15–20 min as compared to the untreated controls. After removal from vinblastine, cell separation is seen in those cells previously inhibited.

Vinblastine treatment results in the disappearance of longitudinal microtubules. Although the kinetosomes are not affected by vinblastine treatment, paracrystalline structures are seen frequently in the longitudinal ridges and aggregations of membranous vesicles are also seen in the surface region of the treated cells (Fig. 10). The paracrystalline structures appear comparable to those described by other workers (Bensch & Malowista, 1969; Bryan, 1971). Paracrystalline structures are not seen in the cortical region of non-dividing cells treated with vinblastine.

Similar to the effect of vinblastine, colcemid and colchicine treatments also result in rounding of cells and significant division arrest (Fig. 11). In colchicine, cytokinesis progresses but final cell separation is inhibited in more than 80% of the treated cells. Although an inhibitory effect on binary fission is seen with 1 mg/ml colcemid, the predividers treated with 10 mg/ml or lower concentrations of colchicine behave and complete fission as normally as the controls. Concentrations between 15–20 mg/ml of colchicine are required to achieve an inhibitory effect on cell division. The longitudinal microtubules are found to be sensitive to colcemid and colchicine treatment. In colchicine treated cells, 2–4 microtubules are seen in some of the

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PLATE 2

Fig. 5. Cross-section through a cell immediately after cell separation. × 80000.

Fig. 6. Cross-section through a cell 15 min after separation of the fission products. The longitudinal microtubules have already disappeared. × 47000.

Fig. 7. Longitudinal section through the surface of a dividing animal. The longitudinal microtubules (*lm*) extend continuously throughout the visible length of the ridge. × 33000.

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ridges, while in most of the ridges they are not seen (Fig. 12). In these, filamentous materials are seen instead of the microtubules.

The longitudinal microtubules are also found to be very sensitive to mercaptoethanol and cold treatment (Fig. 15). Further studies with mercaptoethanol will be reported in a subsequent publication.

4. DISCUSSION

Ehret, Albinger & Savage first described longitudinal microtubules in *P. bursaria* and termed them 'suprakinetodesmal microtubules'. They were thought to be progenitors of kinetodesmata (Ehret *et al.*, 1964; Ehret & McArdle, 1974) since they were seen only in those ridges where fewer kinetodesmata were present. But in *P. tetraurelia* they are observed in all the longitudinal ridges at the time of asexual reproduction. Although Jurand & Selman (1969) observed these microtubules only during fission in *P. aurelia*, they did not mention any chronological or functional relation to the fission process. In another ciliate, *Nassula*, subpellicular microtubular bundles extend along the length of each kinety at the start of fission (Tucker, 1971), and in *Tetrahymena* longitudinal arrays of microtubules are always present between the kinetodesmata and the cell membranes (Allen, 1967). These microtubules have not been seen in *P. caudatum* (Allen, 1971).

The fact that a specific correlation exists between the longitudinal microtubules in *P. tetraurelia* and binary fission suggests that they are significant for the normal completion of cell division in *Paramecium*. Furthermore, the experiments with chemicals known to affect microtubules confirm that suggestion. The following discussion will therefore focus on the possible role of microtubules in asexual reproduction, a time when a single cell undergoes its phenotypic transformation into two cells. Cellular elongation, morphogenesis, and separation will be considered in turn.

(i) *Elongation*

Predividers undergo a rapid increase in length just prior to and during cytokinesis. This does not correlate well with increase in number of ciliary units (Kaneda & Hanson, 1974). Hence, the question is open as to what achieves cellular elongation at this time. The role of microtubules in elongation of mitotic figures suggests an answer as does their probable role in cellular elongation (Burnside, 1971; Bryan, 1974). In support of that view, our results are confirmatory from three points of

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PLATE 3

Fig. 8. Light micrograph of dividing control animals.

Fig. 9. Light micrograph of vinblastine treated cells. Furrowing has progressed but cell separation is inhibited.

Fig. 10. Longitudinal section through the surface area of vinblastine treated cell. Paracrystalline structures (*p*) are seen in the longitudinal ridge area, longitudinal microtubules are absent. Note, also, the aggregation of membraneous material (arrow). × 36000.

Fig. 11. Colchicine treatment. Cells are rounded and furrowing is arrested at about this stage.

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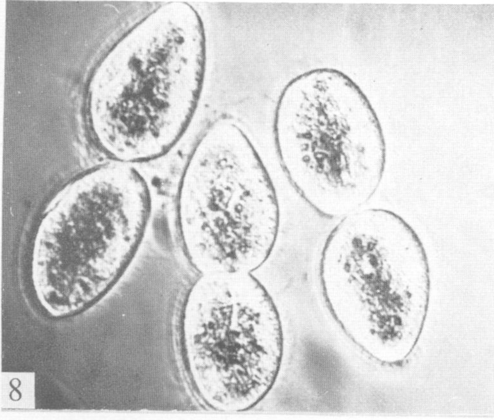

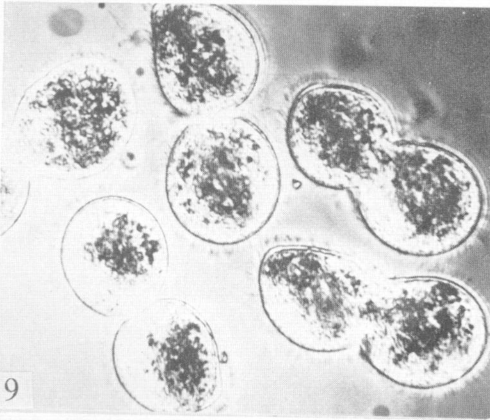

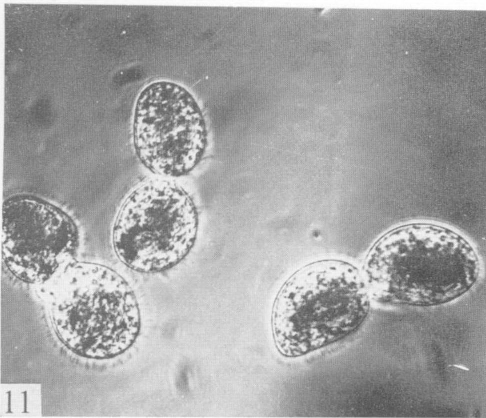

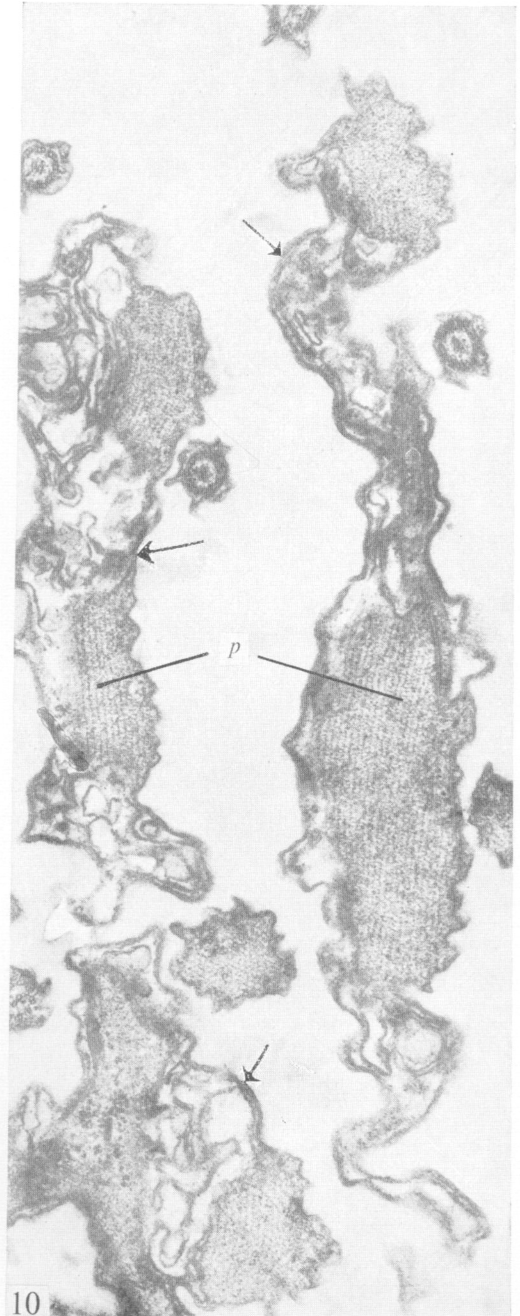

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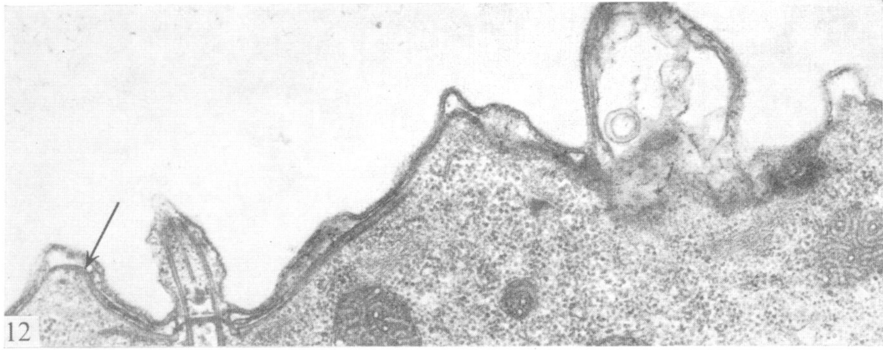

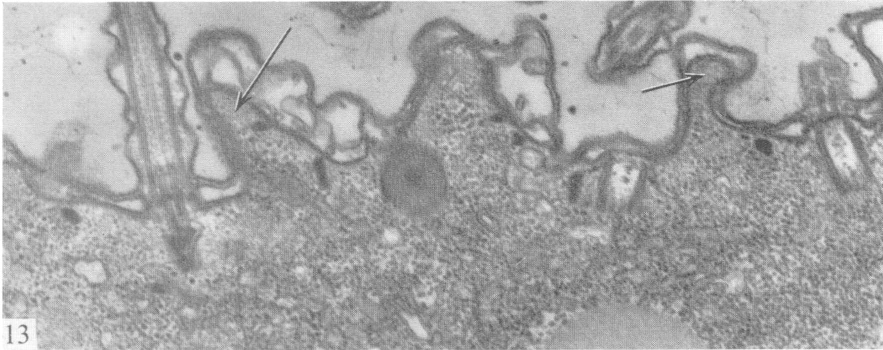

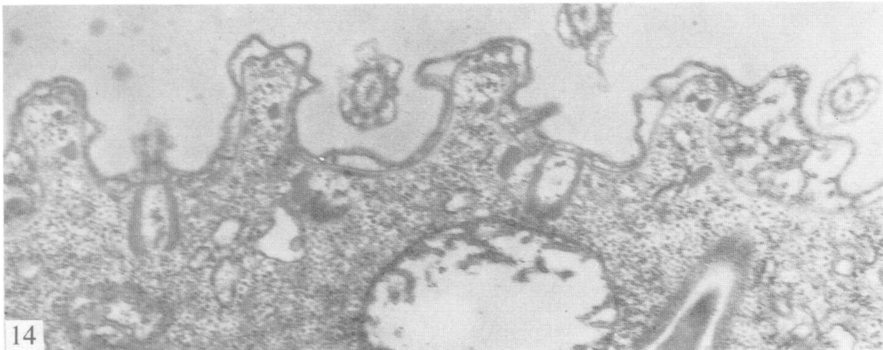

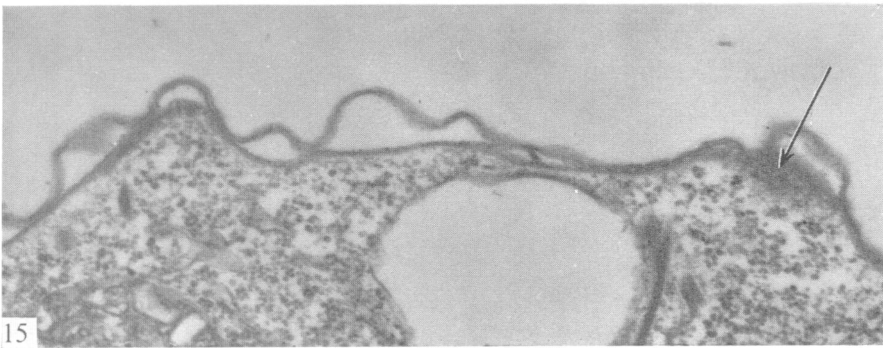

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view: (*a*) the occurrence of the longitudinal microtubules at times of maximal cellular elongation correlates perfectly with the postulated function; (*b*) their position, as longitudinal, cortical structures, is also consistent with the postulated function; and (*c*) the destruction of the tubules by vinblastine, colchicine and colcemid, results in suppression of elongation, which is predictable if the proposed function is correct.

As to how the elongation is carried out, there is no clear answer yet. Our observations are consistent with the mechanism preferred by Burnside (1971), i.e. transport of cytoplasmic constitutents from one part of the cell to another which lengthens the cell and allows for further terminal growth of the tubules. (See, also, review by Bryan, 1974.) Evidence is presently accumulating that suggests membrane transportation, with subsequent insertion into the surface ciliary units, may be the mechanism which achieves this elongation (Fig. 10). This will comprise a subsequent report. Allen (1974) presents convincing evidence for microtubule related transportation of food-vacuole membranes in *Paramecium*.

(ii) *Morphogenesis*

The morphogenetic features to be emphasized here are the moulding of cell form and the new spatial array of cellular components. In particular, the proter obtains a new posterior end with its special components and likewise the opisthe obtains a new anterior. What is the evidence that the longitudinal microtubules play a role in these processes? First, there are reasons from other work to suggest such a role. Kennedy (1969) and Tilney (1971), in particular, have emphasized that microtubules are essential to the determination of cell shape. This has been found in work on the heliozoan, *Actinosphaerium nucleofilum* (Tilney & Porter, 1967) and in the formation of primary mesenchyme in the sea urchin *Arbacia punctulata* (Tilney & Gibbins, 1969). In *Tetrahymena pyriformis*, hydrostatic pressure is known to degrade the longitudinal microtubules with consequent rounding of the cells (Kennedy & Zimmerman, 1970). In *Paramecium tetraurelia* the maintenance of cellular form is demonstrably dependent on the infraciliary lattice (Sibley & Hanson, 1974). The role of this microfilamentous cytoskeleton is problematical during fission. Its presence is not clearly demonstrated in the area of the fission plane and it must degenerate to some extent to allow for constriction of the furrow. It is possible that the longitudinal microtubules support cellular form at this time, acting as ribs between the kineties. This postulated function is consistent with

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PLATE 4

Fig. 12. Cross-section through an animal treated with colchicine. Microtubules are possibly present in one ridge (arrow) while no microtubules are seen in the others. × 37000.

Fig. 13. Cross-section of an animal treated with colcemid. No microtubules are seen; instead filamentous structures are seen, presented here in cross section (arrow). × 38000.

Fig. 14. Cold-treated cells. Microtubules are not seen in any of the ridges. × 40000.

Fig. 15. Mercaptoethanol treated cell. Only a few microtubules are possibly present in the left ridge. Filamentous materials are indicated (arrow). × 48000.

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the results of exposure to chemicals which prevent microtubule formation, i.e. normal cellular form is not preserved, the affected cells round up, and the microtubules in question are absent (Figs. 10–15).

But microtubules are clearly not the whole story in cellular morphogenesis at this time in the cell cycle, for their position and postulated function cannot explain the cytokinetic furrow even though the presence of microtubule inhibitors also inhibits final cell separation.

(iii) *Cytokinesis*

Various workers (Schroeder, 1970; Jurand & Selman, 1969; Selman & Perry, 1970; Szallosi, 1970; Tucker, 1971) have described microfilaments as postulated by Marsland & Landau (1954) in the furrow region of dividing cells. However, in *Paramecium*, Jurand & Selman (1969) find them in the mid-region of the cell whether fission is in progress or not. Our own observations (Sundararaman & Hanson, unpublished) confirm this but we would add that the amount of this material, though it could plausibly initiate and carry out the greater part of the constriction process, would not necessarily account for final separation of the fission products. In fact, the filaments appear to be in the way at this last step. Tucker (1971), in *Nassula*, finds hundreds of microtubules appearing in the furrow region to form a girdle and proposes they are important in the final separation. No such girdle is apparent in *P. tetraurelia*. Final separation must involve elimination of filaments from the furrow at the time of separation and a final placement of membranes to seal off the two new ends of the daughter cells. If, as we suspect, microtubules are important in the placement of new membrane material, then disruption of the tubules will upset placement of membranes and final separation could be inhibited. This is consistent with our observations.

(iv) *Conclusions*

We conclude, then, that the longitudinal microtubules play a role, at the time of fission, in cellular elongation and morphogenesis, and indirectly in cytokinesis itself. Elongation may be achieved by the transportation and guidance of materials for new cell membranes to their sites of insertion in the cortex; the morphogenetic role is thought to be a determinative one with the tubules preserving cellular form in the presumed local absence of the infraciliary lattice which is the normal cytoskeleton; and the cytokinetic function might be associated with the insertion of membranes at the time of final cell separation.

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