MACRONUCLEAR DIVISION WITH AND WITHOUT MICROTUBULES IN *TETRAHYMENA*

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SUMMARY

The effects of cold shocks (o °C) and colchicine (4 mg/ml) on macronuclear microtubules and macronuclear division have been investigated in *Tetrahymena*. Macronuclear division was affected by both treatments, but loss of microtubules occurred only in the presence of colchicine.

When colchicine was applied immediately prior to macronuclear elongation, the nuclei underwent a partial elongation without microtubules, but were unable to constrict and separate into daughter nuclei. Such nuclei were cut in two by the advancing cytoplasmic fission furrow. When colchicine was applied to macronuclei in the fully elongated state, they maintained their elongation in the absence of microtubules, and were subsequently separated into daughter nuclei by the cytoplasmic furrow. It is suggested that macronuclear microtubules, most probably the membrane-associated microtubules, are required for the terminal stages of nuclear elongation and separation. However, the considerable macronuclear elongation which takes place in the absence of microtubules serves to focus attention on molecular mechanisms of force production which must reside elsewhere in the macronucleus.

INTRODUCTION

Microtubules have been reported within the dividing macronucleus of ciliated protozoa, but little is known about their role in the division process. The ciliate macronucleus typically does not show recognizable chromosomes, and conventional mitotic spindles have not been seen. Division of this nucleus, which has been described as a process of 'amitosis', involves an elongation of the nucleus within the persisting nuclear envelope, and an eventual pulling apart into the 2 daughter nuclei. Only a few ultrastructural descriptions of this process have been published. In general, most descriptions report few or no microtubules present in non-dividing macronuclei, then the appearance of microtubules during elongation. They appear individually, sometimes in bundles, and sometimes associated with the nuclear envelope. The most complete descriptions, other than *Tetrahymena*, are of *Nassula* (Tucker, 1967), *Blepharisma* (Inaba & Sotokawa, 1968), and certain peritrichous ciliates (Carasso & Favard, 1965).

In *Tetrahymena*, there is disagreement about the presence of microtubules in the non-dividing macronucleus (see Elliott & Kennedy, 1973). The microtubules are clearly apparent during macronuclear elongation, however, and they have been described by Falk, Wunderlich & Franke (1968), Ito, Lee & Scherbaum (1968), Tamura, Tsuruhara & Watanabe (1969) and Wunderlich & Speth (1970). An

association of microtubules with the macronuclear envelope was demonstrated by Falk et al. (1968) and Wunderlich & Speth (1970).

One way to obtain information about the role of macronuclear microtubules in division is to prevent their formation, or remove them, with agents to which they are sensitive. Nuclear microtubules are usually sensitive to cold, colchicine, and high hydrostatic pressure. We have explored the effects of cold and colchicine in the present investigation.

There have been 3 previous reports on the effects of colchicine on macronuclear division in Tetrahymena, all concluding that the macronuclear microtubules are sensitive to colchicine (Kennedy, 1969; Tamura et al. 1969; Wunderlich & Speth, 1970). In one study, it was shown that the cells eventually recover in the presence of the drug, reform microtubules and undergo macronuclear and cell division (Wunderlich & Speth, 1970). In the study by Tamura et al. (1969), it was further shown that colchicine applied to synchronized Tetrahymena late in the cell cycle had no blocking or delaying effect on either cell or macronuclear division, even though macronuclear microtubules were not present. However, nuclear cleavage was typically unequal; many daughter cells were produced with nuclei which were either smaller or larger than normal, and some had no nuclei at all. One of the major objectives in the present investigation has been to study the process of macronuclear division without microtubules in detail, in the hope that this might lead to a better understanding of the normal mechanism of macronuclear division in ciliates. It has been shown that significant elongation of the macronucleus can occur in the absence of microtubules, and that microtubules are not required to maintain an elongation once it has occurred normally. However, autonomous nuclear constriction and separation do not occur without nuclear microtubules; the macronucleus is cut in two by the advancing cytoplasmic fission furrow.

MATERIALS AND METHODS

Tetrahymena pyriformis, strain GL, was maintained in a medium containing tryptone, vitamins, salts, and dextrin which has been described by Frankel (1965). For synchronization, cells were grown in 500-ml flasks containing 125 ml of this medium, or in some experiments, 2 % proteose-peptone supplemented with liver fraction L and salts as described by Rasmussen & Zeuthen (1962). The cells were grown at 28 °C to a density of 34000–69000 cells per ml, then synchronized with seven 30-min heat shocks of 34 °C separated by six 30-min periods at 28 °C.

Samples were removed at frequent intervals after the end of the synchronizing treatment (EST) and Feulgen preparations were made in order to determine the time course of macronuclear division. The cells were fixed in 2 % chromic acid (7 parts), formalin (4 parts) and glacial acetic acid (1 part). The samples were washed in distilled water with a trace of detergent, treated according to the Feulgen-staining method described by Humason (1962), counterstained with fast green, and mounted.

Samples for treatment were removed and subjected at various times to cold or colchicine. The cold-treated cells were plunged into an ice bath at 0 °C. Colchicine (Cal Biochem, grade A) was prepared and stored according to Nelsen (1970), and added to the experimental samples to give a final concentration of 4 mg/ml.

Samples for electron microscopy were fixed for 20 min in 2·25 % glutaraldehyde in 0·1 M phosphate buffer, pH 7·0. They were rinsed in phosphate buffer, then postfixed in 1 %

osmium tetroxide in o·1 M phosphate buffer. The cells were embedded in Epon, sectioned on a Reichert OM-2 ultramicrotome, stained with uranyl acetate and lead citrate, and observed with a Philips EM 300 electron microscope.

RESULTS

Macronuclear division in synchronized control cultures

The light micrographs presented in Figs. 1–4 show the major changes which occur in the macronucleus of *Tetrahymena* during division. The nucleus, which is approximately spherical in Feulgen-stained preparations (Fig. 1), begins to divide by undergoing a pronounced elongation (Fig. 2). This occurs at about the time of initiation of the cytoplasmic fission furrow. As elongation continues, a thinning of the nucleus occurs in the mid-region which results in the constricted configuration in Fig. 3. The cytoplasmic fission furrow is still in an early stage of development when this occurs, which suggests that the mechanism for nuclear constriction lies within the nucleus itself. The nucleus finally separates into 2 daughter nuclei, as shown in Fig. 4. As the strand between daughter nuclei breaks, a small extrusion body may be left outside both daughters in the region between them (Fig. 4). In some cells, the thin strand connecting daughter nuclei may persist until the daughter cells are separated.

The time course of nuclear division in synchronized cells was determined by scoring the relative frequencies of each of the 4 nuclear configurations shown in Figs. 1–4 present in Feulgen preparations at various times after the end of the synchronizing treatment. It was found that nuclear division began about 65 min after the end of the synchronizing treatment, and was completed within the next 30 min in the majority of cells. In the course of these experiments it was found that the exact time of cell and macronuclear division in synchronized cells varies slightly (5–10 min) according to the growth medium used and the cell density at EST. For this reason, each of the experiments with colchicine was done on half of a culture while the remaining half was scored as the control for that experiment. The stage distributions for macronuclear division in untreated cells can be seen in 3 different experiments in Tables 1–3 (control cells).

Cells were fixed and prepared for electron microscopy at various times after the end of the synchronizing treatment in order to observe the presence and orientation of nuclear microtubules. Cells fixed shortly after EST were found to have microtubules (1) aligned beneath the nuclear envelope and (2) displayed in a random orientation throughout the interior of the nucleus. They were perhaps fewer in number in these cells than in later stages, but the distribution was similar. The internal tubules are shown in a cell at EST+65 min in Fig. 10. They appear singly, never in bundles, and seem to run in all directions. No changes in these internal nuclear microtubules were observed throughout the course of nuclear division, and it is therefore difficult to imagine what role they may play, if any, in segregation of the genetic material or division of the macronucleus.

Several aspects of the microtubules associated with the nuclear envelope, however, suggest that these microtubules might play a role in the elongation and division of the macronucleus. These microtubules are found in large numbers, often arranged in

bundles (Fig. 11), at the beginning of elongation and at all stages subsequently until the daughter nuclei separate. They are especially prominent in the constricted portion of nuclear material between the daughter nuclei as they move apart (Fig. 14). Several workers have suggested that microtubules and membranes which are connected to each other by cross-bridges may be responsible for the generation of force in various types of cellular motile systems. The microtubules beneath the nuclear envelope in *Tetrahymena* are frequently seen to be connected with the inner layer of the nuclear envelope by means of such cross-bridges (Figs. 12, 13). The presence of these connexions, and the distribution of these microtubules in space and time suggest that they may play some active role in the elongation and separation of the macronucleus during division.

The effect of low temperature on nuclear microtubules and nuclear division

Cells were grown in proteose-peptone medium and synchronized by the application of 7 heat shocks. Feulgen preparations were first made and scored from untreated control cells (Table 1) in order to determine the time at which a cold shock would

Table 1. *Stage distributions of macronuclei during synchronous division in normal and cold-treated Tetrahymena*

	Nuclear configuration			
	Spherical	Elongate	Constricted	Separated
Control cells				
EST+65*	84	16	0	0
EST+70	21	64	12	3
EST+75	11	40	24	25
EST+80	8	10	25	57
Cold-treated†				
EST+70	43	51	6	0
EST+80	52	43	5	0
EST+120	55	41	2	2
EST+130	21	31	30	18

* Time in min, after the end of the synchronizing treatment (EST); 100 nuclei were scored for each sample.
† Temperature lowered to 0 °C at EST+70 min and held there for 50 min. At EST+120 min the culture was returned to 28 °C.

affect the largest number of dividing nuclei. The results showed that the majority of cells were in the elongation stage at EST+70 min, and it was decided to initiate the cold shock at this time. Accordingly, a second culture was synchronized and plunged into an ice bath at this time. The nuclear division stage distribution in this culture at EST+70 min was similar to that in the control culture (Table 1), although not quite as many cells were in the elongated stage. The cells were kept in the cold for 50 min, and sampled after 10 min in the cold (EST+80 min) and again at the end of the cold shock (EST+120 min). The nuclear stage distributions at these times show that

(1) nuclei did not continue to divide during the cold shock, and (2) the elongated configuration of nuclei in the process of dividing was not lost in those cells which had attained this stage.

After 50 min in the cold, the cells were returned to the 28 °C waterbath with shaking. It was found that nuclear division had resumed 10 min after being returned to 28 °C, and was even completed in some cells by this time (Table 1). It appears that macronuclei in all stages of division were halted in their development during the cold shock, then resumed normal development at normal rates upon return to the optimal growth temperature.

Cells were fixed for electron microscopy in order to determine whether the macronuclear microtubules were affected by the cold treatment. Samples were taken at the beginning of the cold shock, 10 min later, and after 50 min in the cold. It was found that both the internal macronuclear microtubules and the membrane-associated macronuclear tubules persisted throughout the entire 50-min cold shock. It can be concluded that the macronuclear microtubules are not sensitive to cold, although they are sensitive to colchicine, as shown below.

The effects of colchicine on nuclear microtubules and nuclear division

The effect of colchicine on the progress of macronuclear division was studied in cells grown in tryptone medium in 2 different experiments. In the first experiment, colchicine was added early, just prior to the elongation of the nuclei, in order to see if this would prevent the ensuing elongation process. Colchicine was added at a final concentration of 4 mg/ml to one half of a synchronized culture at EST + 65 min, and the remaining half was used as the control. The results are presented in Table 2.

Table 2. *The effect of an early addition of colchicine on macronuclear division in synchronized Tetrahymena*

	Nuclear configuration			
	Spherical	Elongate	Constricted	Separated
Control cells				
EST + 65*	99	1	0	0
EST + 70	90	10	0	0
EST + 75	62	35	3	0
EST + 80	27	47	17	9
EST + 90	4	22	26	48
Colchicine added†				
EST + 70	93	7	0	0
EST + 75	79	21	0	0
EST + 80	63	35	2	0
EST + 90	44	35	18	3
EST + 100	27	30	14	29

* Time in min after the end of the synchronizing treatment (EST); 100 nuclei were scored in each sample.

† Colchicine (4 mg/ml) was added to half the initial culture at EST + 65. The other half was used as the control culture above.

It is seen that less than 1 % of the nuclei had begun to elongate at the time colchicine was added. A comparison of the nuclear stage distributions in the colchicine-treated cells and the control cells reveals that nuclear division did proceed to completion in the presence of colchicine, but at a slower rate than in untreated cells. The distributions also suggest that the drug took effect within about 5–10 min after it was added to the experimental culture.

Although nuclear division occurred in the presence of colchicine, it was immediately apparent in the Feulgen preparations that the process was fundamentally different from division in the absence of the drug. The differences are illustrated in Figs. 5–7. First of all, the nuclear elongation was only partial. Early stages in nuclear elongation in the presence of colchicine appeared in Feulgen preparations to be thickened and truncated (Fig. 5), and often actually presented square profiles. Elongation never occurred in colchicine to the extent seen in control cells, with the result that maximally elongated nuclei in these cells appeared in Feulgen preparations to be quite thick with curiously angular corners (Fig. 6). These nuclei are apparently not able to undergo the constriction in the central region which normally separates each nucleus into daughter nuclei. Examination of later stages in Feulgen preparations showed that the partially elongated nuclei remained unconstricted and positioned approximately in the centres of the cells until the cytoplasmic fission furrows had nearly divided the cells in two. It appears as though the advancing cytoplasmic furrow cuts the nucleus in two at a very late stage of cell division in these cells (Fig. 7). As suggested in Fig. 7, the 2 daughter cells frequently received grossly different quantities of nuclear material in this type of division, and a few daughter cells received none.

Samples of the colchicine-treated cells were prepared for electron microscopy in this experiment at EST + 85 and EST + 100 min in order to determine the effect of colchicine on the macronuclear microtubules. Neither the internal nuclear microtubules nor the membrane-associated microtubules were present in any of the many nuclei observed in either sample. The involvement of microtubules in the elongation which took place prior to the first ultrastructural observation at EST + 85 min is considered unlikely, because the second experiment with colchicine (described below) shows that macronuclear microtubules are removed in less than 10 min after addition of the drug. Moreover, the decline in the number of spherical nuclei after EST + 85 min (Table 2), and the corresponding increase in constricted and separated configurations, indicates that a considerable number of spherical macronuclei must have passed through the elongated configuration after EST + 85 min. The only alternative to this requires cytokinesis to proceed in cells with spherical nuclei while becoming arrested in cells with elongated nuclei, which seems unlikely, and the pinching of spherical nuclei into constricted and separated configurations by the cytoplasmic fission furrow, which was not seen in the Feulgen preparations. It is therefore concluded that the macronuclei underwent elongation in the absence of microtubules, then were constricted and separated by the advancing cytoplasmic fission furrow.

Measurements indicate that the average maximum length of the colchicine-treated nuclei prior to the constricting action of the cytoplasmic furrow was 16 μm. The average diameter of spherical nuclei was 9 μm, and the maximum elongation of the

macronuclei prior to constriction in untreated cells was 22 μm. The macronuclear elongation which occurred in colchicine was therefore about 54 % of the extent of macronuclear elongation found in untreated cells at this comparable stage. Further macronuclear elongation occurred during constriction in both untreated and treated cells. Although this is probably autonomous in untreated nuclei (Fig. 3), it may be due entirely to the action of the cytoplasmic furrow in colchicine-treated macronuclei (see Figs. 7, 9).

Table 3. *The effect of a late addition of colchicine on macronuclear division in synchronized Tetrahymena*

	Nuclear configuration			
	Spherical	Elongate	Constricted	Separated
Control cells				
EST + 70*	53	43	4	0
EST + 75	24	46	28	2
EST + 80	17	25	32	26
EST + 85	7	8	27	58
Colchicine added†				
EST + 75	49	40	8	3
EST + 80	36	44	12	8
EST + 85	32	47	13	8
EST + 90	16	41	21	22

* Time in min after the end of the synchronizing treatment (EST); 100 nuclei were scored for each sample.

† Colchicine (4 mg/ml) was added to half the initial culture at EST + 70 min. The other half was used as the control culture scored above.

In the second experiment with colchicine, the drug was added at a later stage in order to determine what effect this would have on nuclei which had already undergone elongation. Colchicine was added at a final concentration of 4 mg/ml to one half of a synchronized culture at EST + 70 min, and the remaining half was used as the control. The results are presented in Table 3. It can be seen that 43 % of the cells were elongated when colchicine was added in this experiment, but only 4 % had undergone constriction and none had separated. As in the previous experiment, comparison of the stage distributions with time in the treated and untreated samples shows that nuclear division continued in the presence of colchicine, but at a slower rate than in the control cells. The stage distributions also indicate that the drug took effect within 10 min, as in the previous experiment.

Two types of elongated nuclei were observed in the Feulgen preparations of these colchicine-treated cells: short truncated forms and long truncated forms. The former were most prevalent in the later samples, and appeared identical to those seen in the earlier experiment. As in the previous experiment, these were most probably nuclei which were spherical at the time of colchicine addition and which underwent partial elongation in the presence of the drug.

Many of the elongated nuclei in the earlier samples in this experiment, however,

were much longer than any seen in the experiment in which colchicine was added prior to elongation. Nuclei of this type, shown in Fig. 8, were presumably derived from those nuclei which were in a maximal state of elongation at the time the drug took effect. In support of this interpretation is the fact that the first nuclei to be cleaved ('Constricted' and 'Separated' categories in the colchicine-treated samples at EST + 80 and 85 min, Table 3) were of this longer type (Fig. 9). For these reasons it appears likely that nuclei which were elongated when treated with colchicine retained their elongation, although they became much more angular at the ends (cf. Figs. 8, 9 with 2, 3). These nuclei also were apparently unable to constrict and separate into daughter nuclei; as in the previous experiment, the Feulgen preparations suggest that they were passively cleaved by the advancing cytoplasmic fission furrow late in cell division (Figs. 8, 9).

Samples of the colchicine-treated cells in this experiment were fixed and prepared for electron microscopy at EST + 80 and 90 min. As in the previous experiment, no microtubules were seen in the macronuclei of cells at any stage in these samples. The colchicine-treated macronuclei in both experiments appeared more amoeboid at the ends than control macronuclei, with several lobose projections often visible in a single section. One such projection is indicated by the arrow in Fig. 15. The nucleoli in colchicine-treated macronuclei often showed detached cortical regions (Fig. 15), but other structures present appeared normal. Fig. 15 also shows a fission furrow very close to an elongated macronucleus. Stages later than this have not been seen.

DISCUSSION

Stability of macronuclear microtubules

The microtubules of the macronucleus of *Tetrahymena* are sensitive to colchicine, but are not sensitive to cold shocks. Although the microtubules of dividing nuclei in most cells are sensitive to low temperatures, a few exceptions are known. In one of these, *Strongylocentrotus droebachiensis*, it was found that a mitotic spindle will form at 0 °C (Stephens, 1972). The cold-insensitivity of the mitotic microtubules in *S. droebachiensis* is undoubtedly adaptive, because this organism occurs in northern seas. The cold-insensitivity of *Tetrahymena* macronuclear microtubules may also be adaptive, at least for those forms which live in northern waters. Handel & Roth (1971) reported that the mid-body microtubules of neural tube cells of chick embryos are not sensitive to cold, although the mitotic apparatus microtubules from which they presumably originate are sensitive to cold. The adaptive significance in this system is not apparent. Microtubules sensitive to colchicine but not to cold were reported by Handel (1971) in the nerve fibres of chick dorsal root nerve explants. The molecular mechanisms by which some microtubule systems are protected against the depolymerizing action of low temperature remain to be discovered.

The role of macronuclear microtubules in nuclear division

There are conflicting reports in the literature regarding the presence of microtubules in non-dividing macronuclei (see Elliott & Kennedy, 1973). We found them in

strain GL shortly after the end of the synchronizing treatment, more than 40 min before nuclear division began. We also have observed them in logarithmically growing cells in abundance, and conclude that they are probably present throughout the cell cycle. This agrees with a previous report by Ito *et al.* (1968). These authors, and also Falk *et al.* (1968), have described attachments of microtubules to chromatin and nucleoli. Both groups have suggested that microtubules may connect these structures with the nuclear envelope, and that this in some way might assure the independence and/or segregation of subnuclei. However, microtubule attachments are difficult to establish with certainty without serial sections, high voltage electron microscopy, or nuclear fractionation. For the present, it must be concluded that we have no clear indication of the function of the internal macronuclear microtubules in *Tetrahymena*. Bundles of microtubules more suggestive of spindles have been reported within the macronuclei of *Nassula* (Tucker, 1967) and certain peritrichous ciliates (Carasso & Favard, 1965).

The membrane-associated microtubules appear to increase in number during macronuclear elongation, and they are predominantly oriented along the axis of elongation. This, together with the fact that they are connected with the nuclear envelope by cross-bridges, suggests that the elongation may be brought about, at least in part, by some sort of interaction between these intranuclear microtubules and the nuclear envelope. Microtubules in close association with the macronuclear envelope during elongation have also been reported in the ciliates *Nassula* (Tucker, 1967), *Diplodinium* (Roth & Shigenaka, 1964), and *Blepharisma* (Inaba & Sotokawa, 1968; Jenkins, 1969). However, the membrane-associated microtubules in the latter 2 ciliates appear on the outside of the nuclear envelope, a situation which also occurs in certain fungi (Heath & Greenwood, 1970; Heath, 1974). A detailed description of mitosis in the fungus, *Thraustotheca clavata*, has led to the suggestion that an interaction between the nuclear envelope and its associated microtubules may be responsible for generation of part of the necessary mitotic forces in this system (Heath, 1974).

The primary effect of removing macronuclear microtubules with colchicine in *Tetrahymena* was to block the terminal phases of macronuclear elongation and the associated constriction and separation into daughter nuclei. Macronuclear microtubules, presumably the membrane-associated group, therefore appear to play an essential role in these processes.

Macronuclear division without microtubules

The ability of *Tetrahymena* cells to cleave their macronuclei without benefit of the normal complement of nuclear microtubules provides for an increased probability that daughter cells will receive adequate genetic material under environmental conditions which favour the depolymerization of microtubules. However, the only agent presently known to depolymerize the macronuclear microtubules is colchicine. For this reason, it is difficult to imagine that this ability is of general adaptive significance in nature. Nevertheless, the process of macronuclear cleavage without microtubules may be instructive with regard to the normal mechanisms which operate during macronuclear division in *Tetrahymena*.

The first requirement for the microtubule-less division is that the macronucleus be positioned and held in the plane of the cytoplasmic fission furrow. This is because the macronucleus, in the absence of its intrinsic mechanism for constricting and separating in two, is forced into 2 parts by the advancing cytoplasmic fission furrow. The molecular basis for this nuclear positioning is not known, but could be due to cytoplasmic actin filaments. Although not reported for *Tetrahymena*, a concentration of actin filaments around the nuclei in other cell types has been demonstrated recently with fluorescent-labelled antibody and fluorescent-labelled heavy meromyosin (Lazarides, 1974; Sanger, 1974).

Perhaps the most interesting feature of macronuclear division in the presence of colchicine is the elongation of the nucleus which can be effected in the absence of microtubules. It is clear that the microtubules are required for complete elongation of the nucleus, but the thick and angular elongation forms seen in colchicine-treated cells must be produced by molecular mechanisms acting independently of microtubules. The forces generated during partial elongation might be due to actin or actin-like substances, or to other 'matrix' components of the nucleus. Forer & Behnke (1972) showed actin-like filaments in glycerinated mitotic spindles of crane fly spermatocytes treated with heavy meromyosin, and Hinkley & Telser (1974) have obtained similar results with neuroblastoma mitotic spindles. No such studies have yet been done with *Tetrahymena*, or other ciliates, but it is possible that actin-like filaments are present and might be responsible for the forces generated in macronuclei which elongate without microtubules. Once the macronucleus of *Tetrahymena* has undergone a nearly complete elongation with microtubules present in the nucleus, removal of the microtubules with colchicine does not cause collapse of the elongated state. Some nuclear matrix components, perhaps actin-like filaments, are presumably also responsible for the maintenance of the elongated state in the absence of macronuclear microtubules.

In the absence of the microtubule-dependent mechanism for autonomous nuclear constriction and separation, the macronuclei appeared to be cleaved by the advancing cytoplasmic furrow. This apparently does not occur in untreated cells, because the cytoplasmic furrow is always at an early stage of development when macronuclear constriction occurs. Additional evidence for the normal independence of nuclear constriction and cytoplasmic furrowing is provided by Doerder, Frankel, Jenkins & DeBault (1975); in a *Tetrahymena* mutant called 'conical', the plane of nuclear division is different from the plane of cytoplasmic division.

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Figs. 1–9. Light micrographs of Feulgen preparations of *Tetrahymena* cells in various stages of macronuclear division in the absence of colchicine (Figs. 1–4), and in the presence of colchicine (Figs. 5–9). × 900.

Fig. 1. The appearance of the macronucleus at the end of the synchronizing treatment. There is no sign of division at this time or for the next 60–70 min.

Fig. 2. Elongation of the macronucleus, shown here, begins about 65–70 min after the end of the synchronizing treatment (EST) and coincides with the beginning of cytoplasmic furrow formation.

Fig. 3. Macronuclear constriction, shown here, follows elongation. Note that the nuclear constriction and the cytoplasmic furrow are well separated from each other, which suggests that macronuclear constriction normally occurs by a mechanism which is independent of cytoplasmic furrow formation.

Fig. 4. Separation of daughter macronuclei. This may occur, as shown here, before the late stages of cytokinesis. However, a very thin strand of nuclear material connecting the daughter nuclei may persist in some cells until cytoplasmic division is completed.

Fig. 5. Partial elongation of the macronucleus in a cell 10 min after the addition of colchicine. The drug was added just prior to the time of normal elongation (EST + 65 min).

Fig. 6. Later stage in the partial elongation which occurs in the presence of colchicine. Colchicine was added just prior to the onset of elongation in untreated cultures and the cells were fixed 15 min later.

Fig. 7. Cleavage of the macronucleus in a colchicine-treated cell by the cytoplasmic fission furrow. The partially elongated nuclei in colchicine-treated cells show no ability to constrict autonomously, but instead are cut in two by the advancing cytoplasmic furrow. As suggested by this micrograph, the daughter cells often get abnormally unequal amounts of nuclear material in this type of division.

Fig. 8. Nucleus in a cell treated with colchicine while in the fully elongated state indicated in Fig. 2. As shown here, nuclei treated at this time retain their elongated configuration, but become irregularly shaped at the ends and show no ability to constrict autonomously.

Fig. 9. A later stage in the division of a nucleus treated with colchicine while in the elongated state. The cytoplasmic fission furrow appears to be required to constrict the macronucleus and to separate it into 2 daughter nuclei.
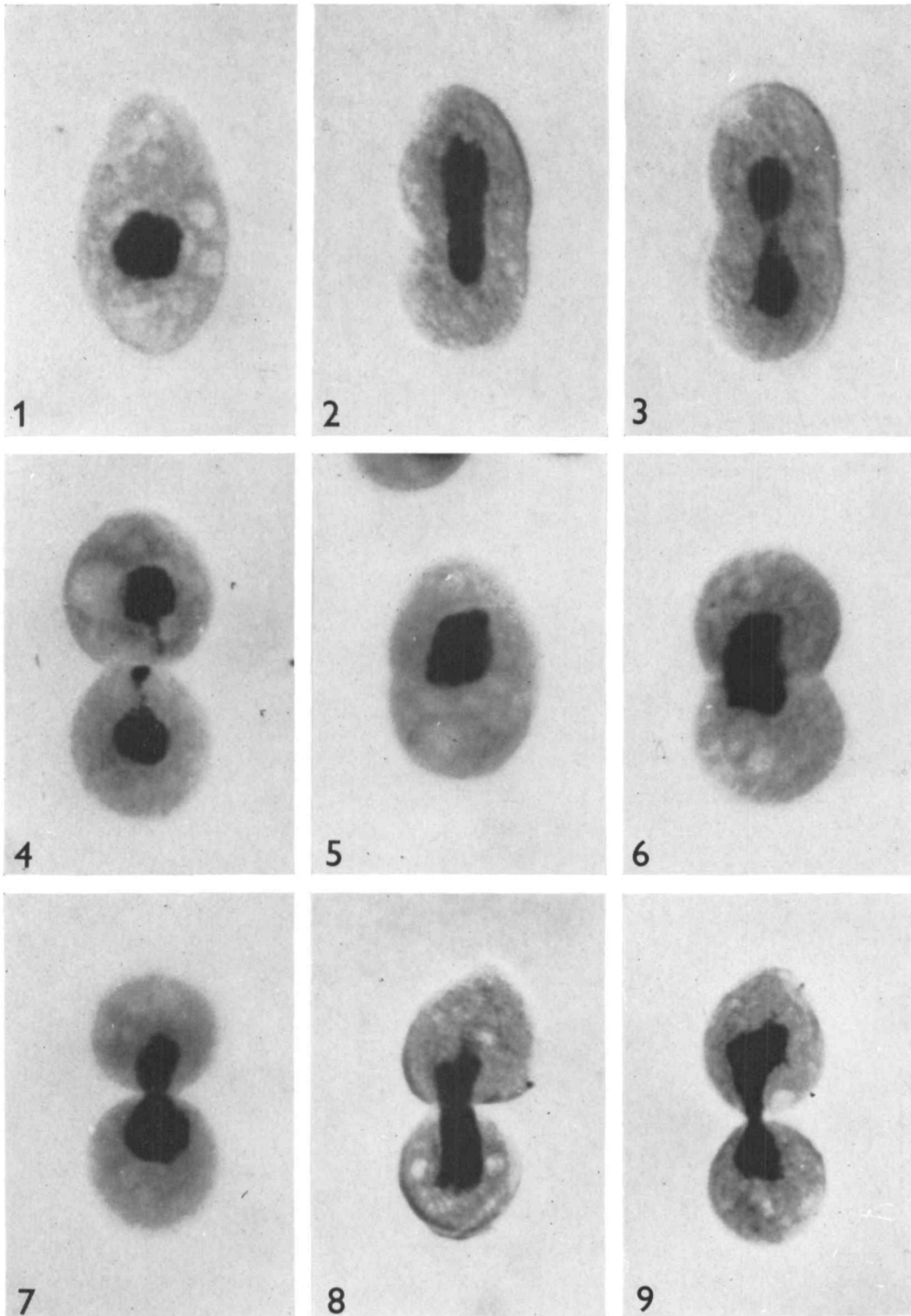
Fig. 10. Portion of a non-dividing macronucleus of *Tetrahymena* showing the distribution of intranuclear microtubules. Note the random orientation of these tubules (arrows). × 51 000.

Fig. 11. Region of the nucleus (bottom) next to cytoplasm (top) in a cell in which macronuclear elongation is probably just beginning (EST + 65 min). Note the band of microtubules just beneath the inner nuclear membrane. × 64 000.

Fig. 12. Cross-section of nuclear membrane-associated microtubules in an elongating *Tetrahymena* macronucleus. Cross-bridges can be seen connecting the 3 microtubules within the nucleus to the nuclear envelope. × 136 000.

Fig. 13. Longitudinal section through a nuclear membrane-associated microtubule showing cross-bridges connecting it to the inner membrane of the macronuclear envelope (2 of these indicated by arrows). × 110 000.
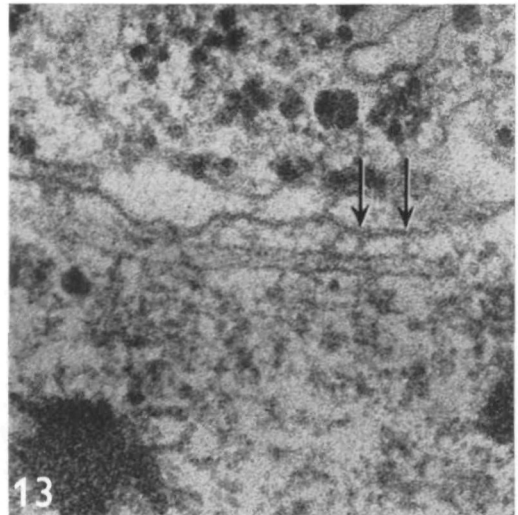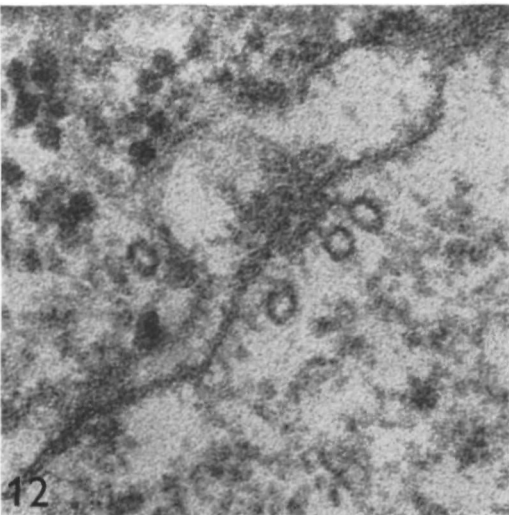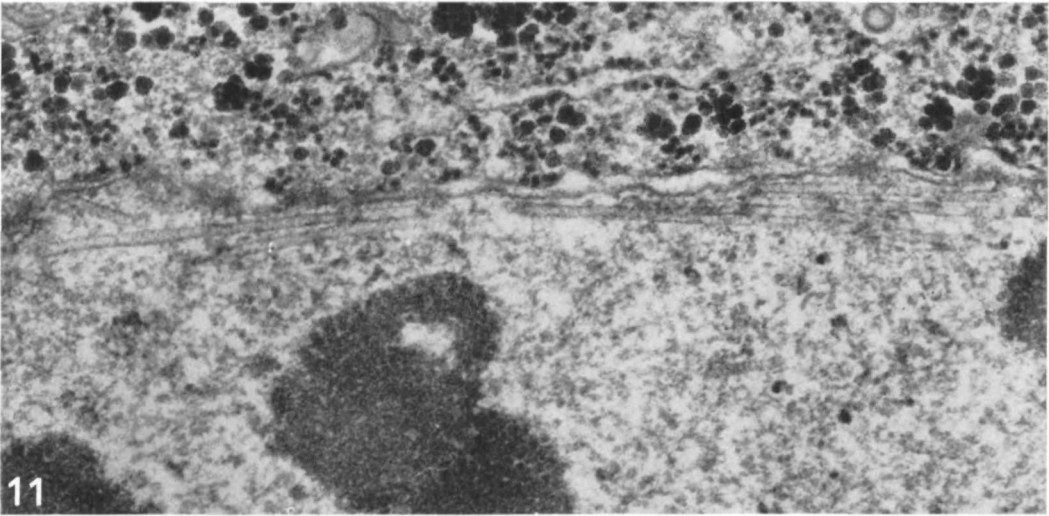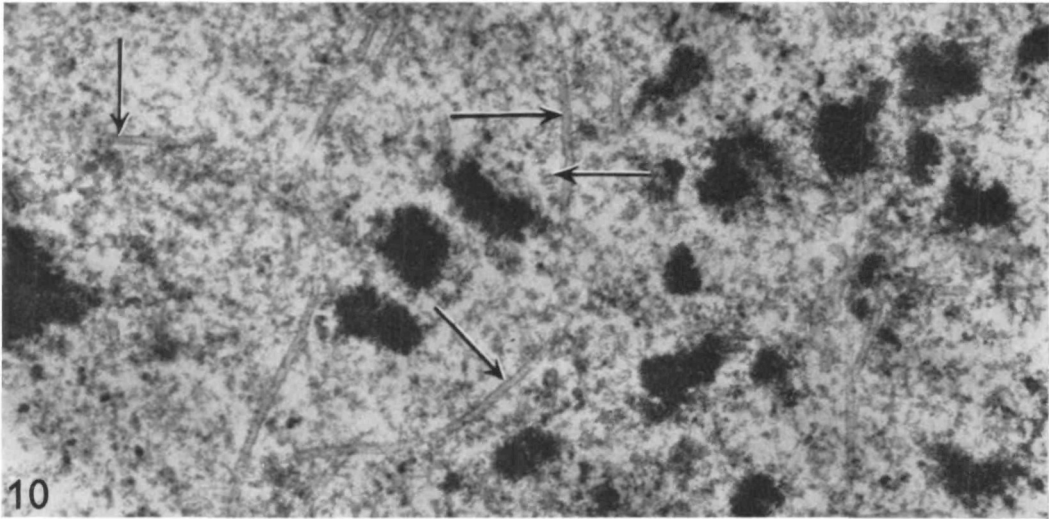
Fig. 14. Section through the constricted zone of a normally dividing macronucleus of *Tetrahymena*. Many microtubules are found in this region (arrows), and they are oriented with their long axes parallel to the axis of nuclear elongation. As this micrograph suggests, most of these microtubules are probably associated with the nuclear envelope. × 30000.

Fig. 15. Section through the region of the cytoplasmic furrow in a cell at EST + 90 min which had been treated with colchicine 20 min earlier. Note that the macronucleus has retained its elongated configuration even though no microtubules are present. The ends of such nuclei are often highly amoeboid (note the branching indicated by the arrow), and none has been seen in a state of constriction. The nucleus is apparently constricted by the advancing cytoplasmic fission furrow. Nucleoli (*n*) frequently show detached cortical regions in colchicine-treated cells. × 11000.