ULTRASTRUCTURE OF NUCLEAR DIVISION IN *PARAMECIUM AURELIA*

II.* AMITOSIS OF THE MACRONUCLEUS

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Abstract

Ultrastructural details of macronuclear amitosis in the ciliate *P. aurelia* are described. During amitosis the nuclear membrane remains intact, and there are no changes in the appearance of the nucleoli and chromatin bodies of the macronucleus. At the commencement of amitosis the nucleus adopts a smoother outline and a position in the cell opposite the gullet. This usually occurs at the time when the micronuclei are in metaphase of mitosis. At this stage, bands of microtubules are apparent in the cytoplasm, close to, and parallel with, the long axis of the nucleus. Microtubules also appear in the fibrous background region of the nucleus. The macronucleus then elongates and narrows; bands of microtubules are evident close to the inside of the nuclear membrane as this occurs. As karyokinesis proceeds (concurrently with cytokinesis) microtubules are found in all regions of the macronucleus, oriented along the long axis. At no stage of division do microtubules appear to be attached to the chromatin bodies of the macronucleus. Several aspects of macronuclear amitosis in ciliates are discussed.

I. INTRODUCTION

The first paper of this series (Stevenson and Lloyd 1971) described the fine structure of mitosis in the micronucleus of *Paramecium aurelia*. We now present observations on the division of the macronucleus in the same organism.

Division of the ciliate macronucleus is a process which has baffled description, as there are very few or no visible kinetic elements involved in the division process, which has been characterized as amitotic. Some authors (e.g. Grell 1964; Raikov 1968; Nilsson 1970) have pointed out that amitosis is an incorrect term to describe the macronuclear division process. Because of the lack of any suitable alternative term, however, it will be retained.

In *Paramecium*, in contrast to some other ciliates (reviewed by Raikov 1968), there is little morphological change at the light microscopical level in the appearance of the macronucleus during division. There appears to be no spiralization of chromosomes and no spindle apparatus. However, using polarizing optics (Schwartz 1957), a birefringent protein substance has been observed in the dividing macronucleus of *P. bursaria*, while electron microscope studies have shown the involvement of microtubules in the division of the macronucleus (Jurand and Selman 1970). The present paper reports observations on the division of the macronucleus in *P. aurelia* and stresses the involvement of microtubules in the division process.

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II. Materials and Methods

These were identical to those of the previous paper of this series (Stevenson and Lloyd 1971).

III. Results

The stages of division of the macronucleus are shown diagrammatically in Figure 1 and in photomicrographs of sections stained with toluidine blue (Figs. 2–5). Hertwig (1889) gave the first detailed description of macronuclear amitosis and Sonneborn (1947) has summarized it in English. Briefly, Hertwig's account states that the macronucleus moves to the side of the cell away from the gullet, elongates and narrows, and divides into halves transversely. These phases may be seen in Figures 2–5.

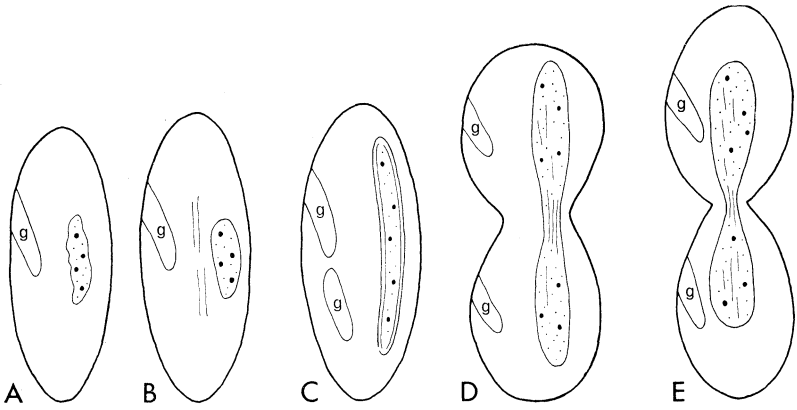


Fig. 1.—Amitosis of the macronucleus (diagrammatic) illustrating the role of microtubules in the division process. Large dots in nucleus represent nucleoli, small dots represent chromatin bodies. *g*, gullet. Thin lines represent microtubules. *A*, interphase; *B*, condensation; *C*, elongation; *D* and *E*, stages of karyokinesis and cytokinesis.

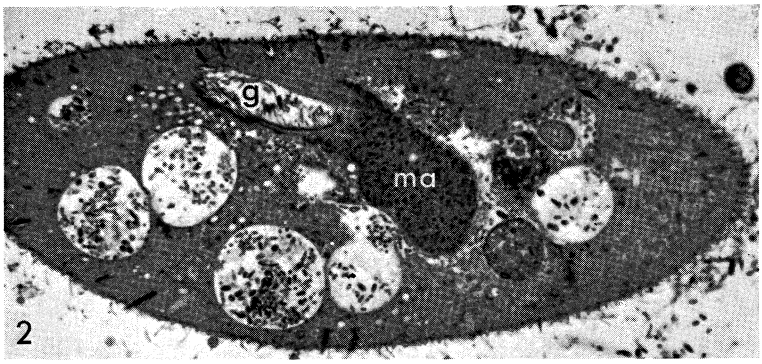
The ultrastructure of the macronucleus of *P. aurelia* in interphase has been described several times previously (Sonneborn 1953; Jurand, Beale, and Young 1962; Wolfe 1967). Briefly, it is 30–40 μm by 10–15 μm in size and approximately ovoid in shape. It is often irregular in outline (Fig. 2) having lobes and projections. In the electron microscope, two distinct types of bodies of high electron density can be seen

Fig. 2.—Longitudinal section of *P. aurelia* stained with toluidine blue. The interphase macronucleus (*ma*) is irregular in shape, lying below the gullet (*g*). ×800.

Fig. 3.—Longitudinal section of *P. aurelia* stained with toluidine blue. Macronucleus (*ma*) is now smoother in outline and has moved to one side of the cell. This stage can be termed condensation. ×800.

Fig. 4.—Longitudinal section of *P. aurelia* stained with toluidine blue. Macronucleus (*ma*) has elongated to fill almost the whole length of the cell. This is the elongation stage, and cytokinesis is just commencing. ×800.

Fig. 5.—Longitudinal section of *P. aurelia* stained with toluidine blue. Macronucleus (*ma*) is still elongated, and karyokinesis is in progress, concurrently with cytokinesis. ×800.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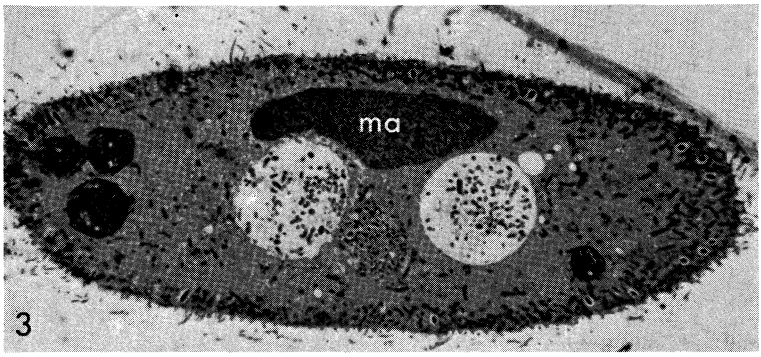
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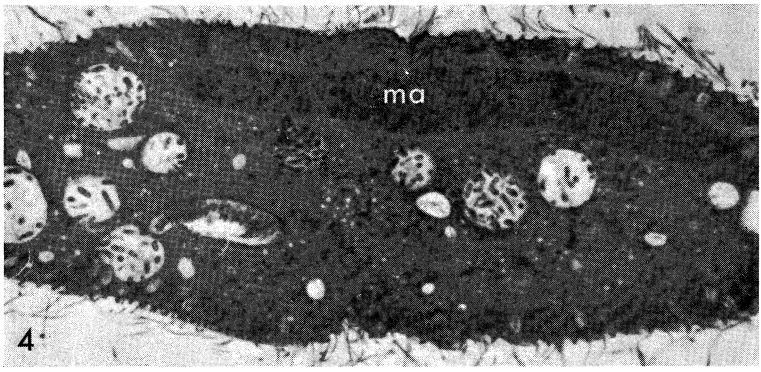
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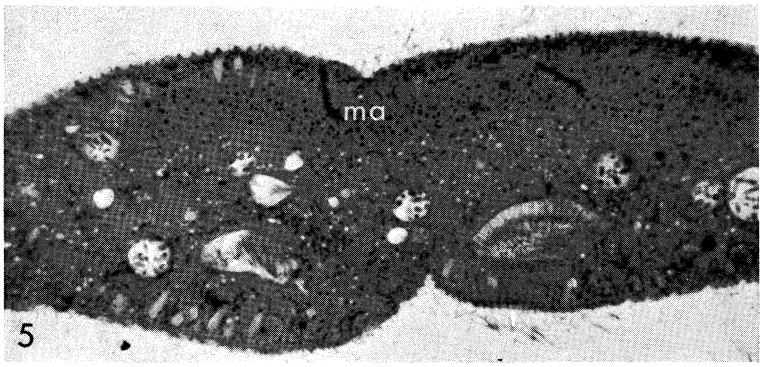
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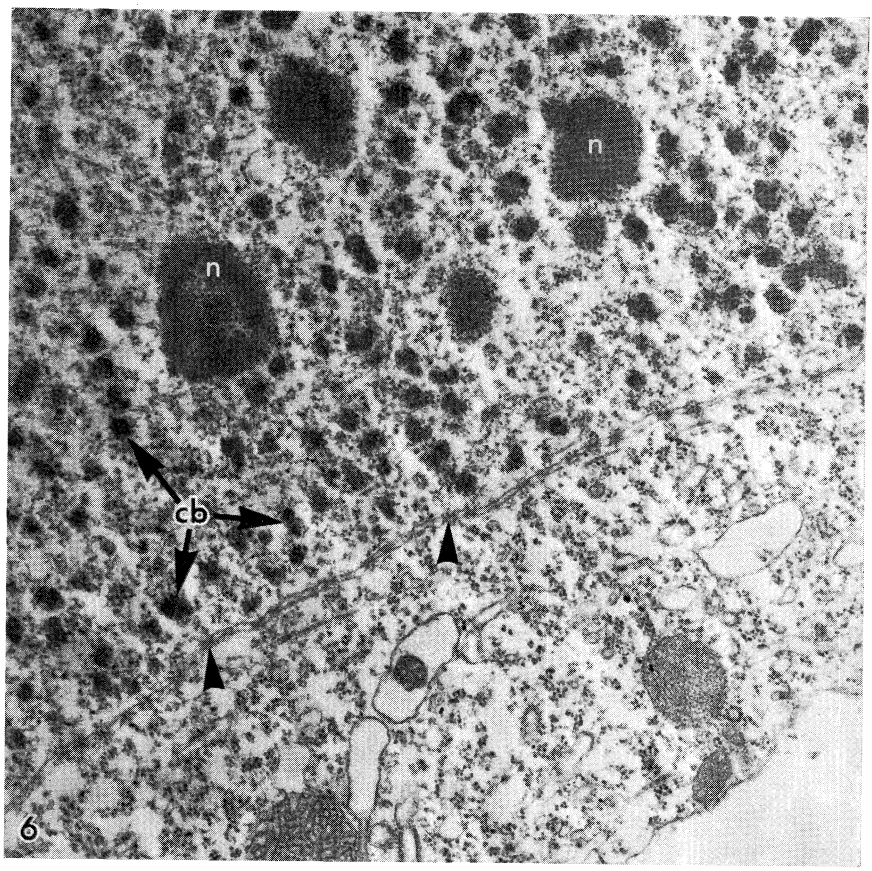
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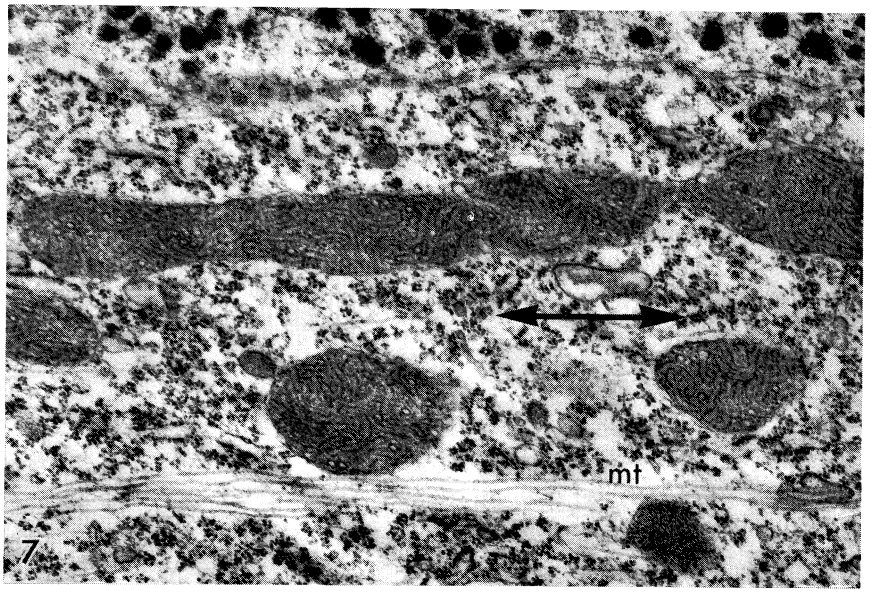
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within it. The larger bodies (Jurand, Beale, and Young 1962) are up to 1 μm in diameter, irregular in outline, and fibrogranular in appearance. They may or may

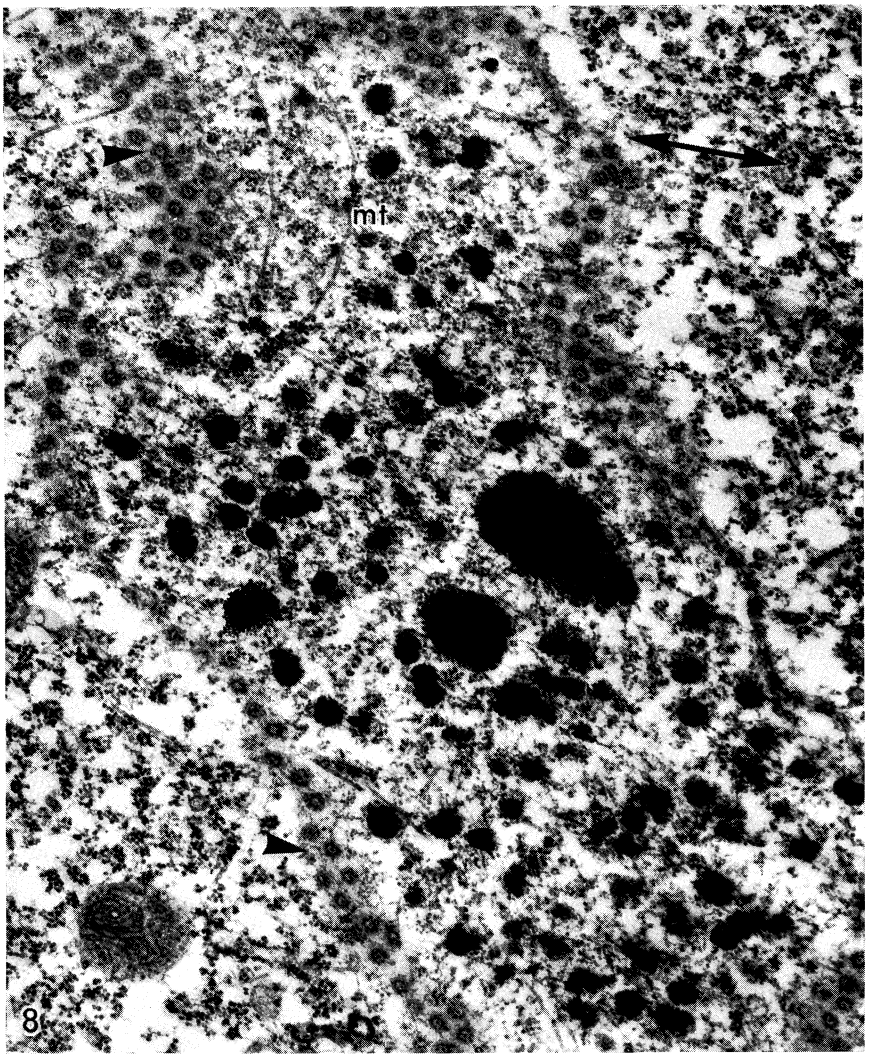


Fig. 8.—Condensation stage of amitosis. Microtubules (*mt*) in various orientations, are appearing in the fibrillar background region of the nucleus. Double-headed arrow indicates long axis of cell. Numerous nuclear pores are also visible (arrowheads). ×29,400.

not have a central core of lower electron density up to 0·5 μm or occasionally larger in diameter. The small granules are up to 20 nm in diameter (Fig. 6). Sonneborn (1953) estimates the number of large bodies at about 1000 in a fully developed macronucleus.

Fig. 6.—Interphase macronucleus. Nucleoli (*n*) with granular fine structure, chromatin bodies (*cb*) in fibrillar background region. Nuclear membrane has frequent pores (arrowheads). ×24,500.

Fig. 7.—Condensation stage of amitosis. Band of microtubules (*mt*) parallel to the macronucleus. Double-headed arrow indicates long axis of cell. ×26,000.

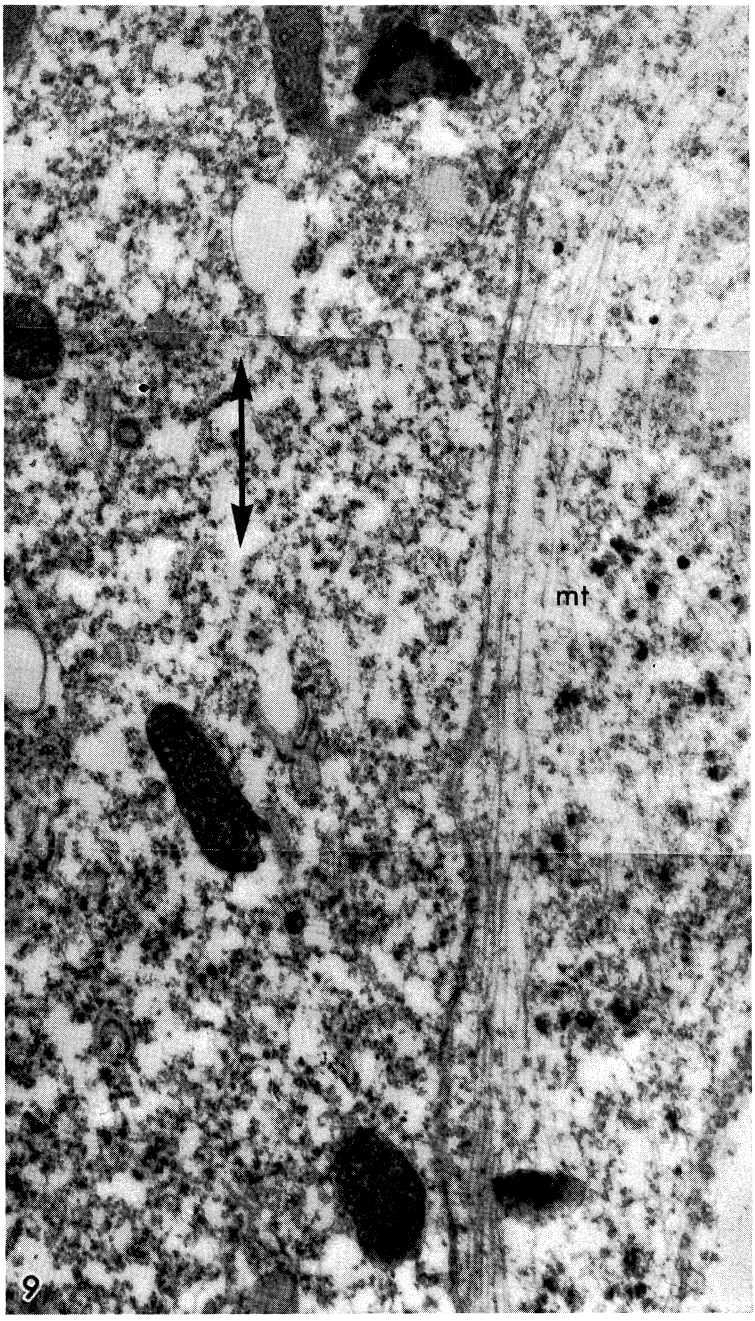


Fig. 9.—Elongation stage of amitosis. A montage of three plates showing band of microtubules (*mt*) immediately beneath nuclear membrane. Double-headed arrow indicates long axis of cell. ×16,000.

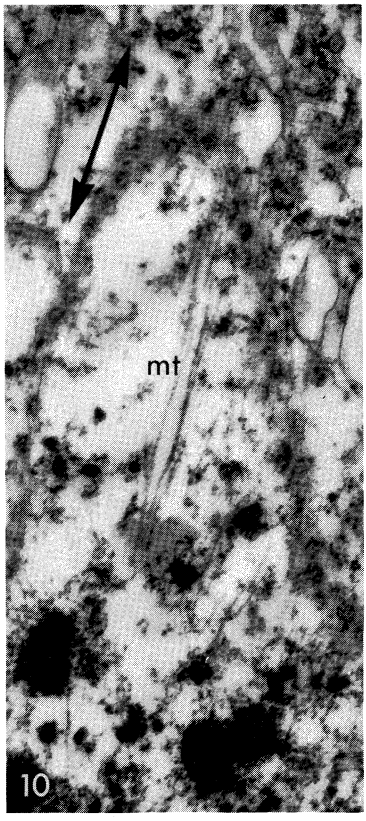
The small bodies (Jurand, Beale, and Young 1962) are about 0·1–0·2 μm in diameter, also irregular in shape, and fibrillar in ultrastructure. Between the large and small bodies are fibrils of low electron density of 5 nm or less in diameter (Fig. 6). The consensus of opinion is that the large bodies are nucleoli and the small bodies centres of condensed chromatin (Nanney and Rudzinska 1960; Wolfe 1967; Jurand and Jacob 1969). The macronucleus is bounded by a typical double membrane with frequent pores (Fig. 6). Usually there are 60–80 pores per 1 μm^2, rather fewer than Franke (1967) reports in *Tetrahymena*. The pores appear similar in structure to nuclear pores in other organisms and consist of a dense ring of diameter 80 nm surrounding a dense central area of 30–40 nm in diameter (Fig. 8).

The first signs of nuclear division are the adoption of a smoother nuclear outline and of movement of the nucleus to the side of the cell away from the gullet (Fig. 3). The micronuclei at this time are usually in metaphase of mitosis. At this time, bands of microtubules appear in the cytoplasm parallel to, but not usually contiguous with, the nuclear membrane (Fig. 7). In Figure 7, a section from the same cell as that shown in Figure 3, microtubules are oriented along the long axis of the cell and it appears that as the microtubules grow the nucleus adopts its longer, narrower, and smoother shape and its position in the area of the cell opposite the gullet. Between the microtubules and the nucleus there is a band of cytoplasm containing mitochondria, vesicles, and a large number of ribosomes (Fig. 7), which presumably provide energy and raw materials for syntheses which occur in the next phase, the elongation phase. Jenkins (1969) also observed microtubules external and adjacent to the nuclear envelope at a corresponding stage of macronuclear division in *Blepharisma*.

Simultaneously with the appearance of microtubules outside the nucleus, microtubules appear inside the nucleus (Fig. 8) in the fibrous background area. Initially, they are not oriented but may lie at any angle and may be curved. They are not linked to chromatin bodies at distinctive sites. Microtubules appear in all areas of the nucleus, though they seem to be concentrated somewhat in regions near the membrane. This appearance of microtubules is the only evident change in the internal contents of the macronucleus during division; the appearance of nucleoli and chromatin bodies does not change in any way during amitosis.

The elongation phase is short-lived but very striking. The nucleus assumes the shape of a long thin sausage (Fig. 4). Extranuclear microtubules are now reduced in number. The intranuclear microtubules become oriented along the long axis of the nucleus. Large numbers are found in bands immediately below the membrane (Fig. 9) which is a section of the cell shown in Figure 4. Microtubules are very long and individual tubules have been followed for 10 μm or more (Fig. 9). During elongation small projections may often be seen on the nucleus (Fig. 10). Microtubules often extend into these projections. From consideration of changes of surface area during elongation, much new membrane material must be provided. Clearly the membrane of the macronucleus is in a dynamic state at this time.

The third phase of macronuclear division, karyokinesis, takes place at the same time as cytokinesis in *Paramecium*. Microtubules, singly or in bundles oriented along the long axis of the cell, are frequent in the isthmus of the dividing nucleus (Figs. 11 and 12). However, the microtubules do not appear to act in the way described by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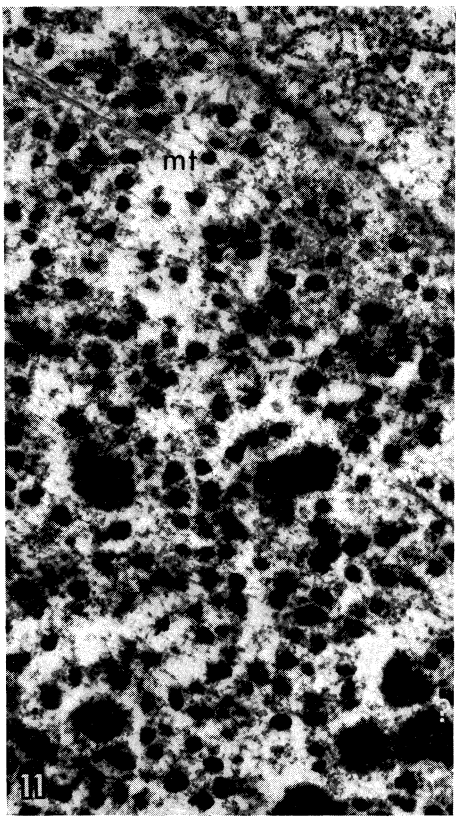
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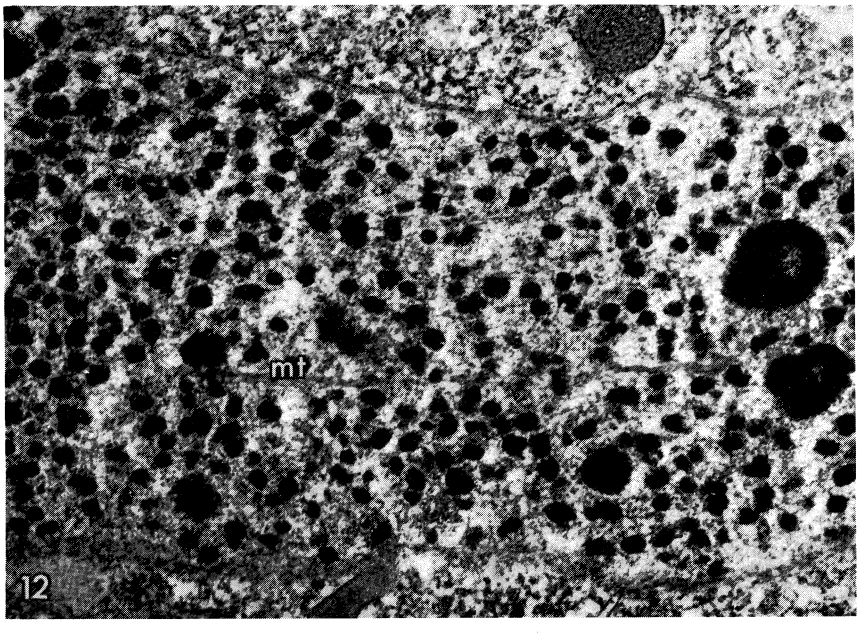
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Raikov (1962, 1966) for *Nassula ornata*, in which they form what he has described as a "pushing body". Tucker (1967) has described the same structure in *Nassula* as a separation spindle. In *P. aurelia* Jurand and Selman (1970) have observed microtubules in the isthmus of the macronucleus at karyokinesis but, as we can confirm, the microtubules do not appear to be organized into a distinctive spindle-like body. Since they are not attached to chromatin bodies, the precise function of the microtubules at this stage of karyokinesis is not apparent. Clearly the previous elongation is important but it seems that the microtubules do not have an exclusion function in addition, i.e. they do not act by filling the nuclear isthmus and excluding chromatin bodies.

As the fission furrow of the cell becomes more marked, the macronucleus is split into two approximately equal parts. The plane of karyokinesis is not necessarily exactly at the cytokinesis furrow and the macronucleus is divided at a rather variable time with relation to cytokinesis.

IV. Discussion

The ciliate macronucleus is a difficult subject for the interpretation of fine structures, because hypotheses must take into account both ultrastructural and genetic considerations. The principle genetic considerations are the polyploidy of the macronucleus and its persistent maintenance of heterozygosity, indicating some precision of the division process. The development of the macronucleus by differentiation from a micronuclear syrkaryon after sexual processes is also an important consideration.

In *P. aurelia* the macronucleus is about 860 *n*-ploid (Woodard, Gelber, and Swift 1961). From genetic considerations it seems established that it is truly polyploid, i.e. contains multiple copies of all genes in the genome (review by Preer 1968). Grell (1953, 1962, 1964) has postulated that all the genetic material of a genome is linked together linearly to form a giant chromosome, a "Sammelchromosome" or composite chromosome, which is segregated in this linked form during nuclear division. If one assumes that linked units are diploid much of the genetic data can be explained (though Grell proposed haploid units) and the model of a composite chromosome is compatible with the cytology of many ciliates (Raikov 1968).

Wolfe (1967) and Nilsson (1970) contend that the macronuclear chromatin granules (small bodies) are chromosome equivalents and that they are either permanently, or at least temporarily, during division, linked up as a tangled string. Two or more genomes may presumably also be interconnected.

In *Paramecium* and many other ciliates in which the macronucleus divides into approximately equal parts, three main stages of macronuclear division can be

Fig. 10.—Elongation stage of amitosis. Projection on macronucleus, containing microtubules (*mt*). Double-headed arrow indicates long axis of cell. ×19,600.

Fig. 11.—Karyokinesis stage of amitosis. Microtubules (*mt*) in body of nucleus, oriented along the long axis. ×19,600.

Fig. 12.—Karyokinesis stage of amitosis. Microtubules (*mt*) in isthmus of dividing macronucleus, with no attachment to chromatin bodies. ×24,500.

recognized: condensation, elongation, and karyokinesis. The first of these stages is not so striking in *Paramecium* as in ciliates with complexly shaped macronuclei, e.g. *Spirostomum*, *Stentor*, *Blepharisma*. In these ciliates a moniliform macronucleus contracts to a smaller cylinder or sphere. The smoothing of the outline of the *Paramecium* nucleus, perhaps by bands of microtubules, and movement of the nucleus to one side of the cell could be a process that facilitates the later genome separation by changes in internal organization. Precisely what these changes may be is not clear, but they do not involve changes in the appearance of the chromatin bodies. Condensation, therefore, is not equivalent to chromatin condensation, and in the *P. aurelia* macronucleus no changes in chromatin condensation are observed.

Elongation is the most striking stage and in *Paramecium* occurs rapidly. During elongation, bands of intranuclear microtubules are found immediately under the membrane, the microtubules often extend into projections of the nucleus. The concept of microtubules exerting physical pressure on membranes has been established in other organisms (Bajer and Molé-Bajer 1969). It is possible that microtubules are in fact being polymerized in the areas of projection, being formed from precursors entering the nucleus from the cytoplasm at these points, which might also be points of membrane growth. Whatever the precise mechanism, elongation could be viewed as a passive genome separation process that is equivalent in function to anaphase of mitosis.

Microtubules in the macronucleus are not attached to chromatin bodies in *Paramecium*, nor are they in other ciliates (Raikov 1962, 1966; Carasso and Favard 1965; Tucker 1967; Inaba and Sotokawa 1968; Tamura, Tsuruhara, and Watanabe 1969). At karyokinesis the isthmus of the macronucleus usually contains large numbers of chromatin bodies but few microtubules. These microtubules could have a role in keeping segregating chromatin bodies from different genomes apart, but it is difficult to postulate an active function for microtubules at karyokinesis.

If the theory of composite chromosomes is accepted, many puzzling features of macronuclear behaviour at division can be explained and a solely skeletal role for microtubules can be postulated. Microtubules would be involved in positioning and shaping the nucleus, but not actively in the separation of genetic material, in contrast to their role in mitosis. Partition of genetic material in amitosis occurs by a still ill-understood mechanism, which, however, has sufficient precision to be repeated for a large number of division cycles. But although amitosis has sufficient precision for partition of genetic complements, it does not offer the possibility of new genetic combinations, as do processes in which subgenomic units are partitioned. Most ciliates have retained the possibility of recombination in the micronucleus while obtaining an efficient synthetic mechanism by polyploidy of the macronucleus. By means of its polyploidy the essentially ephemeral ciliate macronucleus is resistant to selective forces, but when genetic reassortment is carried out it is dismantled and rebuilt from recombined genes.

V. Acknowledgments

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