# CELL DIVISION IN *OEDOGONIUM*

## II.* NUCLEAR DIVISION IN *O. CARDIACUM*

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[*Manuscript received May* 5, 1969]

*Summary*

Nuclear division in *O. cardiacum* is described. Before division, the nucleus enlarges considerably. At prophase, the nucleolus starts dispersing and kinetochores appear on the condensing chromatin, situated and oriented apparently at random in the nucleus. By prometaphase, the kinetochore pairs become aligned along the spindle axis before moving into the metaphase-plate configuration; this supports an earlier theory explaining metakinesis. During prophase and metaphase particularly, the nuclear envelope at the poles forms channels that extend for some distance into the cytoplasm; these may also bifurcate. The nucleolus disperses but remains in the intranuclear spindle throughout division as a loosely knit skein of granular material. The kinetochores have a complex structure, up to seven distinct layers being detectable; the kinetochore pairs split, and then migrate polewards at anaphase with the rest of the chromosome trailing behind. Large numbers of microtubules run from the kinetochore into evaginations of the nuclear envelope which increase in size during anaphase. The spindle grows in length considerably during anaphase, this coinciding with a proliferation of interzonal microtubules, first seen amongst the trailing chromosome arms. The nuclear envelope enclosing the spindle becomes severely stretched at this stage; it contracts closely around each of the daughter nuclei, isolating them from the rest of the spindle (including microtubules and the remains of the nucleolus). The spindle then collapses; the nuclei come together and then flatten against one another; between them, vesicles and other components of the septum collect amongst a large number of transversely oriented microtubules.

## I. INTRODUCTION

Development of new processing techniques has enabled the authors to describe the ultrastructure of dividing cells in both *Oedogonium* and *Spirogyra* (Pickett-Heaps and Fowke 1969; Fowke and Pickett-Heaps 1969*a*, 1969*b*). Many unusual or unique features were found. This paper firstly illustrates the importance, alluded to previously (Pickett-Heaps and Fowke 1969), of using young cultures for cytological work, and then it compares the ultrastructure of nuclear division in *O. cardiacum* with that of the unidentified, local species of *Oedogonium* (species A) described earlier.

* Part I, *Aust. J. biol. Sci.*, 1969, **22**, 857–94.

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## II. Methods and Materials

A culture of *O. cardiacum* was kindly supplied by the Botany School, University of Cambridge. The culture methods have been described in detail eslewhere (Pickett-Heaps and Fowke 1969). Best results were obtained from material in the "log-phase" of growth (i.e. a couple of days after subculturing, when release of zoospores was followed by rapid growth of germlings—see below).

The long, involved processes of fixation, embedding, etc. have been given previously (Pickett-Heaps and Fowke 1969). Micrographs are presented of the following material:

(1) Fixed and embedded filaments photographed whole in the Araldite block, with phase optics, before sectioning commenced (Fig. 19c); these light micrographs could be compared with sections taken subsequently.

(2) Thick "blue-green" sections, cut on a glass or diamond knife, and mounted on a clean glass slide; they were stained with toluidine blue (Pickett-Heaps and Fowke 1969) and were then examined in a Leitz Ortholux light microscope.

(3) Thin sections, adjacent to those above, mounted on coated grids. They were stained with uranyl acetate and lead tartrate, and photographed using an Hitachi HU-11E electron microscope.

## III. Results and Discussion

### (*a*) *Use of Young Cultures*

We have previously emphasized how important we have found it to use very young, actively growing cells for this cytological work (Pickett-Heaps and Fowke 1969). The cell in Figure 1* was taken from an older culture (*c.* 3 weeks old) that was still very healthy and viable; it represents the best result obtained. The accumulation of starch is typical, and more important, the cytoplasm generally was very dense, obscuring ultrastructural detail. The reason(s) for this denseness is unknown but our experience with other algae (*Chara*, *Spirogyra*, *Closterium*, and others) indicates that such a phenomenon may be quite generally encoutered with older or inactive cultures.

### (*b*) *Nuclear Division*

In Part I (Pickett-Heaps and Fowke 1969), the main sequence of events observed during cell division in *Oedogonium* (species A) was described. In this paper, we will extend and compare those results by describing mitosis in *O. cardiacum*. Division is essentially similar in both species but many minor differences have been found, and continuing detailed work has revealed much of further interest.

#### (i) *Size, Shape, and Disposition of the Nucleus*

Interphase nuclei in *O. cardiacum* were generally situated against the wall of these long, narrow, and highly vacuolated cells (Fig. 2). During early prophase, a

* All sections in Figures 1–25 are longitudinal. Figures 8*a*, 9, 10, 19*a*, and 19c are light micrographs. All other figures are electron micrographs. Abbreviations used on these figures are as follows: *ch*, chromosome; *k*, kinetochore(s); *m*, mitochondria; *n*, nucleus; *nc*, nucleolus; *ne*, nuclear envelope; *nr*, nucleolar remnants; *p*, pyrenoid; *r*, wall rings; *s*, septum; *st*, starch; *t*, microtubules; *w*, cell wall.

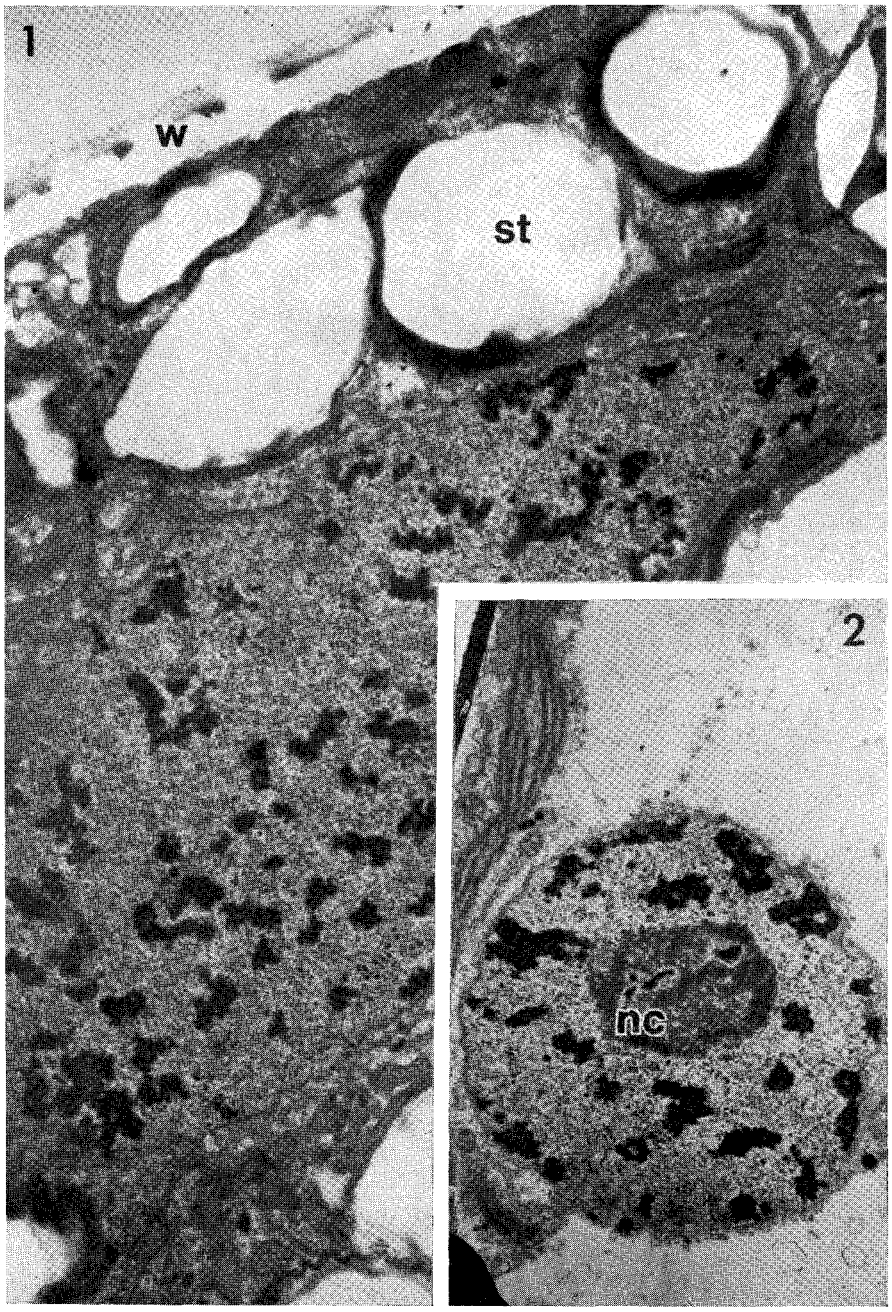



Fig. 1.—Survey view of cell (preprophase) from 3-weeks-old culture. This was the best picture quality obtained from such cultures; whilst cell organelles are visible, the cytoplasm generally was dense, hiding much fine structure. Note typical accumulation of starch (*st*). ×9,600.

Fig. 2.—Typical small, spherical interphase nucleus situated on one side of the cell; some chromatin is present inside nucleolus. ×4,000 approx.

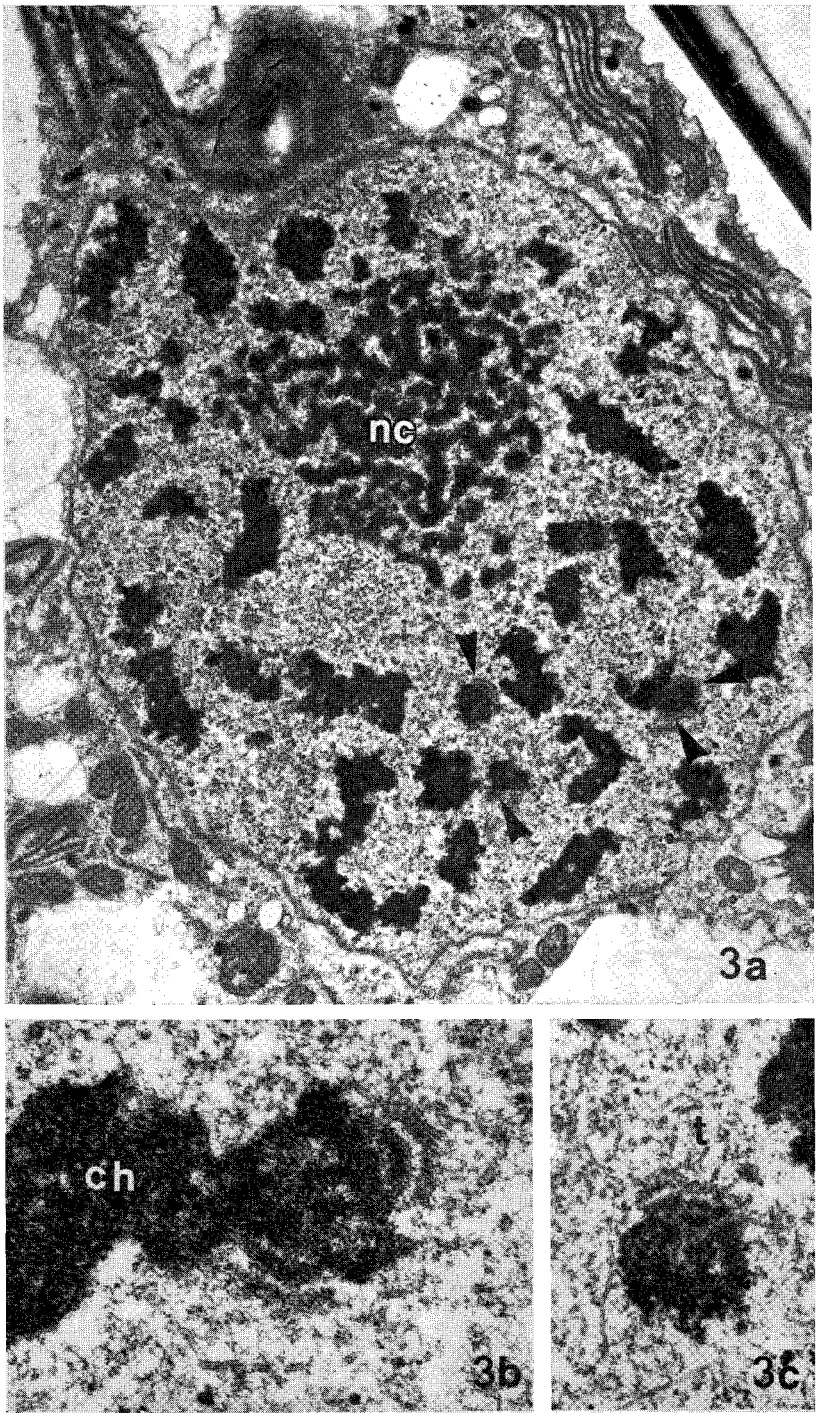



Fig. 3*a*.—Early prophase; nucleolus (*nc*) beginning to disperse. Kinetochores (arrows) were detectable at this early stage—always appearing scattered irregularly within the nucleus. ×6,900.

Fig. 3*b*.—Serial section of the double kinetochore arrowed in Figure 3*a*. Layered substructure (cf. Fig. 7) just becoming visible. ×32,000.

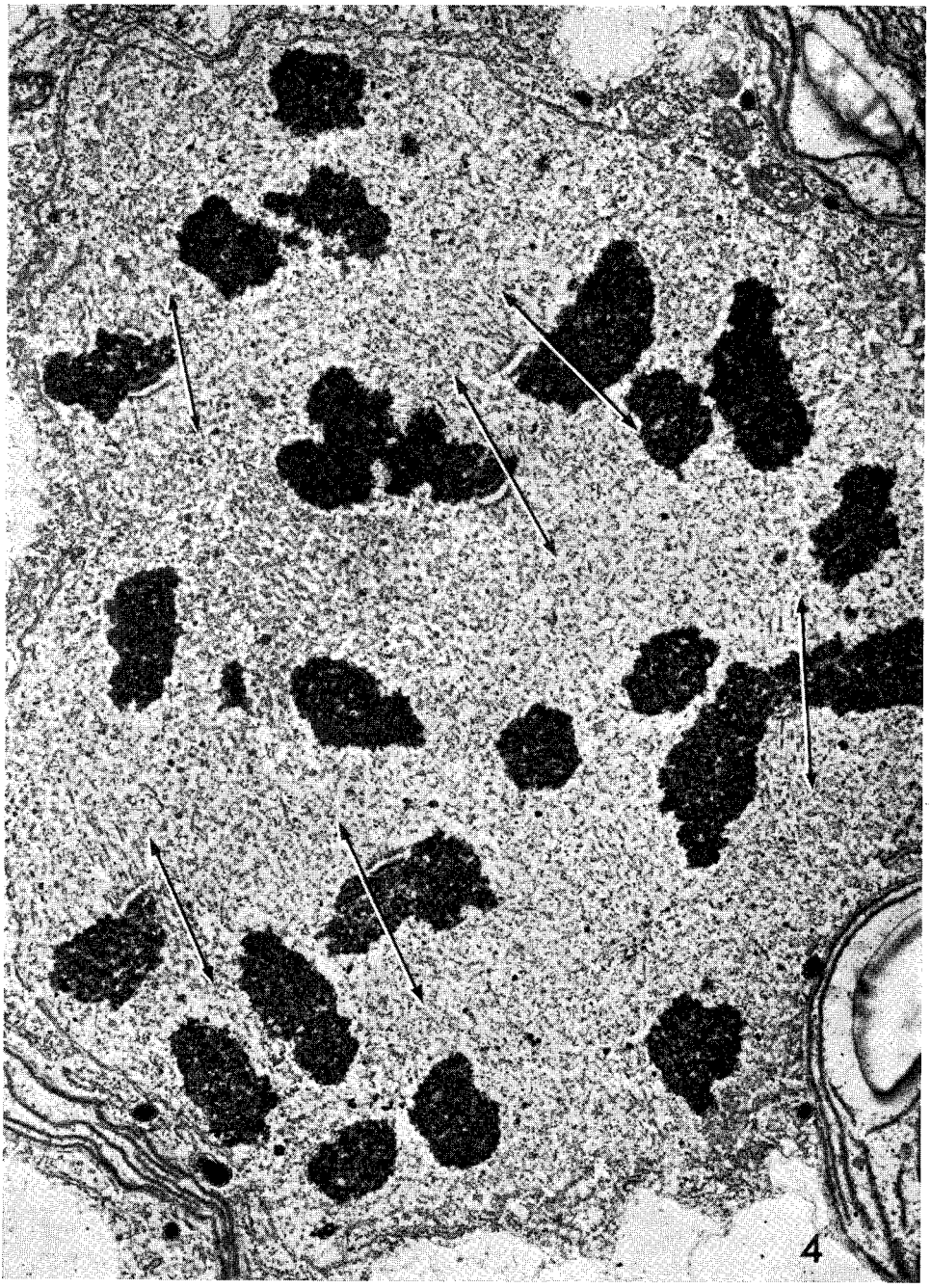
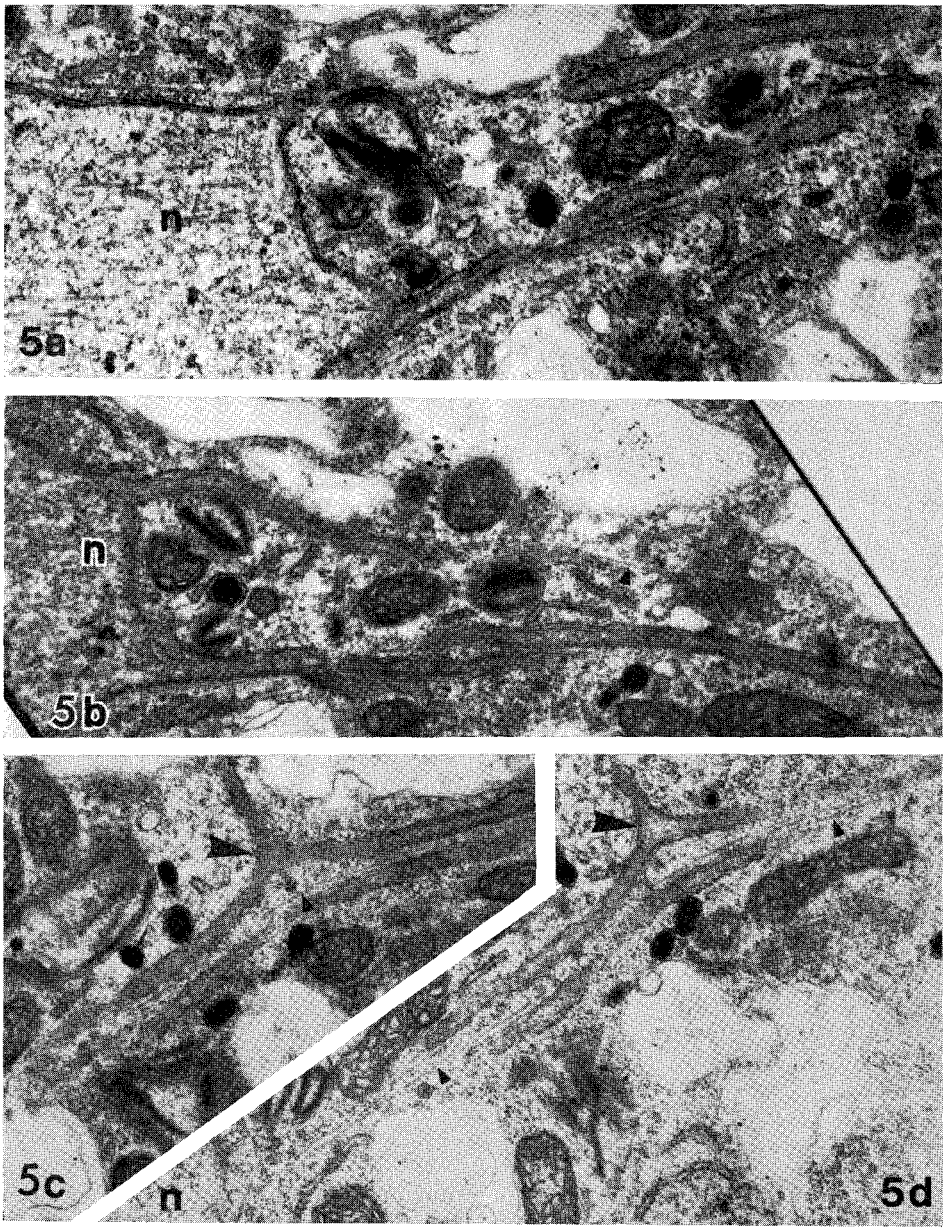



Fig. 4.—Prometaphase. Three pairs and three single (i.e. half-pairs) kinetochores are visible in this one section, widely scattered within the nucleus; they had all become closely oriented along the spindle axis (arrows), in distinct contrast to the situation seen in prophase nuclei (Fig. 3*a*). See also Figure 26. ×8,400 approx.

Fig. 3*c*.—Serial section of uppermost kinetochore arrowed in Figure 3*a*. Some microtubules (*t*) were associated with it. ×18,000.




Figs. 5*a*–5*d*.—Sections being taken at different depths through one pole of a prometaphase cell, showing part of the complex membrane elaborations often detected at this site. The "channels", bounded by membranes continuous with the nuclear envelope at two distinct places (Figs. 5*a*–5*d*), were filled with amorphous material; they bifurcated at one point (large arrow, Figs. 5*c*, 5*d*). Microtubules were closely associated with the membranes (small arrows), as well as being present inside and outside (Fig. 5*a*, arrow) the nucleus (*n*). ×11,000 approx.

considerable increase in nuclear volume coincided with a marked increase in the number and size of cytoplasmic constituents (e.g. chloroplasts, pyrenoids, etc.). The nucleus then extended across the cell lumen; its shape and consequently the shape of the intranuclear spindle was often distorted where it impinged on other large cell organelles.

The spindle was therefore not situated against the wall as in *Oedogonium* (species A), the usual disposition described in the earlier literature (see Pickett-Heaps and Fowke 1969). Furthermore, in that species the very characteristic elongation of the nucleus at preprophase and prophase, also reported in earlier work, was accompanied by the appearance of a sheath of extranuclear microtubules. Such a premitotic change in nuclear shape was not very marked in *O. cardiacum* (Figs. 3*a*, 4), probably due to spatial limitations; however, similar but somewhat fewer extranuclear microtubules were present around the preprophase and prophase nucleus (see Fig. 5*a*).

(ii) *Nuclear Envelope*

The characteristic differentiation of the nuclear envelope at the poles of prophase–metaphase nuclei has been further investigated. Shorter segments of this peculiar double arrangement of the membrane were occasionally also found in other stages of division, generally near the pole (Fig. 18) or the septum (Fig. 25), and rarely in interphase cells. In one case, nine sections at different levels through this differentiated membrane system were obtained at one polar region in a metaphase cell. The four micrographs presented (Figs. 5*a*–5*d*) illustrate at least two and probably three separate channels formed by membranes continuous with the nuclear envelope; these channels might also represent a single-folded system of paired membranes; furthermore, at one point, the paired membranes clearly bifurcated (Figs. 5*c*, 5*d*). Microtubules were associated with these polar membranes (cf. Pickett-Heaps and Fowke 1969). In this same cell, a similar, short segment of such paired membranes was also found near the middle of the nucleus. The amorphous material which is seen between the membranes could be channelled into the nucleus, since in some sections ill-defined bands of similar material were lying amongst the chromosomes. Such material could obviously be microtubule subunits or some other spindle components or both; the membranes therefore could represent either an organelle of sequestration (gathering components from the cytoplasm) or perhaps a specialized site of synthesis of spindle material. These highly speculative ideas are susceptible to experimental trial, and seem to us the most likely of many other possibilities envisaged.

The nuclear membrane remained essentially intact throughout division, although some small gaps were invariably present in it (Figs. 4, 8*b*, 8*c*, *et al.*); during the relatively enormous anaphase elongation of the spindle (see below), the stretched membranes developed some large gaps in the interzonal region, allowing many smaller organelles to enter the spindle (Figs. 19*b*, 20*a*). The membranes then contracted closely around daughter nuclei (Figs. 20*a*, 21, 22*a*), leaving the interzonal microtubules (and nucleolar remnants—see below) loosely enclosed by the rest of

the stretched nuclear envelope (Fig. 20*a*); these interzonal spindle components dispersed as the nuclei approached each other before septum formation (Fig. 22*a*).

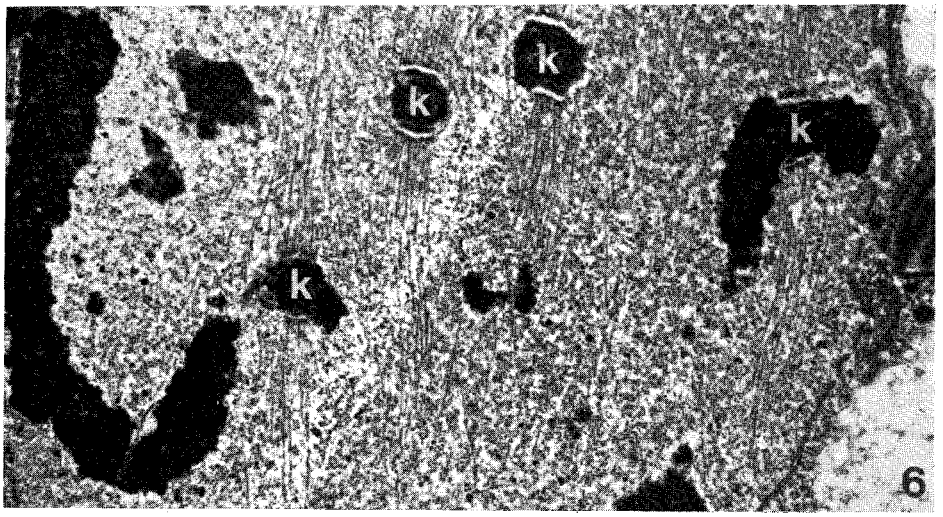
During mitosis, the nuclear envelope at the poles developed evaginations containing diffuse material and microtubules which apparently were connected to the kinetochores (Figs. 8*c*, 14, 17). Whilst metaphase spindles generally contained several smaller blebs in the envelope, at anaphase only one, much larger evagination was apparent at each pole (Fig. 17). By late anaphase, such evaginations were almost devoid of microtubules (Fig. 16) after which they soon disappeared.

These blebs or evaginations would appear to have been formed by the extension of bundles of (kinetochore) microtubules during metaphase and early anaphase. No accumulation of microtubules was apparent (Fig. 16), so microtubule depolymerization must have been steadily occurring within these evaginations during anaphase. Since these evaginations protruded into the cell vacuole, it is doubtful whether they represented some form of anchoring point for the intranuclear spindle. As in other cells, the mechanism of anaphase movement remains puzzling. The comparatively few pole-to-pole microtubules possibly could have provided a framework over which anaphase movement was effected.

(iii) *Nucleolus*

The nucleolus in interphase cells was often closely associated with chromatin (Fig. 2). Dispersal of the nucleolus during prophase (Fig. 3*a*) appeared similar in both species of *Oedogonium*, but subsequent behaviour was somewhat different. In *O. cardiacum* this dispersal was incomplete. The remains of the nucleolus were visible with the light microscope as loosely organized lamellae or strands in the spindle (Fig. 10) being clearly distinguishable from the chromosomes under the electron microscope because of their granular texture and different and variable shape, size, and lack of any direct association with the spindle microtubules (Figs. 11, 12). The nucleolar remnants at metaphase (Fig. 12) and early anaphase were generally seen at the polar regions. However, these lamellae were always moved (Fig. 11) to the central region of the spindle (as in Fig. 8*b*) by late anaphase as they became less conspicuous. They may act as passive bodies possibly indicating by their positions the forces at work inside the spindle. By telophase, they were barely detectable amongst the other components eliminated from the spindle (Figs. 20*a*, 20*b*). In *Oedogonium* (species A), however, the nucleolar material is quite finely dispersed in the spindle, but is eliminated during telophase as a large, densely staining, granular lump between daughter nuclei (Pickett-Heaps and Fowke 1969).

The nucleolus in *Oedogonium* can therefore be thought of as "semi-persistent" during division. This is in contrast with certain algae (e.g. *Chara*, Pickett-Heaps 1967; *Spirogyra*, Fowke and Pickett-Heaps 1969*a*) in which the nucleolar material conspicuously coats the chromosomes, and with other algae and most higher plants where the nucleolus disperses completely during prophase [Lafontaine and Chouinard (1963) and others]. In this regard, *Oedogonium* may resemble *Psilotum nudum* (Fabbri 1960; Allen and Bowen 1966), in which some nucleoli persist throughout division, to be eliminated from the daughter nuclei at telophase.



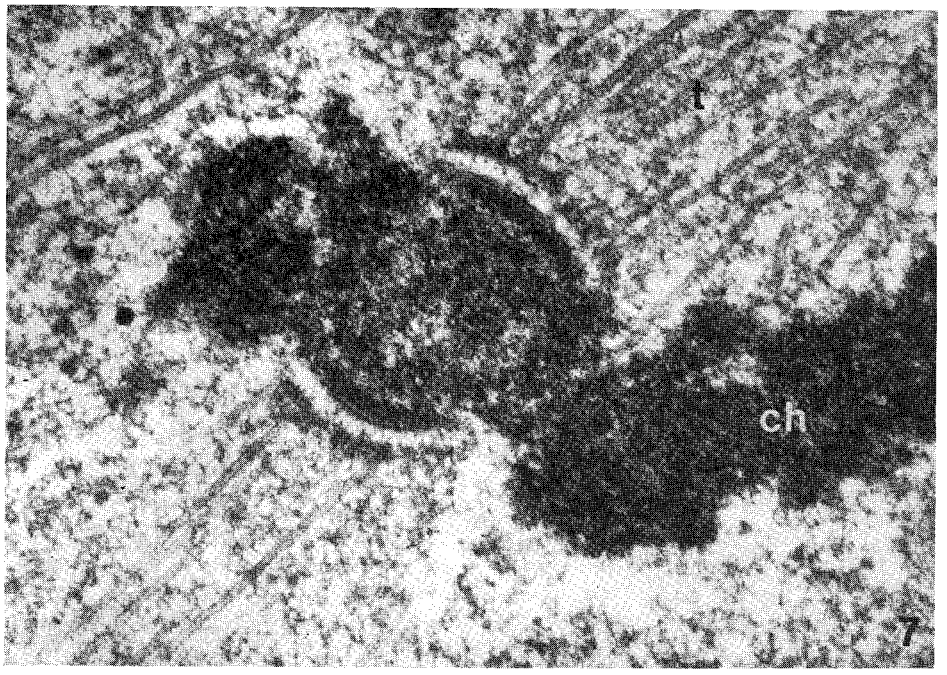




Fig. 6.—Metaphase; paired kinetochores (*k*) with associated microtubules are seen arranged in the typically somewhat irregular metaphase-plate configuration. ×9,000.

Fig. 7.—Paired metaphase kinetochore with attached microtubules (*t*). About six or seven layers can be discerned; between the two kinetochores, a lighter area of the chromosome (*ch*) probably represents the centromere region. Compare with Figures 14 and 16. ×40,000.

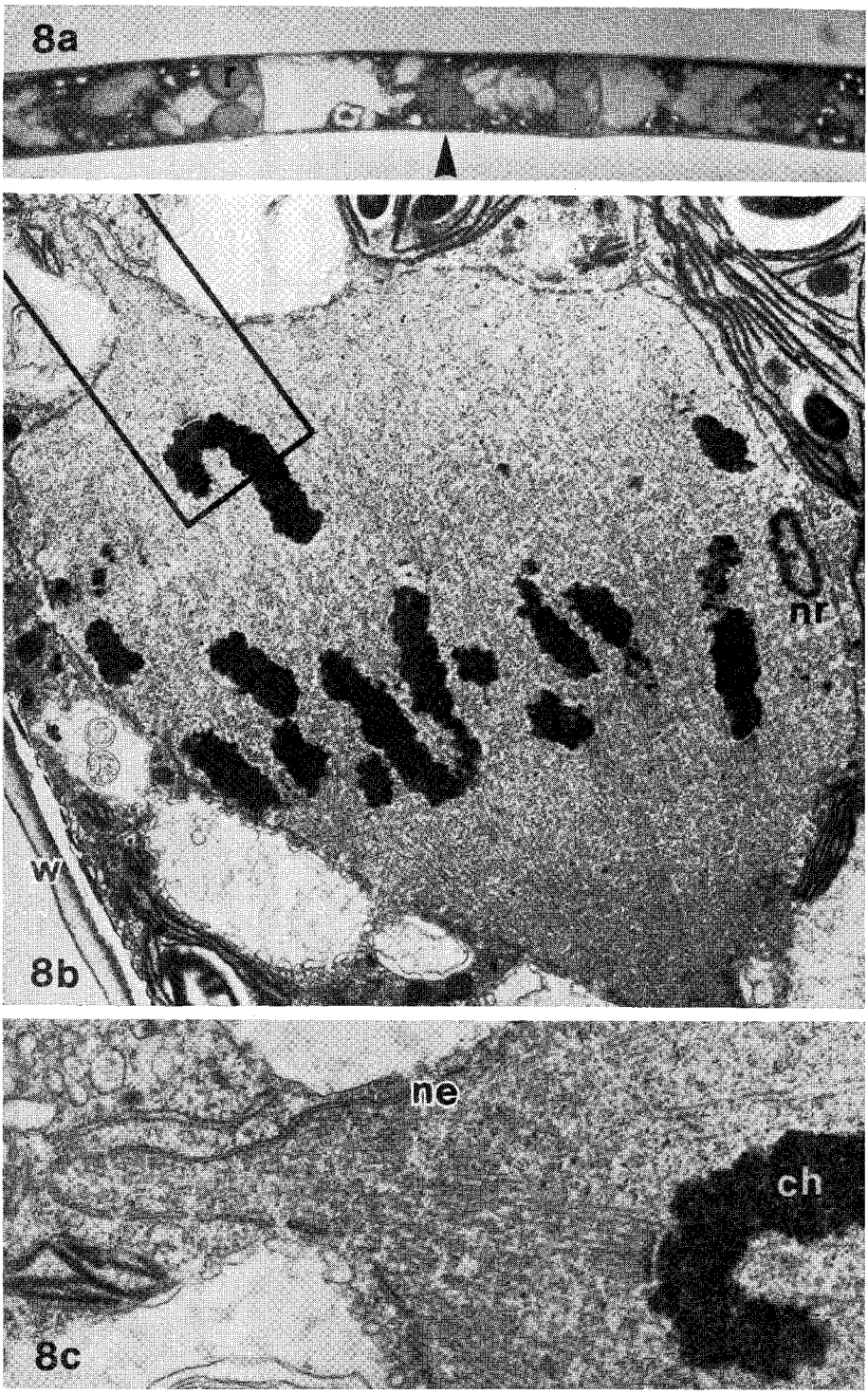



Fig. 8*a*.—Typical series of dividing cells; cell at left was at telophase, with nuclei close together (as in Fig. 24), centre cell (arrowed) seen at early anaphase, and cell on right was at metaphase. ×400.

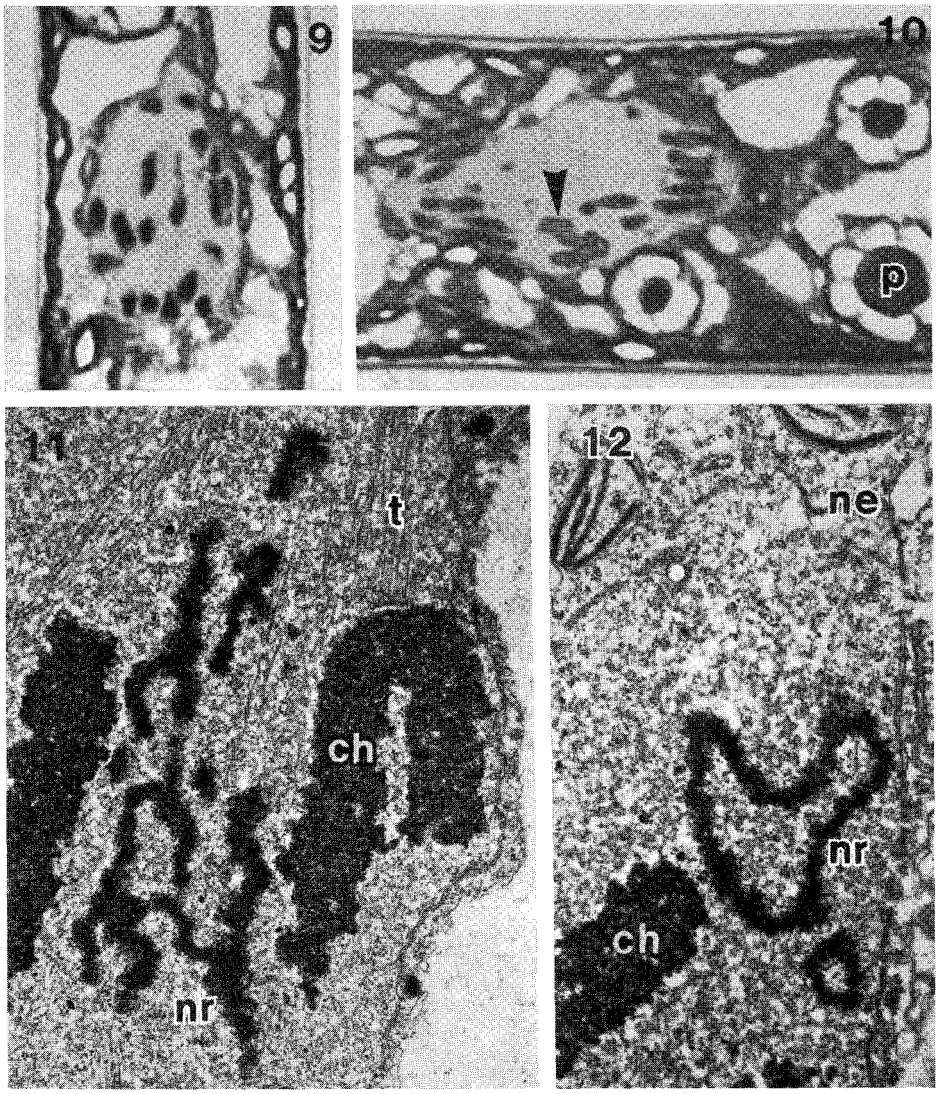



Fig. 9.—This metaphase nucleus contained typical paired chromosomes. The shape of the nucleus was quite variable throughout division (e.g. Fig. 8*b*). ×1,500.

Fig. 10.—Section through a mid-anaphase nucleus. Remains of the nucleolus (arrowed) clearly visible between the chromosome groups. ×1,700 approx.

Fig. 11.—Mid-anaphase nucleus. Remnants of the nucleolus (*nr*) showing typical granular structure, lying amongst chromosomes. ×9,500.

Fig. 12.—As in Figure 11, but in this case the nucleolar remnants (*nr*) were situated at the pole of a metaphase nucleus; cf. Figure 8*b*. ×11,000.

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Fig. 8*b*.—Early anaphase cell from Figure 8*a*. The two sets of chromosomes were distinctly separated. Note irregular shape of nucleus, and nucleolar remnant (*nr*)—see Figures 11 and 12. Area enclosed by black lines shown in Figure 8*c*. ×7,500.

Fig. 8*c*.—Detail of Figure 8*b*. Note microtubules typically running from kinetochore on the chromosome (*ch*) into an evagination of the nuclear envelope. ×21,000.

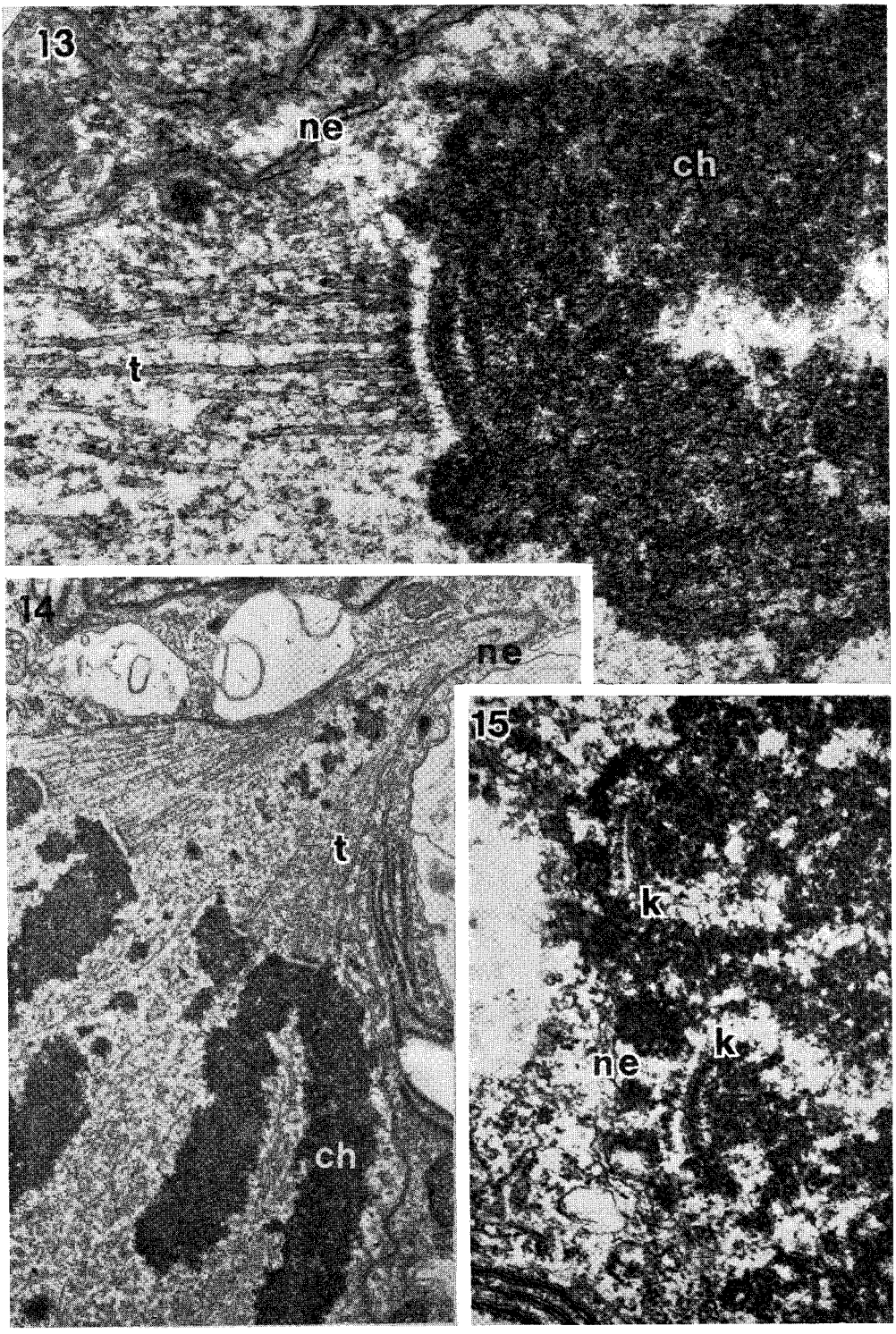


Fig. 13.—Anaphase; structure of the single kinetochore in a chromosome. About seven distinct layers can be discerned in it; microtubules embedded in one layer. ×39,000.

(iv) *Kinetochores*

As in *Oedogonium* (species A) (Pickett-Heaps and Fowke 1969), *O. cardiacum* has very complex kinetochores on the mitotic chromosomes. In favorable sections, up to six or seven separate layered components can be discerned in them (Figs. 7, 13), one layer appearing as a sharply defined line, and another as a distinct, electron-transparent layer (Figs. 4, 7, 8*c*, 13, 16–18) which often became broadened by late anaphase (Fig. 17). A lighter staining region in the chromosome was occasionally detected between the paired metaphase kinetochores (Fig. 7), this probably representing the centromere of the light microscopist. The complexity of these kinetochores is most unusual; to the best of our knowledge, all other algae and higher plant cells described have rather poorly defined, structureless kinetochores if they are detectable at all [e.g. Harris and Bajer (1965), Wilson (1968), and others].

Because of their structure, the kinetochores were visible within early prophase and late telophase nuclei. This has interesting repercussions [see Section III (*b*)(v)].

(v) *Metakinesis: Interaction of Kinetochores and Microtubules*

The mechanism by which the chromosomes move to the metaphase-plate configuration (metakinesis) is puzzling to cytologists. In particular, it has been difficult to decide when and how microtubules interact with the chromosomes. The conspicuous substructure of the kinetochores in *Oedogonium* has enabled us to follow metakinesis in more detail than has been previously possible.

At prophase, paired or single (depending on the relative plane of sectioning with the pair) kinetochores were detectable on partly condensed chromosomes (Figs. 3*a*–3*c*), widely scattered and seldom aligned with the spindle axis. Short segments of microtubules were associated with them (Figs. 3*b*, 3*c*). A few pole-to-pole microtubules were also evident. During prometaphase, some kinetochore pairs were found associated with tufts of microtubules oriented skew to the spindle. However, the next distinct stage in metakinesis was apparently marked by the kinetochore microtubules having become aligned along the spindle axis. This is shown in Figure 4. Six pairs (or parts of pairs) of kinetochores were fortuitously present in one section; although these pairs were still quite widely scattered within the spindle, all six had become oriented parallel to the metaphase-plate configuration. By metaphase, the pairs of kinetochores were aligned approximately in the one central plane (Fig. 6). Tufts of microtubules, representing apparently most of those present, ran from the kinetochores up into the blebs in the nuclear envelope (see above). Paired chromosomes were visible with the light microscope (Fig. 9).

To explain metakinesis Pickett-Heaps (1967) suggested that paired kinetochores, quite possibly randomly oriented, started polymerizing microtubules (see Newcomb 1969) in opposite directions along their common axis. Interaction of these

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Fig. 14.—Later anaphase; chromosomes drawn close to poles. Note microtubules running from kinetochores into the polar evagination of the nuclear envelope. One chromosome arm was also present at the pole, this section grazing its surface. ×9,000.

Fig. 15.—Structure of kinetochores (*k*) still clearly visible on chromatin near nuclear envelope (*ne*) at pole of fully formed, telophase daughter nucleus (as in Fig. 23). ×22,000 approx.

microtubules with the continuous spindle tubules was envisaged as firstly aligning the kinetochore axis with the spindle axis. Next, the increasing length of these kinetochore tubules would cause one or both sets to interact with the polar cytoplasm or membranes or both. If one set reached its polar zone before the other (due to the chromosomes not being centrally placed in the spindle axis), its continued growth would tend to move the chromosome into the equatorial position. Such a growth of kinetochore tubules would also obviously tend to move large passive bodies such as the remains of the nucleolus into the polar regions of the spindle (see above). Depolymerization of microtubules would be occurring to some extent at the poles (as must happen subsequently during anaphase). These latter points were indicated by Forer's (1966) work which showed there was some poleward movement in chromosomal fibres or their components *during metaphase* when the chromosomes are essentially immobile.

The results described above are quite consistent with this theory, which has been summarized in Figure 26. Evidence obtained *in vivo* implicates spindle fibres in metakinesis (Mazia 1961), and microtubules are generally attached to kinetochores by metaphase (e.g. Harris 1965; Harris and Bajer 1965; Bajer 1968; and others).

(vi) *Spindle Microtubules and Anaphase Elongation*

Because cells were easily examined in Araldite blocks, we were able to select many anaphase cells (e.g. Fig. 19*c*) for sectioning, thus confirming that a quite distinct phase of spindle elongation followed separation of chromatids.

At early anaphase (Figs. 8*a*, 8*b*), the nucleus was still quite rotund except for the polar evaginations (see above). By mid-anaphase, the spindle was lens-shaped (Fig. 10) and at late anaphase, it had become markedly elongated (Figs. 19*a*–19*c*). This elongation apparently disrupted the nuclear envelope to some extent (Fig. 19*b*). Coincident with spindle elongation, large numbers of interzonal microtubules appeared; we gained the distinct impression that these proliferated initially amongst the trailing chromosome arms (Fig. 21). This quite strongly suggests that subunits from the kinetochore microtubules, depolymerized at the poles (Fig. 16), were being almost immediately reassembled to form the new interzonal microtubules. The idea is not new, but in *Oedogonium* escape of microtubule subunits from the closed spindle is less likely than with open spindles. These events are summarized in Figure 27.

(vii) *Collapse of the Anaphase Spindle*

By early telophase, the nuclear envelope contracted around the chromosomes (Figs. 20*a et al.*), effectively isolating the interzonal nucleoplasm, containing microtubules and the nucleolar remnants (Fig. 20*b*), from the daughter nuclei. As in *Oedogonium* (species A) interzonal structure then collapsed (Fig. 22*a*), probably conse-

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Fig. 17.—Mid-anaphase; large number of microtubules extending from kinetochores into nuclear evagination; interzonal microtubules were few in number. Often at this particular stage, the clear zone of the kinetochores (arrowed) was particularly conspicuous. ×14,000.

Fig. 18.—Late anaphase; note outgrowth of nuclear envelope at pole (arrowed), normally characteristic of prophase–metaphase (as shown in Figs. 5*a*–5*d*). ×17,000.

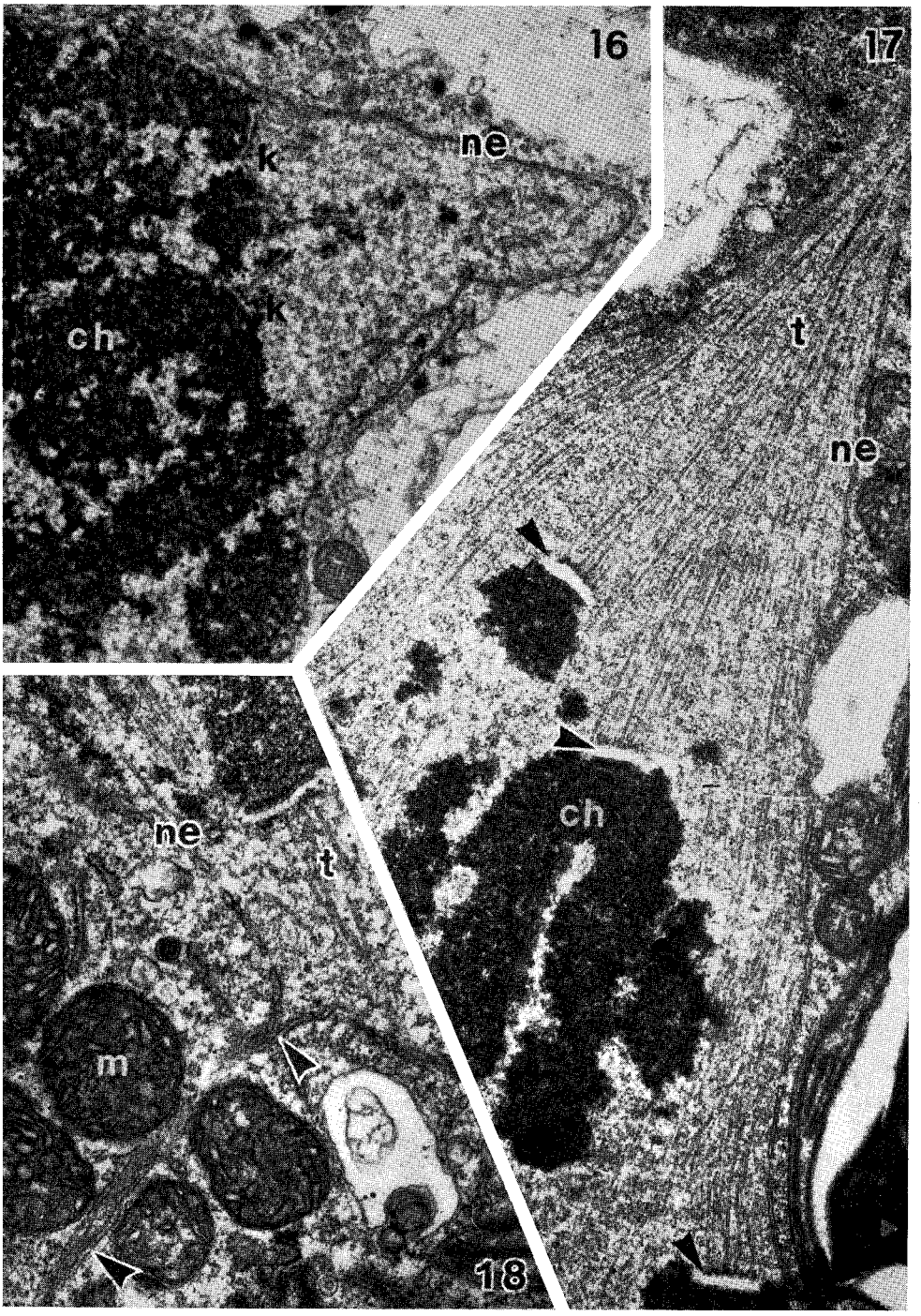



Fig. 16.—Serial section of one polar region of the daughter nuclei seen in Figure 19*b*. Nuclear evagination still present (cf. Figs. 8*c*, 14, 17). but very few microtubules in it; kinetochores remained detectable for some while after this stage (see Fig. 15). ×12,000.

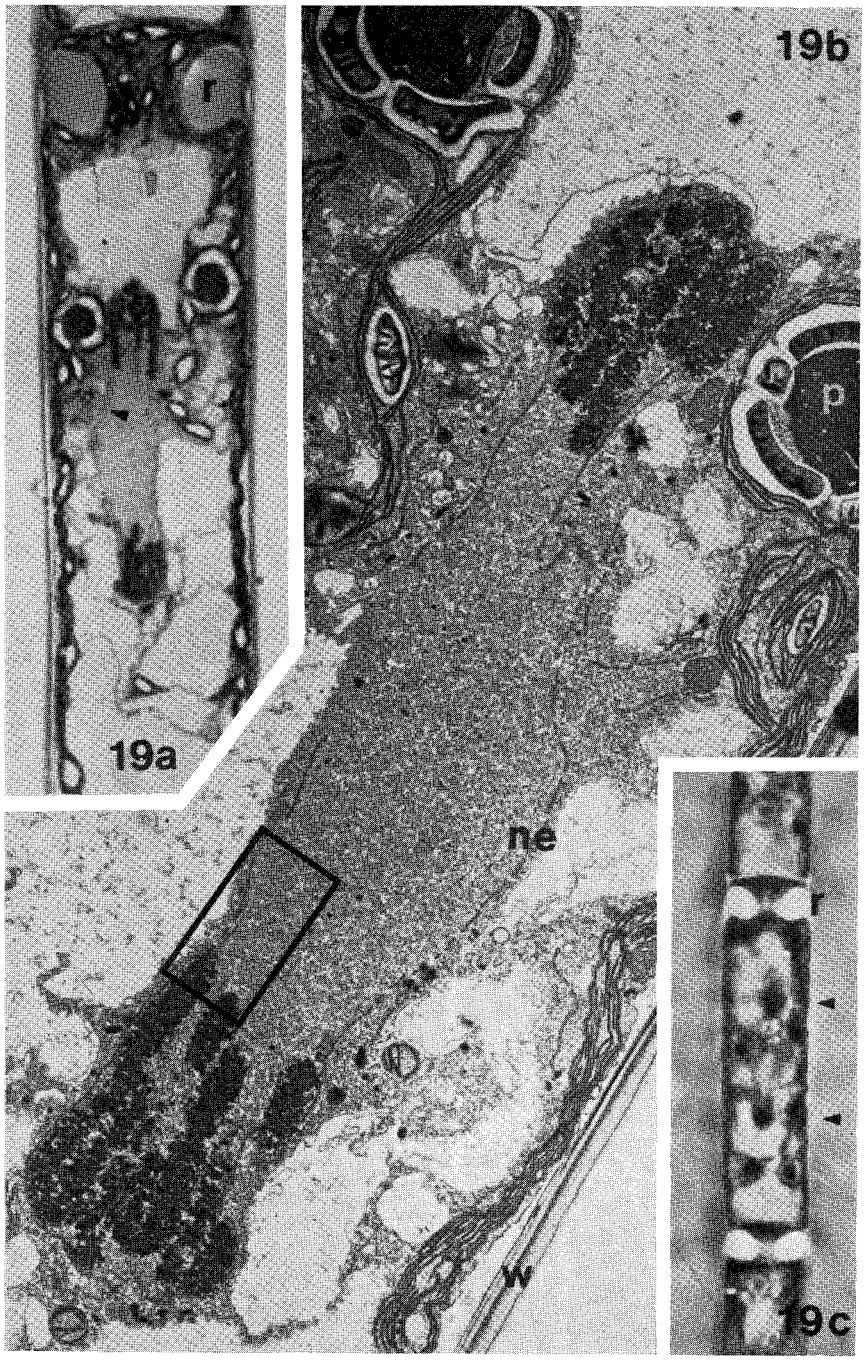



Fig. 19*a*.—Anaphase elongation stage. Note the considerable increase in length of the spindle; nuclear envelope just visible (arrow). ×680.

Fig. 19*b*.—Same cell as that in Figure 19*a*. Nuclear envelope appears stretched and broken, although it was contracting around the chromosomes (Fig. 21). Serial section of area enclosed by rectangle shown in Figure 21. ×2,500.

Fig. 19*c*.—Equivalent cell to that in Figures 19*a* and 19*b*. This was photographed in the Araldite block, using phase optics, before the cell was sectioned. Arrows indicate the two nuclei. This demonstrates how one could choose particular stages of mitosis for sectioning. ×300.

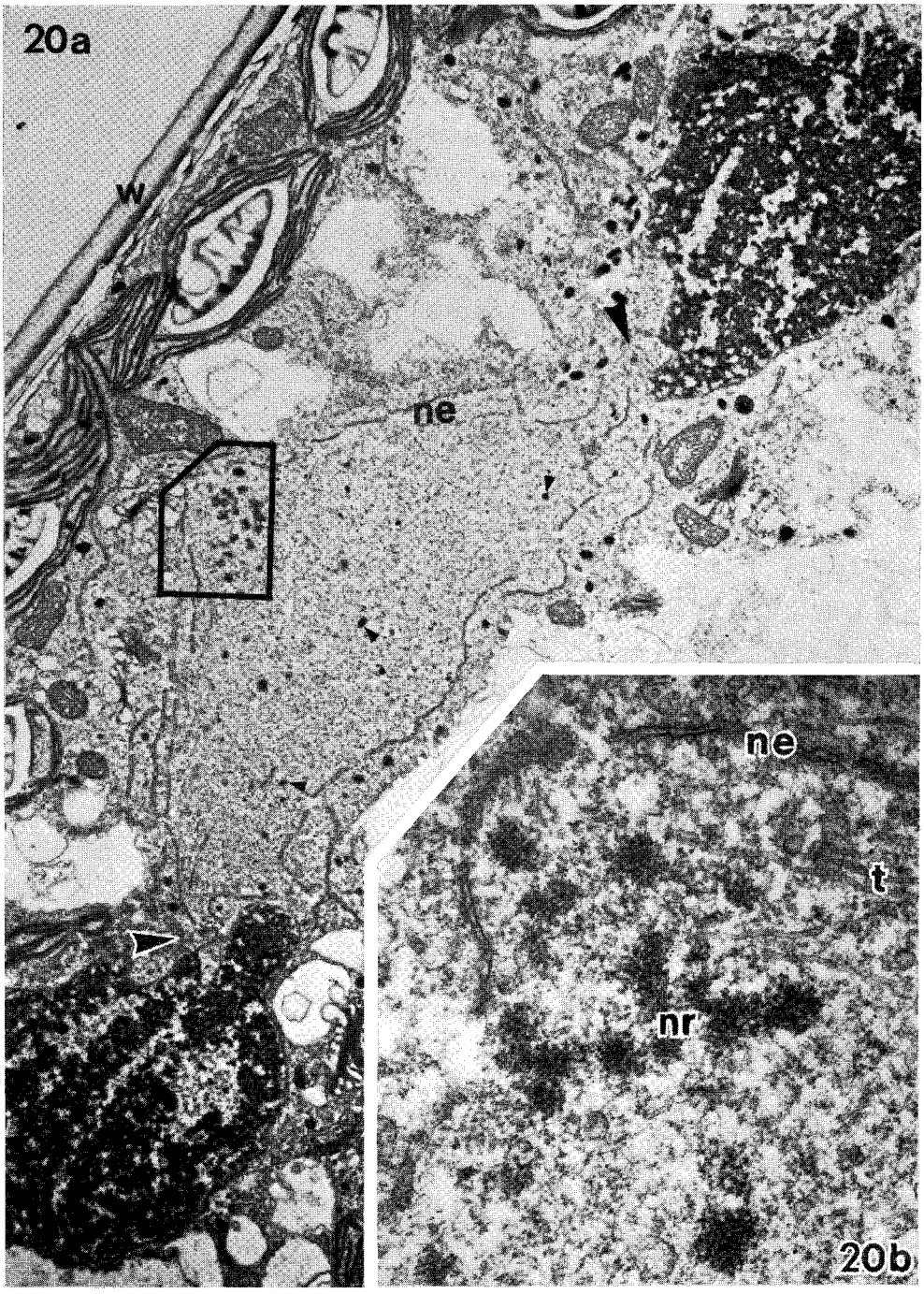
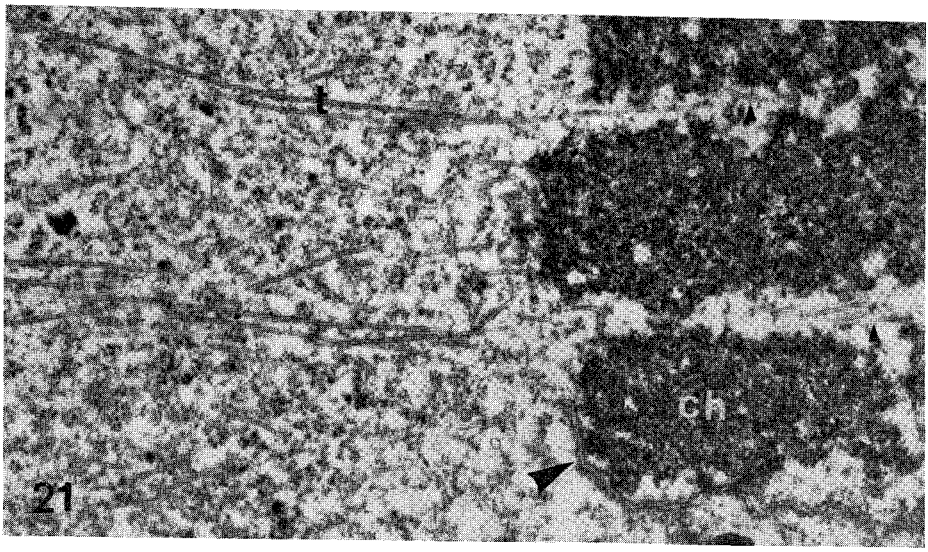



Fig. 20*a*.—Telophase, early stage of spindle collapse (compare with Fig. 19*b*). Nuclear envelope closely coated chromosomes, interzonal region still defined by remains of nuclear envelope; many small organelles (small arrows) had entered this region. Traces of early septum formation at large arrows (see Figs. 22*a*, 22*b*). Region enclosed by lines shown below. ×3,100 approx.

Fig. 20*b*.—Serial section of region delineated in Figure 20*a*. Nucleolar remnants (*nr*—see Figs. 11, 12, and others) inside old nuclear envelope, and interzonal microtubules are visible. ×15,000 approx.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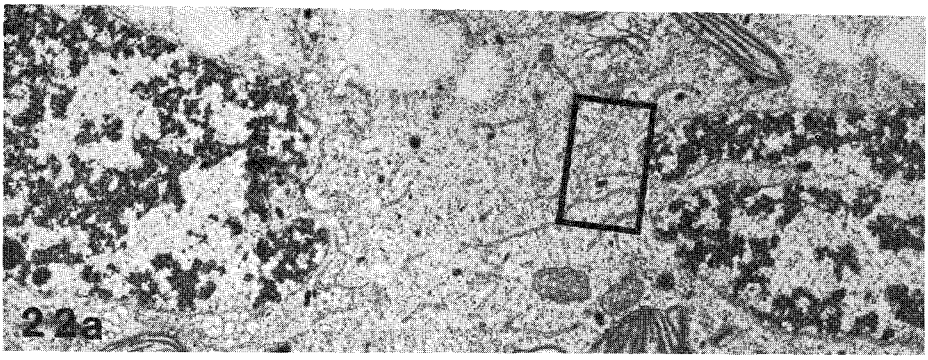
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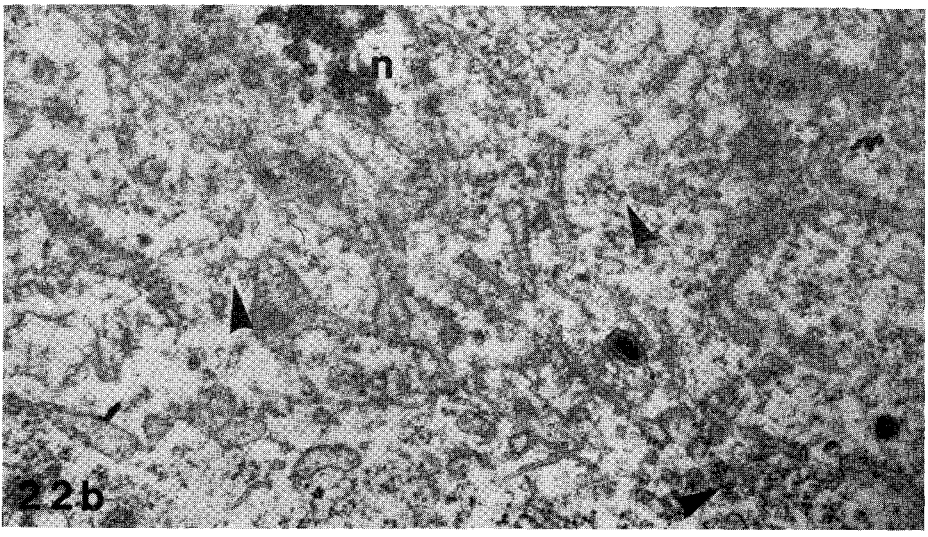
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quent upon breakdown or reorientation or both of the microtubules in it (Fig. 22*b*). The nuclei closely approached one another (Fig. 23) eventually becoming flattened against one another (Fig. 24) whilst septum formation was under way. This of course is in marked contrast to what happens at telophase in the large majority of plant cells which develop a phragmoplast for cell-plate formation (see Pickett-Heaps 1969).

(viii) *The Septum*

The formation of the septum (a structure unique to *Oedogonium*, as far as we know) in *O. cardiacum* resembles that described in *Oedogonium* (species A); with one difference. In the latter species a strand of cytoplasm is extended from between the daughter nuclei across the vacuole, partitioning the cell, the spindle and daughter nuclei being situated on one side of the cell. In *O. cardiacum*, however, the daughter nuclei extended across most of the cell lumen; as they came together, the rounded nuclei (Fig. 23) became flattened (Fig. 24) and between them, a proliferation of small vesicles, membranes, and *transversely oriented* microtubules was seen (Fig. 25). These components extended laterally, eventually forming a clearly defined band across the cell between the daughter nuclei (Fig. 25). As with *Oedogonium* (species A), septum initiation might have occurred near the nuclei in small areas where membranes and microtubules congregated (Figs, 20*a*, 22*a*, 22*b*), whilst the nuclei were still separated (Figs. 20*a*, 22*a*), but the proliferation of septum components did not really commence until the nuclei were flattened together (compare Figs 23 and 24). After the septum had been formed, the sequence of events was very similar to that described previously (Pickett-Heaps and Fowke 1969); in particular, the septum moved up the cell as a coherent unit during cell elongation, the vesicles fusing only when the septum reached the final position to be taken up by the new cross wall. These latter events will be dealt with separately (Pickett-Heaps and Fowke 1970).

Just recently, Hill and Machlis (1968) have presented an ultrastructural study of cell division in *O. borisianum*. They achieved excellent morphological preservation, and though they did not describe mitosis they show the structure of the septum in detail. They consider (p.271) that: "The sequence of events leading to the formation of the transverse wall differs only slightly from that in the vascular plants". We strongly disagree with this interpretation, preferring rather the view that it is quite fundamentally different. In particular, a similar transverse orientation of microtubules between telophase nuclei has been observed in *Chlamydomonas* (Johnson

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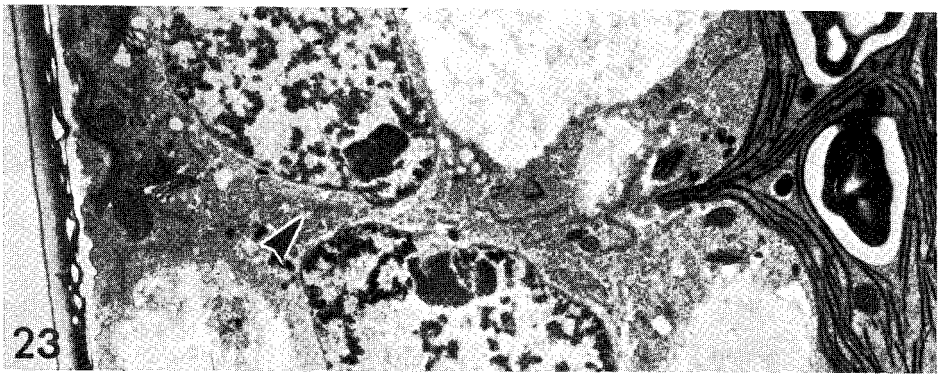
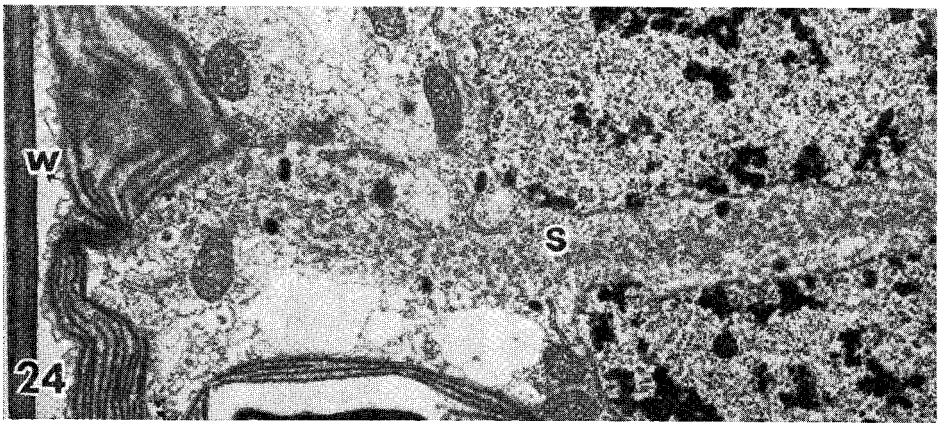
Fig. 21.—Serial section of region delineated in Figure 19*b*. The large number of interzonal microtubules appearing during anaphase elongation penetrated into (and possibly were being formed amongst) the chromosome arms (small arrows). Note nuclear envelope contracting closely around chromosome (large arrow). ×16,000.

Fig. 22*a*.—Further collapse of spindle—compare with Figure 20*a*. Nuclei approaching one another. Septum formation initiated near nuclei—region enclosed by lines shown in more detail below. ×5,000.

Fig. 22*b*.—Serial section of region delineated in Figure 22*a*. Microtubules, formerly longitudinally oriented as in Figure 21, were becoming increasingly rearranged (or repolymerized) into the transverse, septum orientation (see Fig. 25); some are seen in cross-section (large arrows). ×23,000.


23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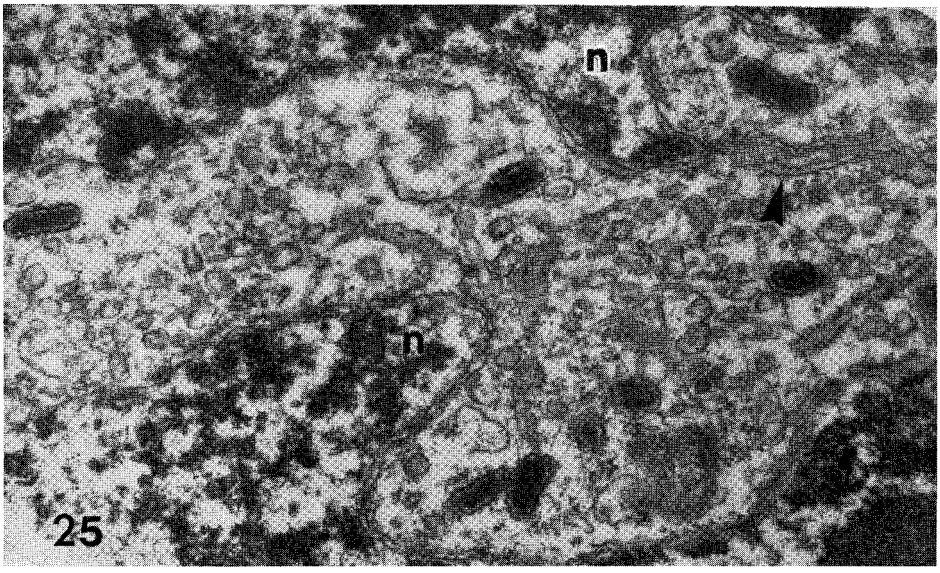
n
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25

and Porter 1968) and *Closterium* (Pickett-Heaps and Fowke, unpublished data). The evolutionary and functional significance of this is discussed by Pickett-Heaps (1969).

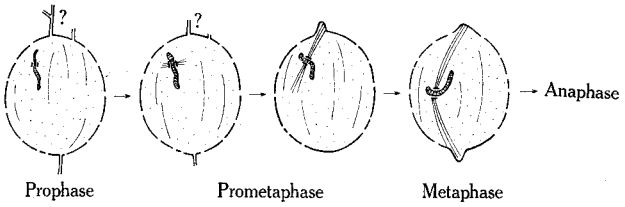


Fig. 26.—Diagrammatic reconstruction of possible sequence of events occurring during metakinesis. At prophase, kinetochores (oriented and situated probably at random) appear on chromosomes (see Fig. 3*a*). Kinetochores polymerize microtubules in two opposite directions; continuous growth of these chromosome fibres is envisaged as interacting with the continuous fibre system of the spindle, resulting first in orientation of the kinetochore pairs across the spindle during prometaphase (see Fig. 4). If one set of the kinetochore fibres impinges on the nuclear membrane before the other, then continuing growth of these fibres would move the kinetochore pair into the metaphase-plate configuration. Channels formed by the nuclear envelope are also represented here (see Figs. 5*a*–5*d*)—their total form and extent are unknown. Kinetochore fibres appear to run into (and probably cause) evaginations of the nuclear envelope (as in Figs. 8*b*, 14, 17).

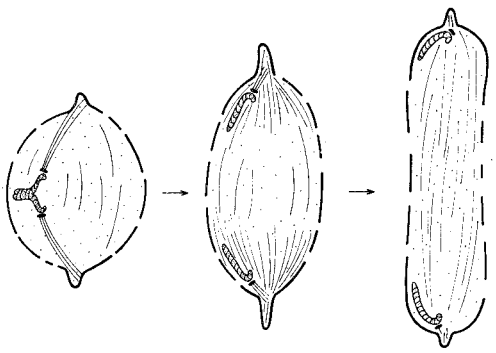

Fig. 27.—Diagrammatic reconstruction of possible sequence of events occurring during anaphase. As before, the kinetochore fibres run into evaginations of the nuclear envelope (see Figs. 8*c*, 17). At early anaphase, the spindle is quite short (Figs. 8*a*, 8*b*), and the interzonal fibre system not very developed. As anaphase continues, there is a progressive increase in the length of the spindle (Figs. 10, 19*a*) as the chromosomes approach the poles; this coincides with the appearance of an increasing number of interzonal microtubules formed perhaps by repolymerization of subunits derived from the kinetochore fibres, which must have been broken down somewhere in the polar evaginations of the nuclear envelope (see Fig. 16). Repolymerization may possibly occur between or near the trailing chromosome arms (Fig. 21).

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Fig. 23.—Last stage of spindle collapse, cell on left in Figure 8*a* (cf. Figs. 19*b*, 20*a*, 22*a*). Nuclei still not flattened; compare with Figure 24. Septum formation visible at arrow, but not extensive at this stage. ×4,000.

Fig. 24.—Septum formation, later than that in Figure 23 above. The complex of vesicles and microtubules extended right across the cell between the nuclei, now flattened together. ×7,500.

Fig. 25.—Components of septum between close-pressed nuclei (*n*), as in Figure 24. Endoplasmic reticulum, vesicles, and some other cell organelles are seen amongst microtubules scattered in the transverse orientation. Occasionally, the nuclear envelope in this region too formed channels (arrow) similar to those seen earlier (Figs. 5*a*–5*d*, 18); continuity between the nuclear envelope and the channel here was confirmed by serial sectioning. ×24,000.

## IV. Acknowledgment

One of the authors (L.C.F.) gratefully acknowledges the receipt of a National Research Council of Canada Postdoctoral Fellowship.

## V. References

Allen, R. D., and Bowen, C. C. (1966).—Fine structure of *Psilotum nudum* cells during mitosis. *Caryologia* **19**, 299–342.

Bajer, A. (1968).—Chromosome movement and fine structure of the mitotic spindle. *Symp. Soc. exp. Biol.* **22**, 285–310.

Fabbri, F. (1960).—Contributo per l'interpretazione della persistenze nucleolare durante la mitosi in *Psilotum nudum*. *Caryologia* **13**, 297–337.

Forer, A. (1966).—Local reduction of spindle fibre birefringence in living *Nephrotoma suturalis* (Loew) spermatocytes induced by ultraviolet microbeam irradiation. *J. Cell Biol.* **25**, 95–117.

Fowke, L. C., and Pickett-Heaps, J. D. (1969*a*).—Cell division in *Spirogyra*. I. Mitosis. *J. Phycol.* **5**, 240–59.

Fowke, L. C., and Pickett-Heaps, J. D. (1969*b*).—Cell division in *Spirogyra*. II. Cytokinesis. *J. Phycol.* **5**, 273–81.

Harris, P. (1965).—Some observations concerning metakineses in sea urchin eggs. *J. Cell Biol.* **25**. 73–7.

Harris, P., and Bajer, A. (1965).—Fine structure studies on mitosis in endosperm metaphase of *Haemanthus katherinae* Bak. *Chromosoma* **16**, 624–36.

Hill, G. J. C., and Machlis, L. (1968).—An ultrastructural study of vegetative cell division in *Oedogonium borisianum*. *J. Phycol.* **4**, 261–71.

Johnson, U. G., and Porter, K. R. (1968).—Fine structure of cell division in *Chlamydomonas reinhardii*. *J. Cell Biol.* **38**, 403–25.

Lafontaine, J. G., and Chouinard, L. A. (1963).—A correlated light and electron microscope study of the nucleolar material during mitosis in *Vicia faba*. *J. Cell Biol.* **17**, 167–201.

Mazia, D. (1961).—Mitosis and the physiology of cell division. In "The Cell". (Eds. J. Brachet and A. E. Mirsky.) Vol. 3. pp. 77–412. (Academic Press, Inc.: New York and London.)

Newcomb, E. H. (1969).—Plant microtubules. *A. Rev. Pl. Physiol.* (In press.)

Pickett-Heaps, J. D. (1967).—Ultrastructure and differentiation in *Chara* sp. II. Mitosis. *Aust. J. biol. Sci.* **20**, 883–94.

Pickett-Heaps, J. D. (1969).—The evolution of the mitotic apparatus. *Cytobios* **20**, 253–88.

Pickett-Heaps, J. D., and Fowke, L. C. (1969).—Cell division in *Oedogonium*. I. Mitosis, cytokinesis, and cell elongation. *Aust. J. biol. Sci.* **22**, 857–94.

Pickett-Heaps, J. D., and Fowke, L. C. (1970).—Cell division in *Oedogonium*. III. Golgi bodies, wall structure, and wall formation in *O. cardiacum*. *Aust. J. biol. Sci.* **23**, 93–113.

Wilson, H. J. (1968).—The fine structure of the kinetochore in meiotic cells of *Tradescantia*. *Planta* **78**, 379–85.