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Formation Of Centriole And Centriole-like Structures During Meiosis And Mitosis In Labyrinthula Sp. (Rhizopodea, Labyrinthulida) - An Electron Microscope Study

Frank O. Perkins

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FORMATION OF CENTRIOLE AND CENTRIOLE-LIKE STRUCTURES DURING MEIOSIS AND MITOSIS IN *LABYRINTHULA* SP. (RHIZOPODEA, LABYRINTHULIDA)

AN ELECTRON-MICROSCOPE STUDY*

F. O. PERKINS
Virginia Institute of Marine Science, Gloucester Point, Virginia 23062, *U.S.A.*

SUMMARY

The fine structure of mitosis in vegetative cells of the marine protist *Labyrinthula* was found to involve formation of two approximately spherical, electron-dense aggregates (termed protocentrioles) 200–300 nm in diameter. Spindle microtubules were directly attached to the structures. The aggregates contained centrally located cartwheel structures but no microtubular elements in the form of a centriole-like cylinder. In non-sporulating cells the aggregates occurred only during mitosis or possibly in late interphase cells. During meiotic zoosporulation *de novo* centriole formation was observed. Vegetative spindle cells, which contained no centrioles, procentrioles, or centriolar plaques, aggregated then changed into approximately round or oval presporangia within sori. Two protocentrioles were formed in the cytoplasm a few hundred nanometres from each nucleus. Microtubules, oriented in astral ray patterns, were attached directly to each of the protocentrioles. Following migration to opposite sides of the nucleus, each of the protocentrioles differentiated into two centrioles attached at the cartwheel or proximal ends in a longitudinally continuous orientation. Binary fission of the paired centrioles (termed bicentrioles) and reorientation yielded a diplosome or an approximate orthogonal orientation of the organelles. Each mature centriole consisted of the usual cylinder of 9 triplet microtubular blades with a cartwheel complex at the proximal end consisting of 5 or 6 tiers of cartwheels. Further centriole replication appeared to occur by orthogonal budding from mature centrioles.

INTRODUCTION

In recent years the nearly ubiquitous involvement of microtubules in nuclear division has become more evident. In most organisms microtubules converge on discrete, specialized organelles located at the poles of the division figures. The organelles may be comprised of microtubules, electron-dense granular aggregates, or fibrogranular aggregates. The microtubular forms (centrioles) are cylinders formed by parallel arrays of microtubules grouped in either triplet blades (Stubblefield & Brinkley, 1967; de Harven, 1968), doublet blades (Phillips, 1966), or singlets (Phillips, 1966). Centrioles are always located in the cytoplasm. Granular or fibrogranular aggregates have been found in the nuclear envelope (Robinow & Marak, 1966), the cytoplasm (Lu, 1967; Motta, 1967), and the nucleoplasm (Perkins, 1968, 1969). It

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has not been determined whether the aggregates are permanent structures in the cell or transient, being formed just before or during nuclear division.

The ultrastructure of centriole and basal body formation by orthogonal budding from pre-existing centrioles (Gall, 1961; Renaud & Swift, 1964; Stubblefield & Brinkley, 1967; Robbins, Jentzsch & Micali, 1968; Sorokin, 1968), by formation from granular or fibrogranular aggregates (Randall *et al.* 1963; Stockinger & Cireli, 1965; Sorokin & Adelstein, 1967; Sorokin, 1968), and by formation from procentriole complexes or blepharoplasts (Mizukami & Gall, 1966) has been described in some detail. Most studies have involved centriole or basal body formation in the presence of pre-existing centrioles, basal bodies, or procentrioles, supporting the belief that the presence of mature organelles or metameric fragments of the organelles is required for formation to occur (de Harven, 1968).

Other studies have been conducted utilizing Protozoa and cryptogams where recognizable centrioles or basal bodies are found only in one segment of the life-cycle (Schuster, 1963; Dingle & Fulton, 1966; de Harven, 1968; Turner, 1968). Cellular stages in other segments of the life-cycle contained no detectable precursor organelles such as procentrioles. In none of the studies of such organisms has the *de novo* formation or differentiation of centrioles or basal bodies been described in detail. The authors noted only the appearance of fully formed centrioles.

Sporulation in *Labyrinthula* sp. (Amon & Perkins, 1968; Perkins & Amon, 1969; Moens & Perkins, 1969), a protist of uncertain taxonomic affinities (Porkorny, 1967), serves as an excellent model system for following, in mitosis, granular aggregate formation and, in meiosis, centriole formation. In each case no apparent precursor organelle exists prior to initiation of formation. The newly formed centrioles are utilized first in meiosis, not simply as basal bodies in flagellar formation; therefore, the following study is unique in that the details of true centriole formation from undetectable precursor structures are described, and not basal body formation. Whether such a distinction is significant is uncertain, however, since the ultrastructure of basal bodies (kinetosomes) and centrioles within cells of the same species is apparently identical.

MATERIALS AND METHODS

The techniques used to induce sporulation in *Labyrinthula* sp. (isolate no. 67) have been described (Perkins & Amon, 1969). Cells were fixed in 2·5 % glutaraldehyde buffered at pH 7·4 with 0·2 M Millonig's phosphate buffer (Millonig, 1961). Commercial glutaraldehyde (50 %) was altered by exposure to a suspension of about 1 g/50 ml of barium carbonate. Four buffer rinses were then accomplished in 0·2 M Millonig's phosphate buffer at pH 7·4 with 0·15 M NaCl over a period of 1–12 h, followed by post-fixation in 1 % OsO_4 for 1 h. The post-fixative was buffered at pH 7·4 with 0·1 M phosphate buffer and contained 0·2 M NaCl. The tonicity of the fixative and buffer rinse was 700 m-osmole, and of the post-fixative 615 m-osmole. All solutions were held at 22–24 °C. Epon 812 with a 0·60 anhydride:epoxy ratio (Coulter, 1967) was the embedding medium. Sections were mounted on Mason and Morton slot grids (Ernest F. Fullam, Inc., Schenectady, N.Y.) with a carbon and Formvar substrate and stained with both 2 % aqueous uranyl acetate at pH 4·8 and lead citrate (Reynolds, 1963). Electron micrographs were taken with an Hitachi HU-11B microscope.

Since the nuclei were very small (2–3·8 μm diameter) and chromosomes were difficult to see,

it was not possible to categorize accurately various stages of the meiotic and mitotic sequences from observations of cells prepared for either light or electron microscopy. Therefore, the terms prophase, metaphase, anaphase, and telophase are used to express only an approximation of the stage under consideration.

Rotation micrographs of centriole cross-sections were obtained by using the methods of Markham, Frey & Hills (1963).

RESULTS

The fine structure of vegetative cells of *Labyrinthula* sp. is fairly typical of uninucleate eucaryotic cells (Fig. 3). Numerous membrane-free cytoplasmic ribosomes, Golgi bodies, smooth endoplasmic reticulum, a nuclear envelope with annular pores, a Feulgen-negative endosome or nucleolus containing ribosome-like substructure, large Sudan IV-staining inclusion bodies, presumed to be lipid, and microtubular mitochondria are present in each cell. Unusual fine structure includes the unit-membrane-limited 'slime way' through which the cells glide (Hohl, 1966; Porter, 1969) and the bothrosomes (Stey, 1968; Porter, 1969), which appear to be sites for slime-way formation.

Even though the fine structure of vegetative spindle cells was examined in cells from isolates which periodically exhibited sporulation, as well as isolates where sporulation was not observed, centrioles were not found. Porter (1969) likewise did not observe microtubular centrioles in his isolates of *Labyrinthula*.

Spindle-cell mitosis was accompanied, probably just before or during prophase, by formation of one or two electron-dense, granular aggregates, herein termed protocentrioles. The earliest detected stage in protocentriole formation was an oval-shaped, electron-dense aggregate 100 × 200 nm (Fig. 4). Further protocentriole differentiation involved increase in size to form one or two approximately spherical granular aggregates 200–300 nm in diameter located a few hundred nanometres from the nuclear envelope (Fig. 5). A cartwheel with 7–9 spokes was observed in the centre of each protocentriole (Fig. 6); however, further differentiation into a mature centriole, as in zoosporulation, never occurred (see below). Numbers and lengths of microtubules attached to the protocentrioles increased until a prominent astral ray pattern was found around each organelle (Fig. 5). Either one protocentriole then divided and the sub-units migrated to opposite sides of the nucleus during prophase, or two protocentrioles, which had been formed *de novo*, migrated apart. Nuclear-envelope breakdown occurred, followed by invasion of microtubules into the nucleoplasm by means of extension away from the two protocentrioles.

During the remaining stages of mitosis the protocentrioles retained their size and structure (Fig. 7). After telophase and before cytokinesis the microtubules and protocentrioles disappeared. Binucleated spindle cells with daughter nuclei in interphase were frequently observed, thereby indicating frequent delay in cytokinesis.

Spindle cells with apparent interphase nuclei were never observed to have centrioles, protocentrioles, or any detectable precursor organelles. Microtubules were also absent from the cytoplasm of cells not engaged in mitosis. During zoosporulation centrioles were synthesized. Spindle cells initiated sporulation by forming sori or aggregates in which the cells became round or oval and were compartmentalized into individual

presporangia by a delimiting 4-nm thick wall and portions of the slime way (Perkins & Amon, 1969). Two astral ray clusters of microtubules were first observed at one side of the nucleus 0·4–0·6 μm from the nuclear envelope (measured from the centre of the aggregate to the outside nuclear membrane). The microtubules of each cluster were attached at one end to a centrally located granular aggregate or protocentriole (Perkins & Amon, 1969; Fig. 8). It was not determined whether the protocentrioles formed during or just after microtubule formation, nor was it determined whether they were formed initially against or away from the nuclear envelope. The microtubules radiated out in all directions, but primarily toward the nucleus. They extended as far as 2 μm from each of the protocentrioles and reached about halfway around the nucleus.

Numerous Golgi bodies and vesicles derived from the bodies were found clustered around protocentrioles, centrioles, and associated microtubules. However, the role of Golgi vesicles, if any, in microtubule, protocentriole, or centriole formation was not determined. Attempts to find Golgi vesicles continuous with microtubules did not yield conclusive results. Elongated vesicles were occasionally observed, but no continuities were found as convincing as those presented by Robbins *et al.* (1968).

Further differentiation of the protocentrioles involved formation of a cartwheel structure within the centre of each aggregate (Fig. 9; Perkins & Amon, 1969). The cartwheel consisted of a centrally located hub with radiating spokes, initially less than 9 (Fig. 9) but ultimately 9. An electron-dense rod or granule was found in the centre of the hub. The spokes terminated in the matrix of the granular aggregate.

The protocentrioles then migrated to positions on opposite sides of the nucleus (Figs. 10, 11). At this stage microtubules were still found around each protocentriole in an astral ray pattern and not continuous between the two organelles. No microtubules were present in the nucleoplasm and the nuclear envelope was intact. The presence of synaptinemal complexes in the nucleoplasm indicated that the nucleus was in prophase I (zygonema or pachynema) (Perkins & Amon, 1969; Moens & Perkins, 1969). Complexes were not present in the nucleus when protocentrioles were closely juxtaposed.

The next stage observed in centriole development consisted of a bicentriole, also found during zygonema or pachynema. The double organelle consisted of 2 centrioles joined at the cartwheel (proximal) ends (Figs. 12–14) in a linear relationship (Fig. 12) or slightly skewed (Figs. 13, 14). Five or 6 tiers of cartwheels were present in each centriole (Fig. 13). Presumably additional tiers of cartwheels were added to the protocentrioles, followed or accompanied by formation of a ring of 9 single microtubules (the A or innermost microtubules of the centriole triplet blades) to which the spokes of the cartwheel were attached. The microtubules probably lengthened bilaterally from the cartwheel region, resulting in formation of an immature bicentriole. An alternative interpretation is that 2 sets of 9 microtubules were formed. Each set then lengthened in opposite directions to yield an immature bicentriole. Lengthening of microtubules away from the cartwheel region of procentrioles and basal bodies has been reported (Renaud & Swift, 1964; Sorokin, 1968), but never bilateral growth.

The B microtubules of the triplet blades were then apparently synthesized, followed

by the C units. Figure 15 shows a presumptive developing centriole with at least 2 doublets and 5 singlets visible. The possibility exists, however, that Fig. 15 represents a section through the region between 2 centrioles of a bicentriole, since serial sections through bicentrioles (Figs. 16–22) revealed that the C and possibly the B microtubules do not extend the full length of each centriole. Sections through the extreme proximal and distal ends revealed doublets, triplets, and some indication of singlets.

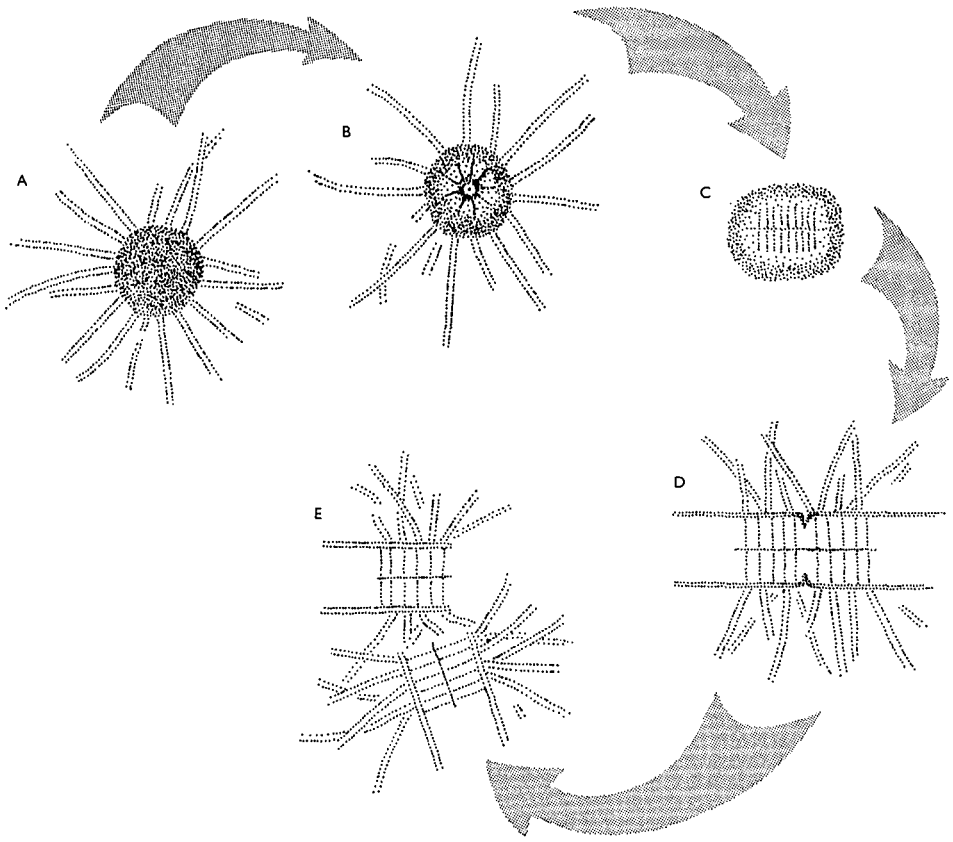

Fig. 1. Diagrammatic representation of centriole formation during prophase I. Granular aggregates (protocentrioles) with attached spindle microtubules were formed next to nuclei prior to or early in prophase I (A). Tiers of cartwheel complexes were then formed in the medulla of each protocentriole (cross-section, B; longitudinal section, C). Bicentrioles (longitudinal section, D) were probably formed by bilateral growth of a ring of 9 microtubules away from the cartwheel regions. Two additional microtubules were then added to each of the 9 singlets to form the 9 triplet blades of mature centrioles. Binary fission then yielded a diplosome consisting of 2 mature centrioles oriented at 40–90° with respect to each other (E).

Spindle microtubules were attached to bicentrioles only in the region of the cartwheels (Figs. 12–14). Some spindle microtubules appeared to attach directly to the microtubules of the centrioles (Fig. 15) rather than to electron-dense, granular satellites adjacent to centrioles as in mouse spleen cells (de Harven, 1968), or to a triplet base

between triplet blades as in Chinese hamster cells (Stubblefield & Brinkley, 1967).

About the end of prophase I the bicentriole divided in half and the two centrioles became oriented at 40–90° with respect to each other, thus forming a diplosome. The term diplosome is reserved, herein, for pairs of centrioles in which there are two distinct, separate entities, as opposed to bicentrioles in which the two entities are either connected or continuous with each other at their cartwheel ends in a longitudinally continuous orientation. Figure 1 illustrates the presumptive sequence of centriole formation and binary fission. Partial nuclear envelope breakdown occurred, followed by invasion of spindle microtubules into the nucleoplasm at the end of prophase I. Perkins & Amon (1969) have presented structural details of nuclear division.

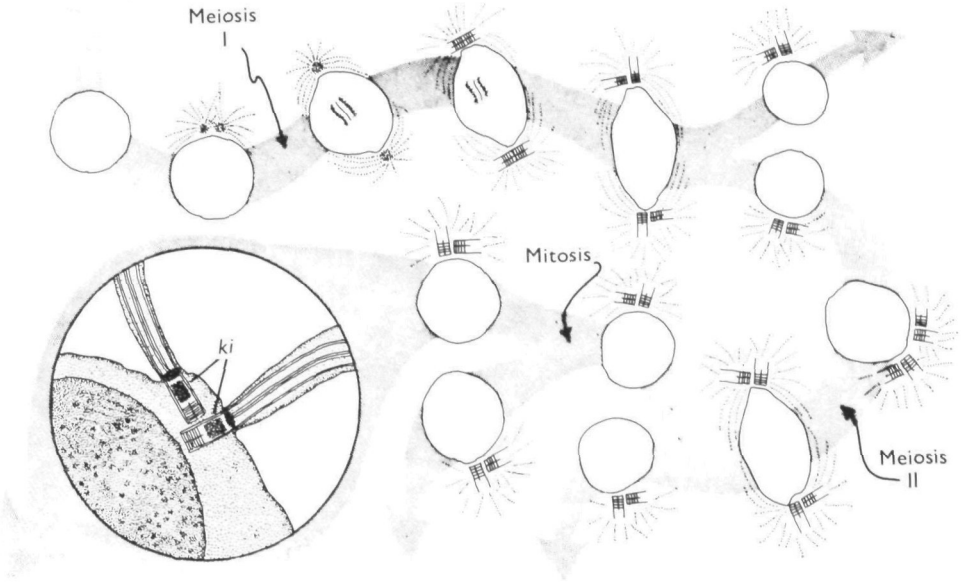

Fig. 2. Diagrammatic representation of centriole formation during zoosporulation. Two protocentrioles were formed on one side of each nucleus, followed by migration to opposite sides of the nucleus. Bicentrioles were then formed and binary fission occurred, followed by reorientation of the daughter centrioles, all during prophase I. Following nuclear division the pair of centrioles in each daughter nucleus then replicated, presumably by budding from the proximal end of each mature centriole, to form 4 centrioles. Migration of the 2 diplosomes to opposite sides of the nucleus occurred followed by meiosis II. Centriole replication by budding presumably occurred in each daughter cell followed by mitosis and then differentiation of eight biflagellated zoospores per sporangium. Pairs of kinetosomes (*ki*) were derived directly from centrioles.

The mature centriole was structurally similar to centrioles described from a variety of other organisms (Stubblefield & Brinkley, 1967; de Harven, 1968) in that it was comprised of a cylinder of 9 triplet microtubules. The cartwheel complex (Figs. 23–25) was typical of protozoan ciliates (Gibbons & Grimstone, 1960) and like that of *Nitella* (Turner, 1968) in that it consisted of tiers of cartwheel structures rather than

only one as in some mammalian centrioles (Stubblefield & Brinkley, 1967). Each cartwheel spoke consisted of a T-shaped structure attached to an electron-dense knob mounted on the A microtubule. A slender rod connected the T to a centrally located hub which encircled a dense rod (Figs. 23, 24). There was some indication that each A microtubule was connected to the B microtubule of each neighbouring triplet by electron-dense granular material. Longitudinal sections revealed 5 or 6 tiers of cartwheels as well as electron-dense material in the lumen at the extreme distal end of the centrioles (Fig. 25). One to three vesicles were found in the lumen of most centrioles, rather than a single vesicle as in Chinese-hamster centrioles (Stubblefield & Brinkley, 1967). The internal helix described from hamster centrioles was not observed.

The distal half of each centriole was comprised of 9 triplet blades connected by bridges between the juxtaposed A–B microtubule interfaces (Fig. 26). The rotation micrograph in Fig. 27 shows the bridges more clearly.

Following telophase I the nuclear membrane was fully reorganized and most spindle microtubules disappeared. Replication of centrioles then occurred, presumably by procentriole budding from the cartwheel portion of each centriole. Budding was never observed, however, and neither was the bicentriole pattern in any stage after prophase I. Therefore, binary fission probably occurred only during prophase I. Migration of the diplosomes to opposite sides of the nucleus then occurred, followed by meiosis II to yield 4 daughter cells per sporangium. The ultrastructural changes were similar to those in meiosis I except that synaptinemal complexes were absent.

The third set of divisions was mitotic, leading to the formation of 8 zoospores. Centrioles probably replicated in the same manner as before meiosis II. During telophase, while spindle microtubules were still attached to the centrioles, flagellar bud formation was initiated (Perkins & Amon, 1969). Figure 2 summarizes centriole formation and changes during zoosporulation.

DISCUSSION

The suggestion that *de novo* centriole formation may occur in some cells has been questioned by students of mitosis and meiosis (Lwoff, 1950; Mazia, 1961; de Harven, 1968). Went (1966) noted that there are 3 hypotheses, any one of which could possibly explain observations of supposed *de novo* centriole formation: (1) the organelle is formed from 'non-specific precursors that contain no inherited information'; (2) 'reaggregation of specific dispersed units which bear information' occurs to form the organelle; or (3) precursor centrioles which contain genetic information 'direct the incorporation of non-specific precursors into a definitive centriole'. An obvious fourth suggestion is that *de novo* formation does not occur, but rather undetected, pre-existing centrioles replicate and form the centrioles. In the strictest interpretation of the *de novo* concept only a mechanism of centriole formation postulated by the first hypothesis could be considered to represent *de novo* formation.

Either the first or the second hypothesis appears most reasonable as a model to explain centriole formation in *Labyrinthula* sp. The large number of electron-microscope observations of vegetative spindle cells made by D. Porter (personal communication)

and myself have failed to reveal centrioles; therefore, it is unlikely that they were overlooked. No precursor centrioles other than the granular aggregates, recognized to be a centriole developmental stage, were observed. The available morphological information does not permit one to determine whether hypothesis number (1) or (2) is correct. It does appear more likely, however, that non-specific precursors are involved, because one would not expect that the cell would maintain specific dispersed centriole units in the cytoplasm through millions of cell generations and then assemble them in the form of a centriole when sporulation occurred. Precise cell counts were not made but it is known that the inocula used during subculture of *Labyrinthula* sp. spindle cells consisted of hundreds of cells and the resulting stationary phase cultures consisted of millions of cells. Quite often 5–10 subcultures of stationary phase cultures were made before sporulation occurred.

In studies of parthenogenetically activated eggs various workers have observed formation of centrioles in cytasters prior to which no centrioles could be found (Yatsu, 1905; Dirksen, 1961; Went, 1966). In such eggs it is easier to visualize a mechanism of reaggregation of dispersed centriole subunits or development from presumptive centriole granules containing genetic information, because in each case the somatic cells of the invertebrate contained centrioles and centrioles were detected either just before or during oogenesis. A span of millions of cell generations in which no centrioles were formed was not interjected between successive centriole biogeneses, only one or several cell generations. There is an alternative hypothesis which could accommodate the suggestion that specific dispersed units bearing genetic information reaggregate to form centrioles. In *Labyrinthula* sp. the granular aggregates which appear at the beginning of each mitotic cycle could represent reaggregation of information-containing units. Only during meiotic zoosporulation would the process be permitted or induced to proceed to formation of mature centrioles. The assumption is made that protocentrioles of mitotic cells and those of meiotic cells are formed by the same mechanism. Only in the meiotic cells is the synthesis allowed to proceed to formation of the microtubular units of the mature centriole. The ultrastructural evidence supports such an assumption. The fact that cartwheels are formed during mitosis is inconsistent; however, it should be noted that cartwheels were not observed in all granular aggregates. Possibly in some examples cell control over the biogenetic process was not complete enough to prevent continuation of the process beyond the steps required to form a functional mitotic component.

The theoretical model of Satir & Satir (1964), which explains the origins of 9-fold symmetry in cilia and centrioles, is appealing in the light of the developmental picture observed in *Labyrinthula* sp. They suggested that 9-fold symmetry is generated from the α-helix of a protein represented by the hub of the centriole or cilium cartwheel complex. The cartwheel spokes are visualized as being extensions of 9 binding sites along the α-helix. Since centriole biogenesis in *Labyrinthula* sp. occurs by formation of a cartwheel complex as the first organized structure in the granular aggregate, followed by formation of the cylinder of triplet microtubules, the cartwheel may be or may contain the organizing centre for centriole formation.

The importance of the cartwheel complex in determining symmetry in centrioles

is further suggested when one considers centriole formation in other organisms. When orthogonal 'budding' occurs from a centriole, formation occurs from the cartwheel end and the cartwheel end is the first to be formed in the developing procentriole (Gall, 1961; Went, 1966). Procentrioles formed by budding from fibrogranular aggregates (Sorokin, 1968) or by fragmentation of blepharoplasts (Mizukami & Gall, 1966) contain cartwheel complexes before developing centriole microtubules can be clearly seen. Admittedly cartwheels have not been demonstrated in centrioles of some cells (Szollosi, 1964); but there is a possiblity that they have been overlooked, particularly if only one cartwheel tier is present, as in Chinese-hamster cells (Stubblefield & Brinkley, 1967). There is also a possibility that the cartwheel will be lost if the centriole becomes a basal body. Turner (1968) observed tiers of cartwheels in *Nitella* centrioles during spermatogenesis, but could not find them when the centrioles had become basal bodies. As one would expect those arthropod 'centrioles' which lack 9-fold symmetry also appear to lack cartwheels (Phillips, 1966).

Labyrinthula is apparently the first organism in which any semblance of centriole binary fission has been observed at the ultrastructural level (Mizukami & Gall, 1966; de Harven, 1968). Several light-microscope studies have provided evidence of binary fission, but verification by electron-microscope studies is lacking. Payne (1927) observed growth and transverse division in centrioles of the hemipteran *Gelastocoris oculatus*, and Costello (1961) observed binary fission in the flatworm *Polychoerus*. Both observations were made during gametogenesis. Lwoff (1950) believed that ciliate kinetosomes were always generated by division of a pre-existing kinetosome; however, Dippell's (1968) ultrastructural studies indicate synthesis from fibrogranular aggregates formed near mature kinetosomes.

It is not known whether the bicentrioles in *Albugo candida* (Berlin & Bowen, 1964) represent end-to-end orientation of two mature centrioles or a stage in binary fission. *A. candida* differs from *Labyrinthula* in that centrioles are present in all stages of the life-cycle. The division of *Labyrinthula* bicentrioles must be considered a special case of binary fission, because once a mature centriole is established in the cell, orthogonal budding appears to be the mode of replication as in other cells (Mizukami & Gall, 1966; Stubblefield & Brinkley, 1967).

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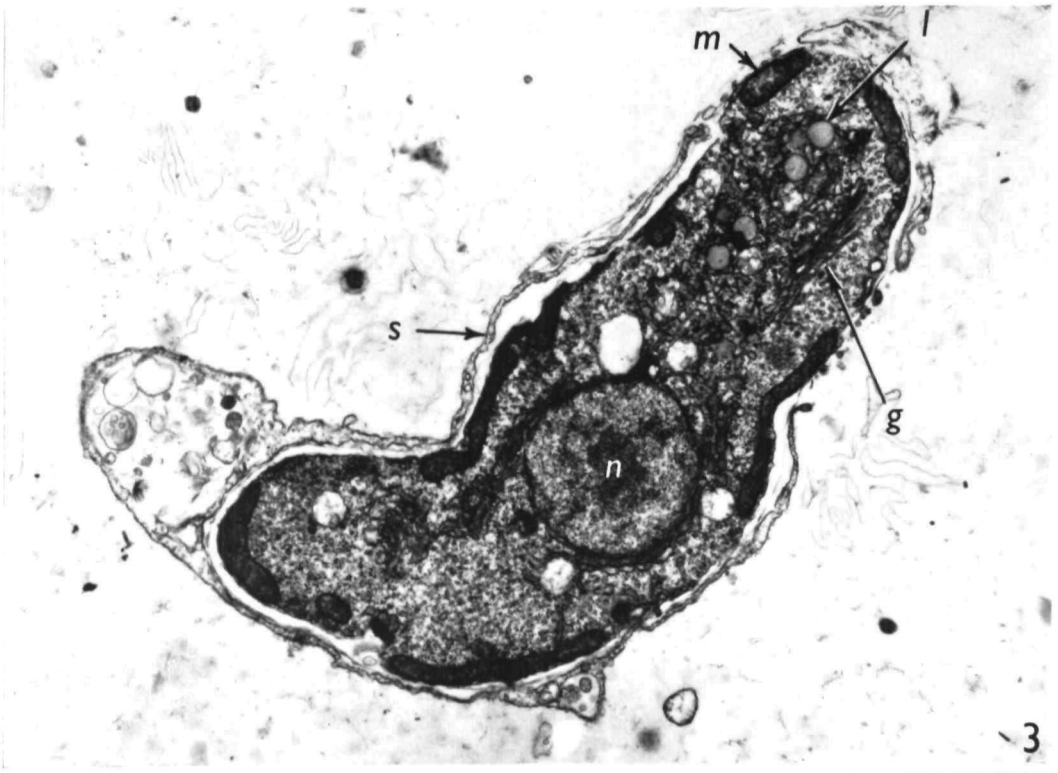
Fig. 3. Vegetative spindle cell of *Labyrinthula* sp. *g*, Golgi body; *l*, presumptive lipid; *m*, mitochondrion; *n*, nucleolus; *s*, 'slimeway'. × 13000.

Fig. 4. Granular aggregate or protocentriole (*p*) forming next to pre-mitotic or early prophase nucleus (*nu*). *mt*, spindle microtubule. × 53000.


m
l
s
g
n
3

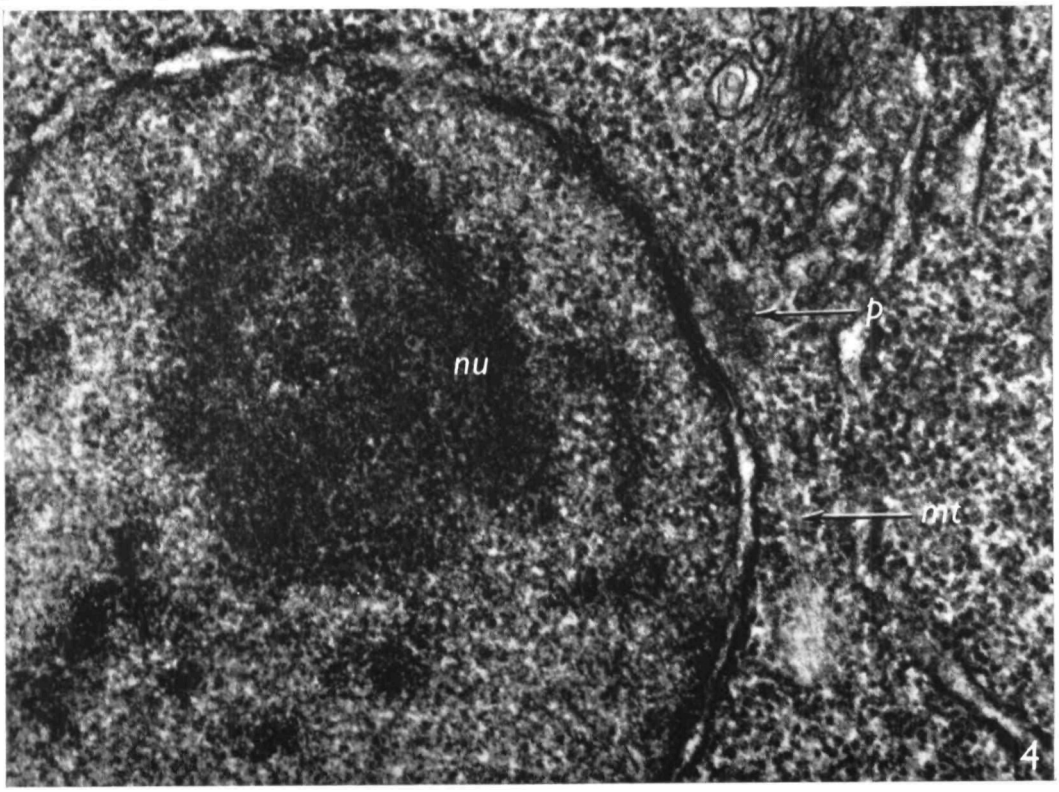

nu
p
mt
4

Fig. 5. Protocentriole (*p*) in cytoplasm next to mitotic nucleus. Spindle microtubules (*mt*) are directly attached to a granular aggregate. × 75 000.

Fig. 6. Fully differentiated protocentriole in which a cartwheel structure (*c*) is visible. *mt*, spindle microtubules. × 94 000.

Fig. 7. Probable prometaphase mitotic figure in which both protocentrioles (*p*) are visible. Nuclear envelope breakdown has occurred. *ch*, chromosome; *n*, nucleolus. × 33 000.

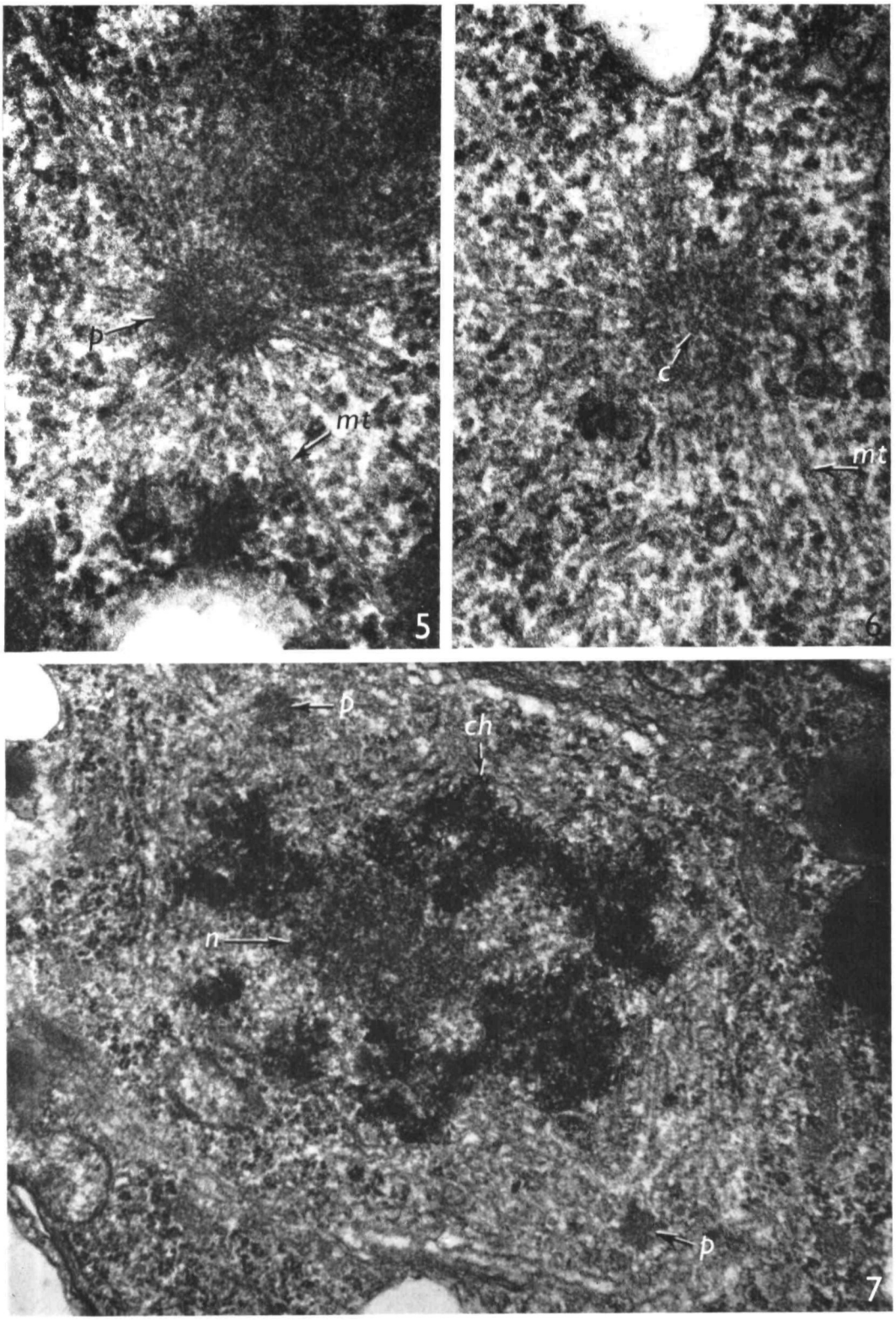

p
mt
5
c
mt
6
p
ch
n
p
7

Fig. 8. Protocentriole (*p*) and associated microtubules (*mt*) next to prophase I nucleus. × 108000.

Fig. 9. Differentiating protocentriole in which cartwheel complex (*c*) is being formed. *g*, Golgi body. × 100000.

Fig. 10. Prophase I nucleus in zygonema or pachynema showing one of a pair of protocentrioles (*p*). × 32000.

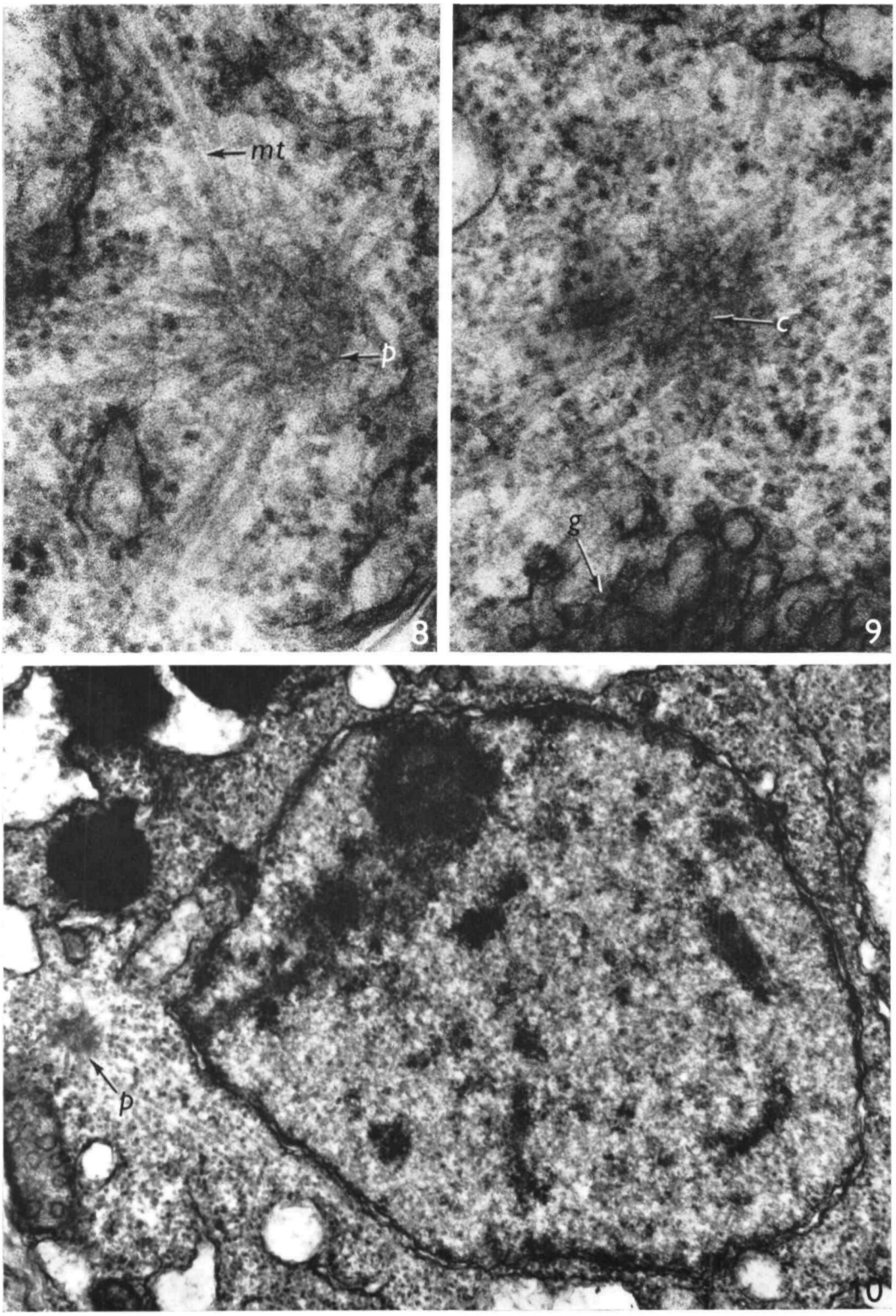
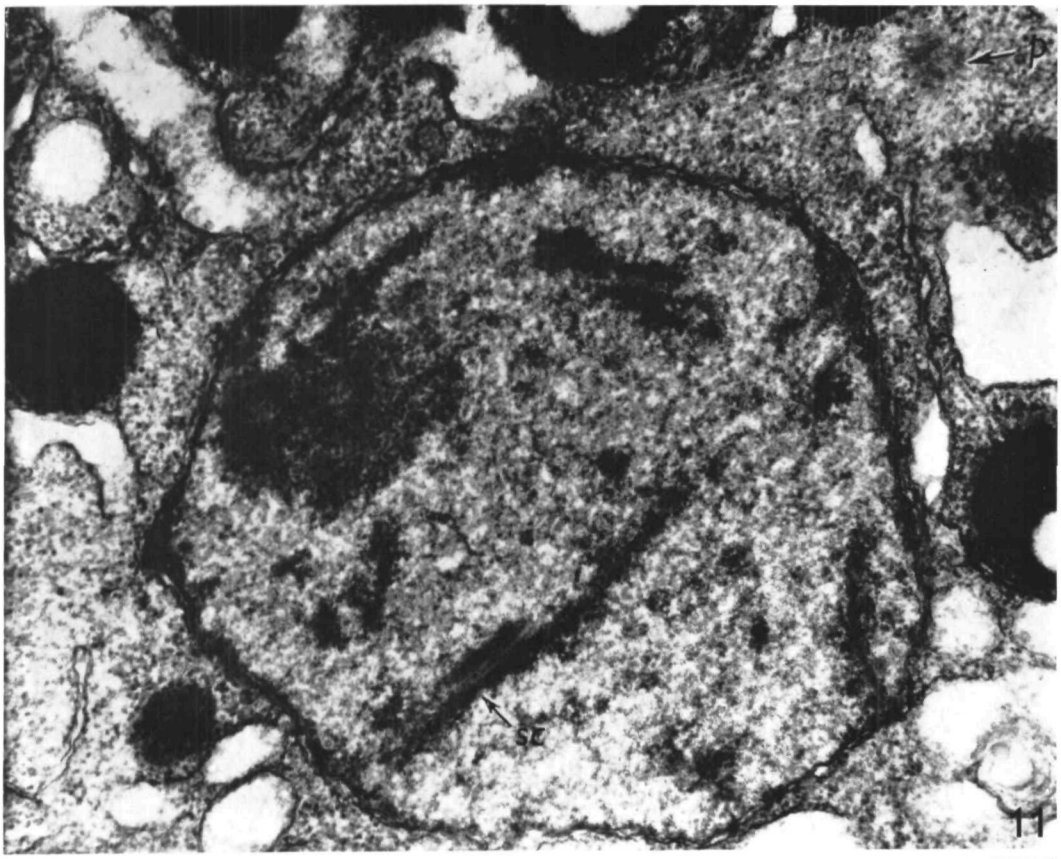
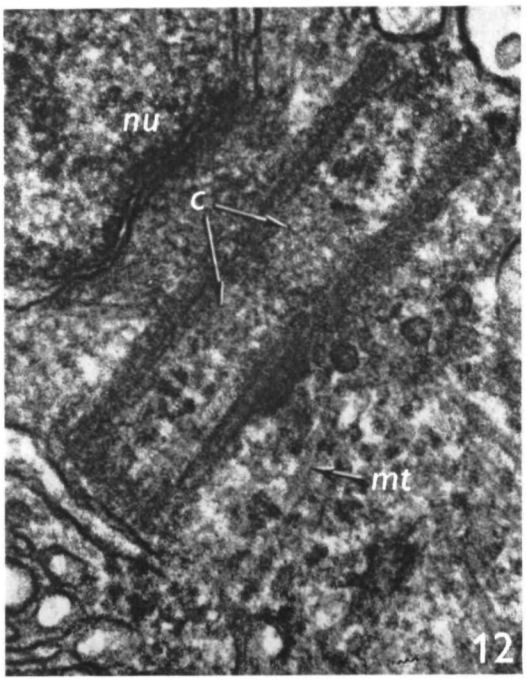

mt
p
8
c
g
9
p
10

Fig. 11. Same nucleus as in Fig. 10 showing second protocentriole (*p*). Sections in Figs. 10 and 11 were separated by a specimen thickness of 0·4 μm. *sc*, synaptinemal complex. × 32000.

Fig. 12. Bicentriole comprised of 2 centrioles attached at proximal ends. Microtubules (*mt*) converge at cartwheel regions (*c*) of each centriole. *nu*, nucleus. × 96000.

Fig. 13. Bicentriole showing hub (*h*) of cartwheel complex and about 5 tiers of cartwheels. × 91000.


p
sz
11


nu
c
mt
12

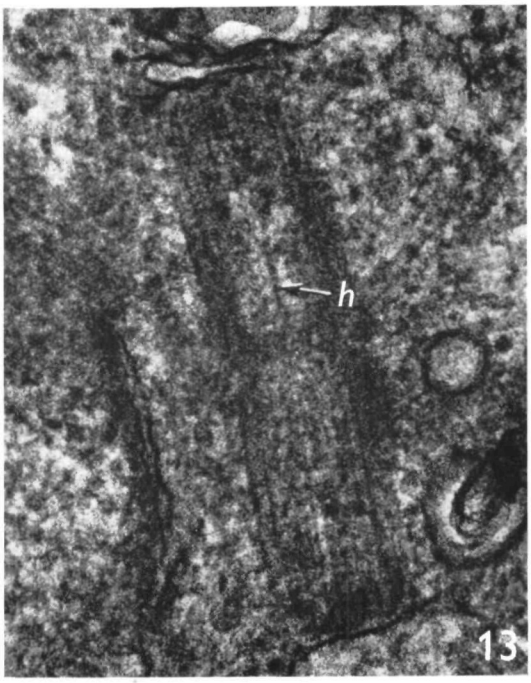

h
13

Fig. 14. Bicentriole showing either direct attachment of microtubules to centriole (arrow) or attachment to electron-dense material (*e*) which is closely juxtaposed to centriole microtubules. × 104000.

Fig. 15. Cross-section through either a presumptive immature centriole or the interface region between two centrioles of a bicentriole (see Figs. 18 and 22). An arc of 5 singlet or A microtubules (*A*) and two doublet or A and B microtubules (*d*) are resolved. One spindle microtubule appears to be connected directly to a B microtubule (unlabelled arrow). × 130000.

Figs. 16–21. Serial section through bicentriole showing triplet blades (*t*) of centriole walls and cartwheel complexes (*c*). Figure 18 shows the extreme proximal end of one centriole of the bicentriole in which doublet (*d*) and triplet (*t*) microtubules and a cartwheel complex are visible. Distal (Fig. 16), proximal (Fig. 17), extreme proximal (Fig. 18), proximal (Figs. 19, 20), and distal portions (Fig. 21) are shown. × 88000.

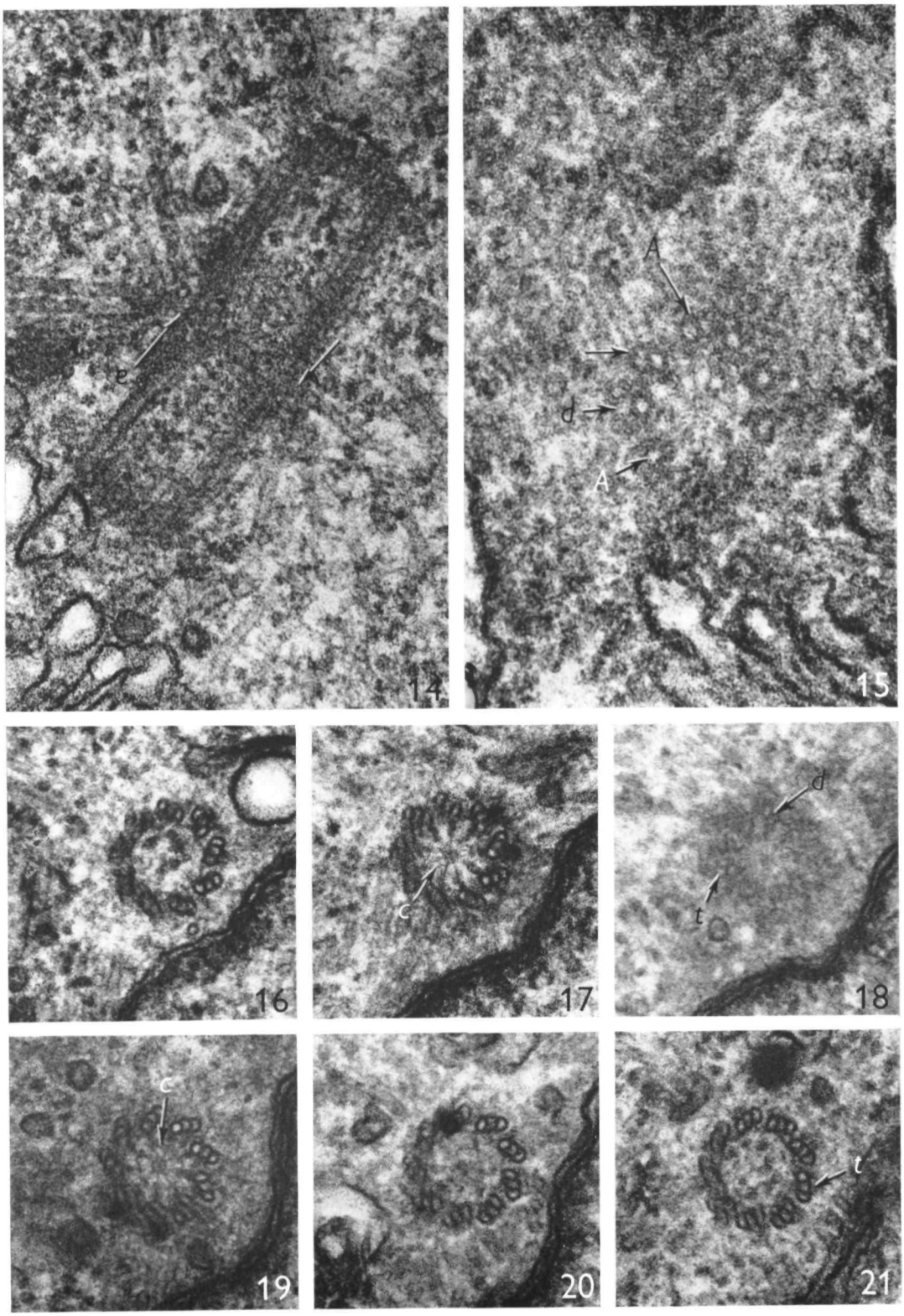
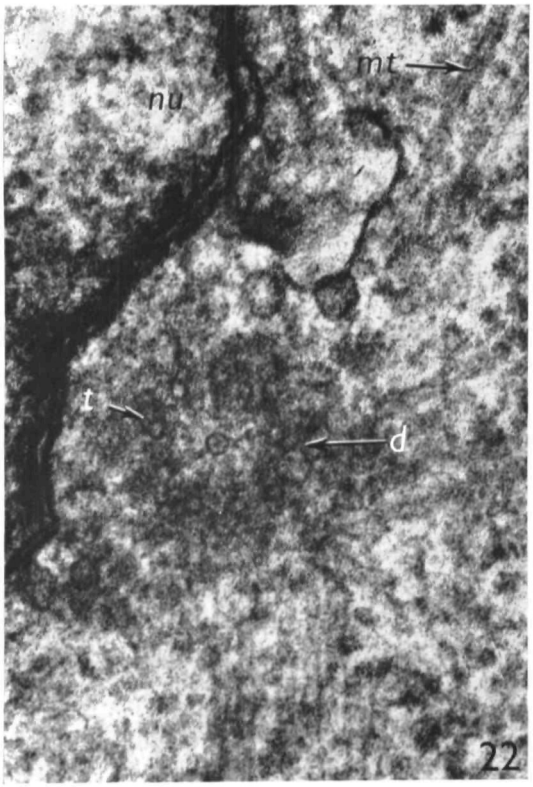
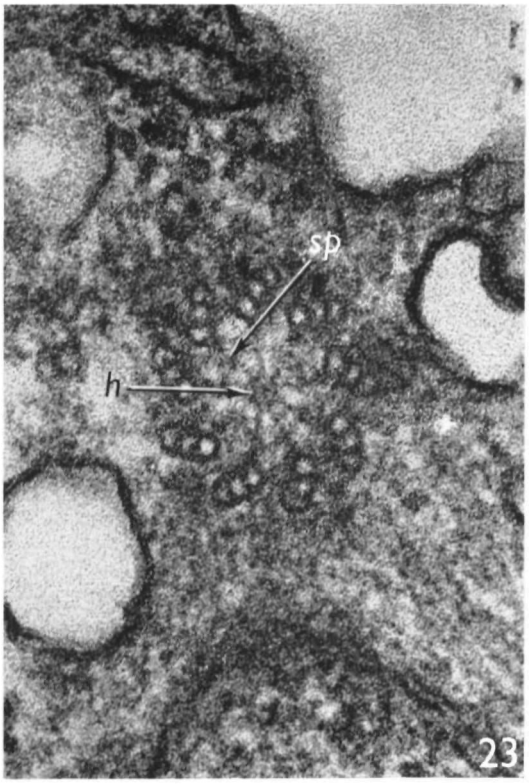

14
15
A
d
A
16
17
c
18
d
t
19
c
20
21
t

Fig. 22. Section through extreme proximal end of one centriole of a bicentriole; obtained from a series of sections through entire bicentriole. Apparent doublet (*d*) and triplet (*t*) microtubules are present. *mt*, spindle microtubules; *nu*, nucleus. × 111000.

Fig. 23. Cross-section through proximal end of mature centriole showing substructure of cartwheel complex. Hub (*h*) with centrally located rod and radiating spokes (*sp*) of cartwheel. × 158000.

Fig. 24. Rotation micrograph of centriole shown in Fig. 23. Spokes are resolved into T-shaped structures (*ts*) attached to knobs (*k*) of electron-dense material. Slender connecting rods (*r*) are attached to the hub. × 370000.


nu
mt
t
d
22


sp
h
23

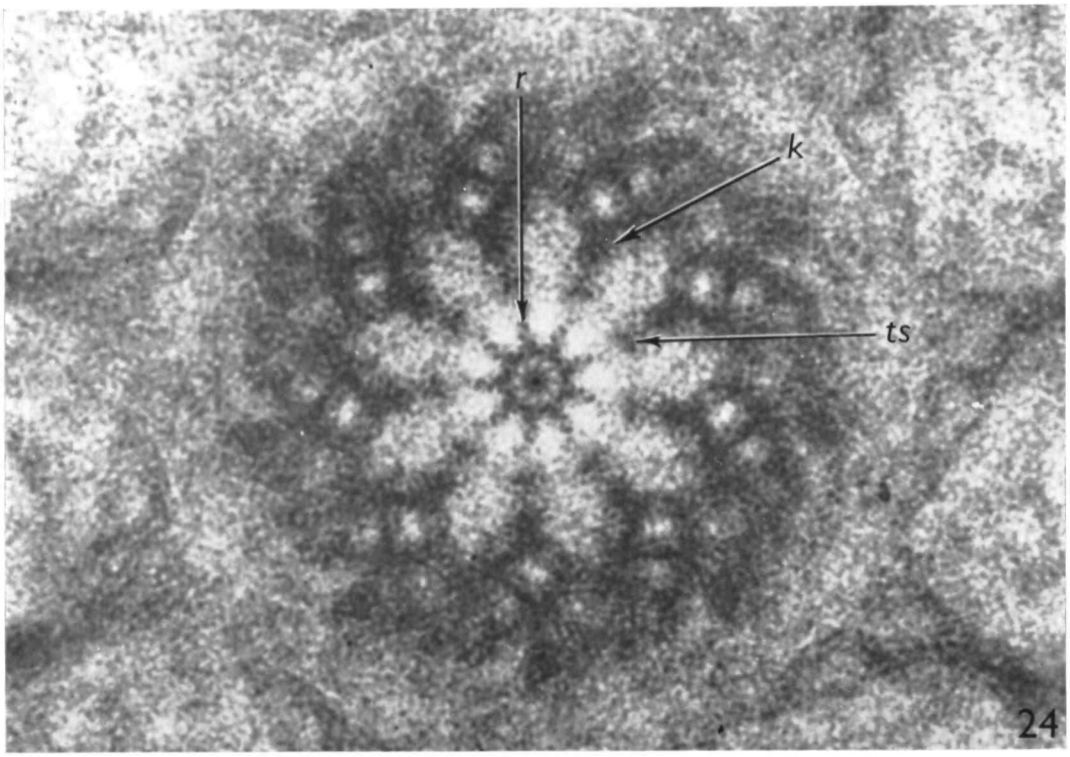
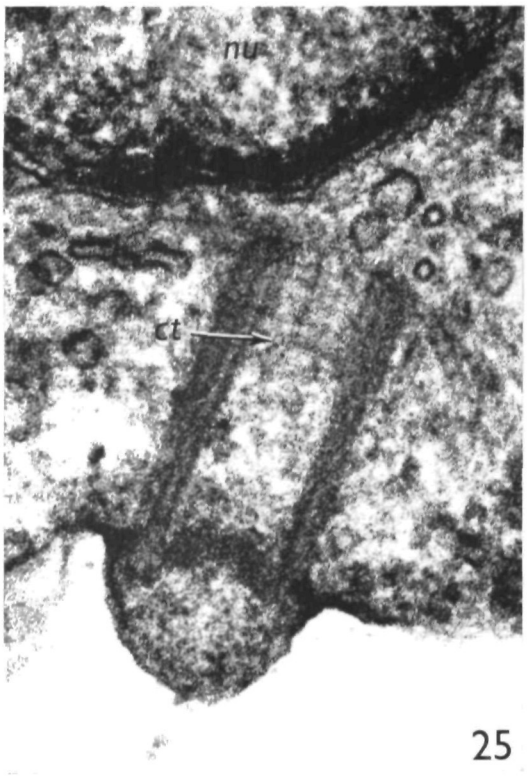

r
k
ts
24

Fig. 25. Longitudinal section through mature centriole. Six tiers of cartwheels (*ct*) are resolved. An electron-dense plaque is found in the distal end of the centriole lumen. The cytoplasmic bulge at the distal end of the centriole is supposedly not a budding flagellum. The cell was known to be between meiosis I and II. Flagella were not normally formed until after metaphase of zoosporulation mitosis. *nu*, nucleus. × 117000.

Fig. 26. Cross-section through distal portion of mature centriole showing bridges (*br*) between adjacent triplet blades. The bridges are attached at the junction between the A and B microtubules. *nu*, nucleus. × 163000.

Fig. 27. Rotation micrograph of a cross-section through distal portion of mature centriole Connecting bridges (*br*) are resolved into a chain of beaded subunits. × 300000.



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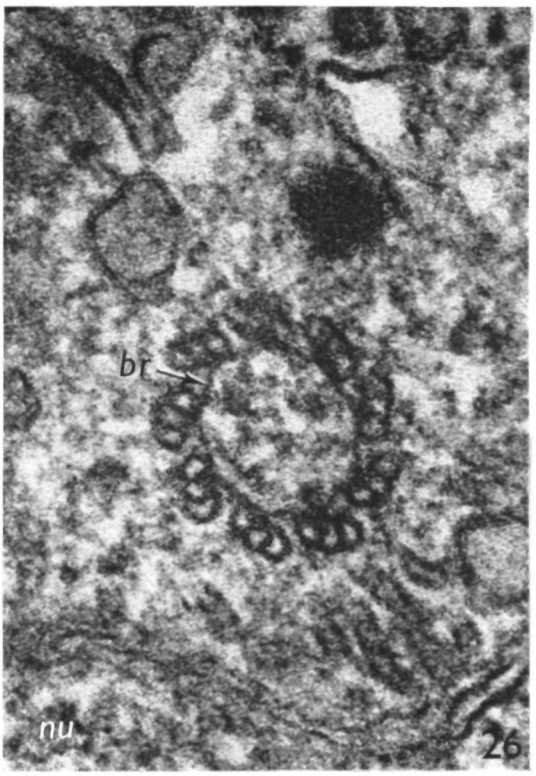

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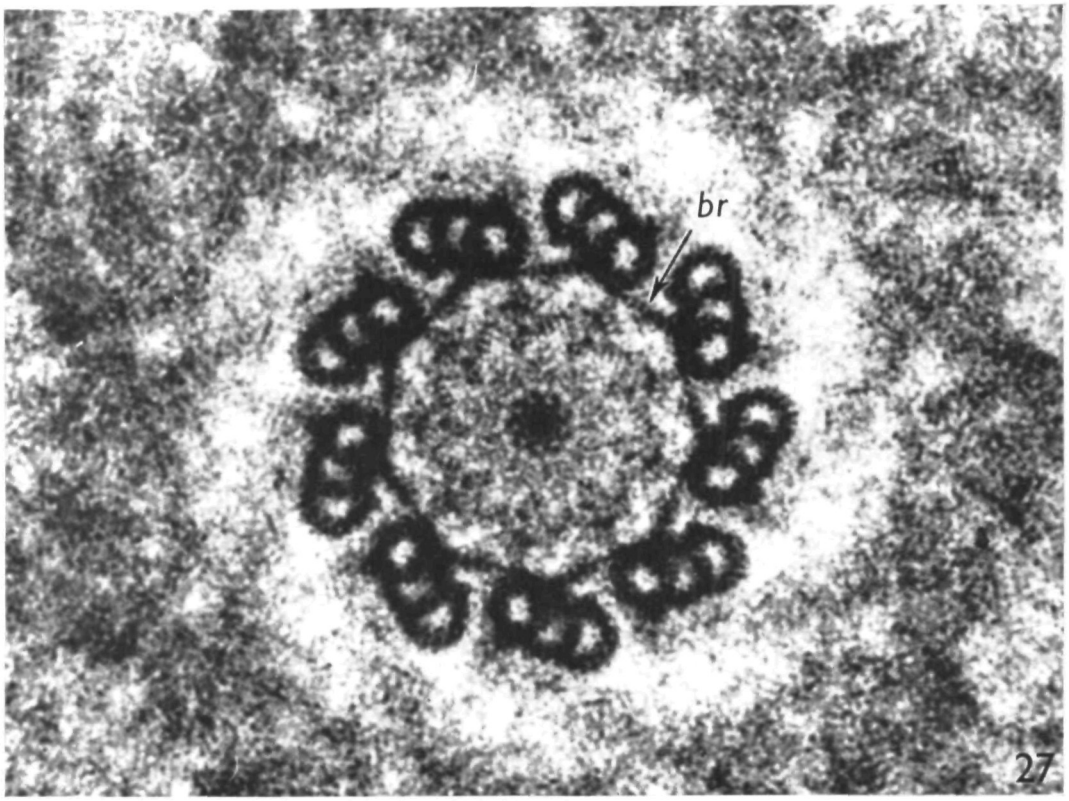

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