

Centrioles in the Cell Cycle. I. Epithelial Cells

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ABSTRACT A study was made of the structure of the centrosome in the cell cycle in a nonsynchronous culture of pig kidney embryo (PE) cells. In the spindle pole of the metaphase cell there are two mutually perpendicular centrioles (mother and daughter) which differ in their ultrastructure. An electron-dense halo, which surrounds only the mother centriole and is the site where spindle microtubules converge, disappears at the end of telophase. In metaphase and anaphase, the mother centriole is situated perpendicular to the spindle axis. At the beginning of the G_1 period, pericentriolar satellites are formed on the mother centriole with microtubules attached to them; the two centrioles diverge. The structures of the two centrioles differ throughout interphase; the mother centriole has appendages, the daughter does not. Replication of the centrioles occurs approximately in the middle of the S period. The structure of the procentrioles differs sharply from that of the mature centriole. Elongation of procentrioles is completed in prometaphase, and their structure undergoes a number of successive changes. In the G_2 period, pericentriolar satellites disappear and some time later a fibrillar halo is formed on both mother centrioles, i.e., spindle poles begin to form. In the cells that have left the mitotic cycle (G_0 period), replication of centrioles does not take place; in many cells, a cilium is formed on the mother centriole. In a small number of cells a cilium is formed in the S and G_2 periods, but unlike the cilium in the G_0 period it does not reach the surface of the cell. In all cases, it locates on the centriole with appendages. At the beginning of the G_1 period, during the G_2 period, and in nonciliated cells in the G_0 period, one of the centrioles is situated perpendicular to the substrate. On the whole, it takes a mature centriole a cycle and a half to form in PE cells.

The behavior of centrioles in the cell cycle has been described in a number of works (13, 21, 22, 24, 29, 30), but the majority of authors have confined themselves to a description of some individual cycle phases rather than making a comprehensive analysis of all the periods of interphase and mitosis. The emphasis in these works is on the location of centrioles in the cell and on the time of their duplication, whereas the interaction between interphase centrioles and cytoplasmic microtubules and the dynamics of all centrosome structures are hardly mentioned. Very little is known about the structure of the growing centriole in unciliated cells from the moment of its emergence near the mother centriole. Of great interest is the verification of the assumption about the oriented location of centrioles in mitosis and interphase.

In the present work we have tried to elucidate the changes in centriole structure and general composition of the centriolar apparatus during and at completion of the mitotic cycle. The method was essentially as follows: we marked the individual cell under the light microscope and then examined the serial sections of this cell under the electron microscope.

MATERIALS AND METHODS

Pig kidney embryo cells (PE cells) were the main cell line used, though the orientation of centrioles in metaphase was studied in Chinese hamster cells (line B11d, clone 237c).

The cells were prepared for electron microscopy by standard procedures (36). For identification of the cells at different stages of the mitotic cycle, the culture was harvested in the exponential growth phase, and before fixation the culture medium was supplemented with radioactive thymidine:

- (a) [3 H]thymidine in a concentration of 1 μ Ci/ml was used for 20 min to label the cells in the S period;
- (b) [3 H]thymidine in the same concentration was added for 5 h, which is the average maximum time of the G_2 period in PE cells (thus, it was only the cells in the G_1 period that remained unlabeled);
- (c) [3 H]thymidine was added to the medium for 20 min in the same concentration, then, after two changes of the culture medium lacking the isotope, 4 h and 20 min later [14 C]thymidine was added for 20 min in a concentration of 5 μ Ci/ml, after which the cells were fixed. The latter technique allows one to identify cells in all phases of the cell cycle (18). It is indispensable for identification of cells in G_2 in a nonsynchronous culture, but it is too sophisticated for general use. Therefore, for labeling the cells in the G_1 and S periods, we used simpler techniques. For studying the cells that have left the proliferative pool, we used a 6-d-old culture grown without replacement of the medium. 1 d before fixation, the medium was supplemented with [3 H]thymidine in a concentration of 1 μ Ci/

ml. The unlabeled cells were considered by us as being released from the cell cycle, which is 20 h long in PE cells.

For a detailed study of the fine structure of centrioles, the cells in some experiments were pretreated for 60–90 s at room temperature with a solution containing 50 mM triethanolamine, 3 mM MgCl₂, and 0.1% Triton X-100, pH 7.4.

After the cells were embedded in Epon-812 and coated, cover slips were removed, the labeled preparations were coated with a nuclear emulsion of the "M" type (NIIKHIMPHOTOPROEKT) and exposed for 15–25 d. After the autoradiograph was developed, the cells were selected at different stages of interphase under the phase-contrast microscope, marked with a special marker attached to the microscope objective 10×, and photographed. Serial ultrathin sections of the selected cells were prepared with LKB and LKB-III ultratomes, mounted onto Formvar-coated grids, and stained with lead citrate according to Reynolds (23). The preparations were examined and photographed in Hitachi HU-IIB and HU-12 electron microscopes operating at 75 kV. Selected interphase cells were identified in light micrographs. Rotational photos were prepared as described previously (34). To measure the centriole tilt towards the section plane, we made, for comparison, photos of the perpendicular section of the centriole using a microscope with a goniometer (Philips-401) at various tilt angles of the section plane.

RESULTS

In metaphase, at each pole of the division spindle in PE cells there is a diplosome typical of vertebrate cells (Fig. 1*a*). The mother centriole is surrounded by a halo consisting of thin fibrillar material which shows more distinctly after the cells are treated with a detergent (Fig. 4). The width of the halo is 100–200 nm, and the diameter of its fibrils is ~5 nm. One of the ends of the daughter centriole is immersed into this halo. The halo is the zone where microtubules converge in the spindle pole. Microtubules radiate from it in all directions.

The ultrastructure of the mother centriole in mitosis is almost the same as that described previously (see references 8 and 34). The only difference is that all along the centriole cylinder the tilt of the triplets relative to the radius varies from 55° in the proximal part to 85° at the distal end (Figs. 1 and 3). Two mother centrioles situated in the two poles of a cell differ somewhat in structure: one contains distinctly visible appendages, whereas the other lacks them (Figs. 1 and 2).

The daughter centriole in metaphase differs from the mother. It has no appendages, its triplets are equally tilted with respect to the radius (at ~60°) over the entire length of the centriolar cylinder. All triplet microtubules are complete. Inside the centriolar cylinder, at the proximal end there is an axis with spokes (the cartwheel) (Figs. 6*a* and 7*a*). The diameter of the axis is 25 nm. Nine spokes radiate from the axis and are attached to the protrusions (knobs) of triplet A-tubules. The length of the cartwheel is not more than one-third of the centriole. There are no electron-dense structures in the middle part of the centriolar cylinder (Fig. 6*b*). The linkages between the triplets that can be seen in the mother centriole are also absent here (cf. Figs. 6*b* and 1*b*). In the distal part of the daughter centriole, there are peculiar outgrowths (ribs) (Fig. 6*c*). Unlike appendages, these ribs remain unaltered in two to four serial sections. The above structure of the daughter centriole remains constant to the end of telophase.

In anaphase, the general structure of spindle poles hardly changes. But by this time both mother centrioles have appendages which are less distinct than in metaphase. The pericentriolar halo is seen better than in metaphase, which can probably be explained by the decrease in the number of microtubules entering it.

There is a certain regularity in the location of mother centrioles in division spindle poles in metaphase and anaphase; their long axes are mainly perpendicular to the spindle axis

(Fig. 8 and Table I). The same orientation was observed in the metaphase cells of the Chinese hamster and also in numerous figures of metaphase cells published previously (8).

In telophase, the orientation of mother centrioles towards the spindle axis becomes random. The halo around the mother centriole remains intact (Fig. 9); however, after being treated with the detergent, it remains less intense than in metaphase (cf. Figs. 4 and 10). Towards interphase, the halo disappears and both centrioles are surrounded, until the end of the G₂ period, only with a rim of osmiophilic substance 40-nm wide, i.e., a matrix (Fig. 11). In mother centrioles of some cells, pericentriolar satellites begin to be formed; in most cases, they appear only after divergence of the centrioles. At the beginning of the G₁ phase, the cells are linked by a residual body, which allows them to be distinguished from other interphase cells. In seven of the eleven interphase cells examined, the mother centriole was tilted at an angle of not less than 75° with respect to the plane of the substrate on which the cells were grown (the probability of random distribution, $P < 0.0015$). No orientation of the centriole towards the substrate is observed during the next interphase up to the G₂ period and in mitosis.

In G₁, when cells are no longer linked, the diplosome, as a whole unit, breaks down. The orientation of the centrioles becomes random. Two centrioles may be several microns apart. The structural differences between them persist: one (the former mother centriole) has appendages, the other (daughter centriole) lacks them and has ribs at the distal end. The structure of the latter centriole changes somewhat at this time. It loses its axis with spokes; in the middle and distal part in the lumen of the centriole cylinder, electron-dense material appears in the form of an amorphous bush ~100 nm in diameter with a 35-nm opening (Fig. 15).

In all cells in the G₁ period, pericentriolar satellites are formed only on mother centrioles (i.e., having appendages; see Fig. 13). These are protrusions consisting of a conical striated stem and a small round head (Fig. 12). The stem is probably formed by comparatively thin fibrils (15). It is attached to two or three triplets. Pericentriolar satellites are the centers where microtubules converge in interphase PE cells. The microtubules are in contact with the satellite head and hardly ever approach its stem (Figs. 12 and 13). The number of satellites is usually two or three, five at the most; they are situated in the distal part of the mother centriole. There are no foci of convergence of microtubules around the second (daughter) centriole in interphase until the middle of the G₂ period. At the same time, some microtubules may approach the matrix of both centrioles.

The main event in the centriolar cycle in the S period is centriole replication (10, 13, 24). Replicated centrioles were found in 15 of the 28 cells examined at this stage. Since we chose the cells in the S period randomly, it may be that centriole replication in PE cells begins either in the middle of the S period or at different times in different cells.

Throughout the S period, differences are observed in the structure of the two mother centrioles, i.e., one of them contains satellites and appendages, while the other has none. The structure of the second centriole changes somewhat in the S period: it loses ribs situated in the distal part. The structure of the centriole with satellites undergoes no changes. The centrioles are located close to each other.

Daughter procentrioles are formed near the proximal end of the mother ones, i.e., the end containing no bush (Fig. 16) (5). At the earliest stage that can be detected by electron microscopy, the procentriole looks like a short cylinder with uneven

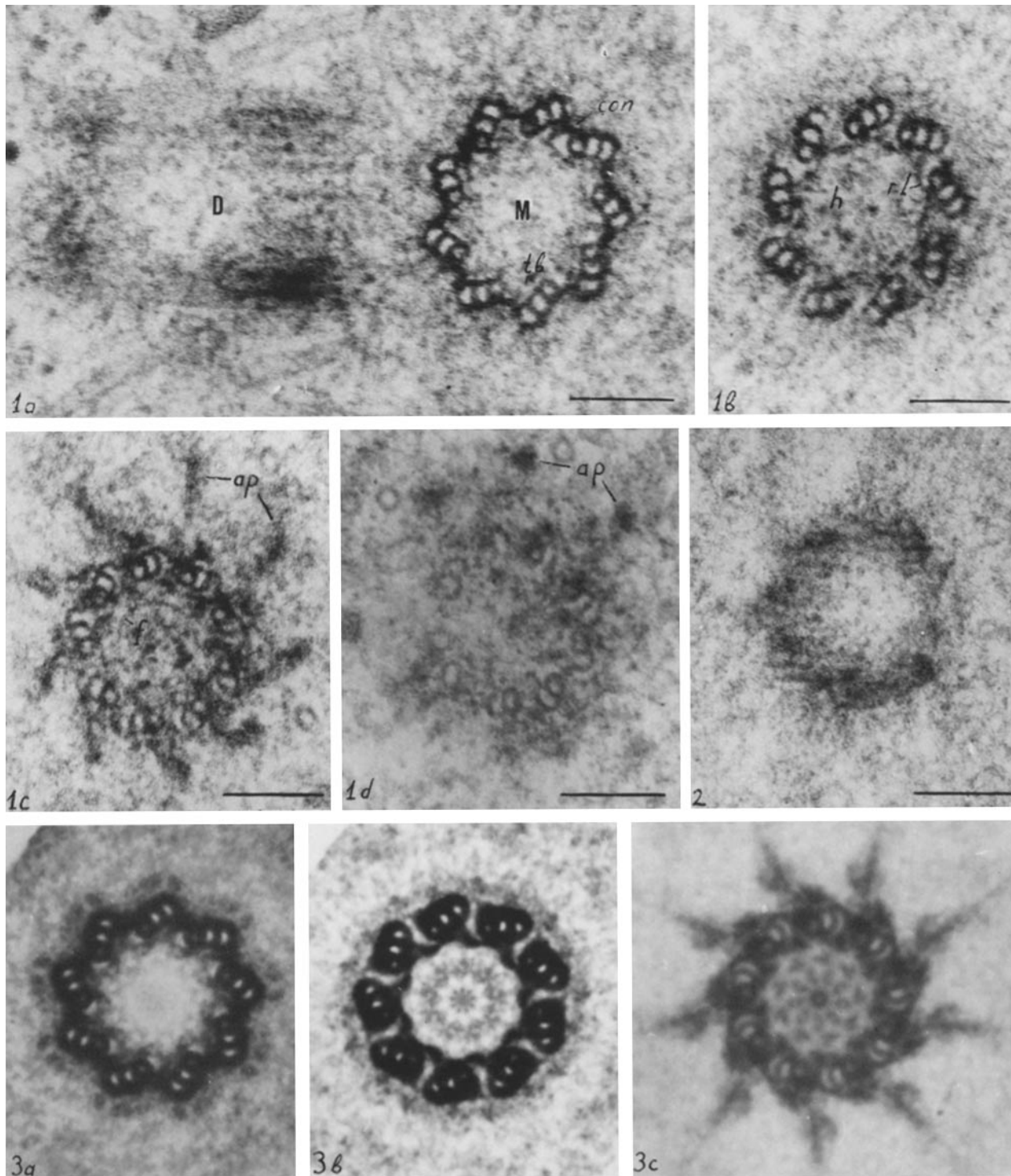


FIGURE 1 (a-d) Serial sections of the diplosome in metaphase PE cells. Sections 1, 4, 7, and 8 (1st, proximal; 8th, distal end). *M*, Mother centriole; *D*, daughter centriole; *ap*, appendages, *tb*, triplet bases; *con*, connectives (A-C links); *h*, bush, *f*, fiber, *rl*, ring of links in the middle part. Bar, 0.1 μm . $\times 160,000$.

FIGURE 2 Distal part of the mother centriole from the other spindle pole in the same cell. Note the absence of appendages. Bar, 0.1 μm . $\times 160,000$.

FIGURE 3 (a-c) Rotation images of sections viewed in Fig. 1 a-c.

stubs 0.15 μm in diameter and $\sim 0.1 \mu\text{m}$ long. The procentriole cylinder begins at a distance of 50–60 nm from the surface of the mother centriole and is in direct contact with its matrix (10, 17). The procentriole wall is made up of amorphous material

in which nine single or double microtubules are immersed. These microtubules are not located symmetrically, and that is why they can hardly be detected in rotation images (Figs. 17 and 18). Inside the procentriole, there is an axis with spokes

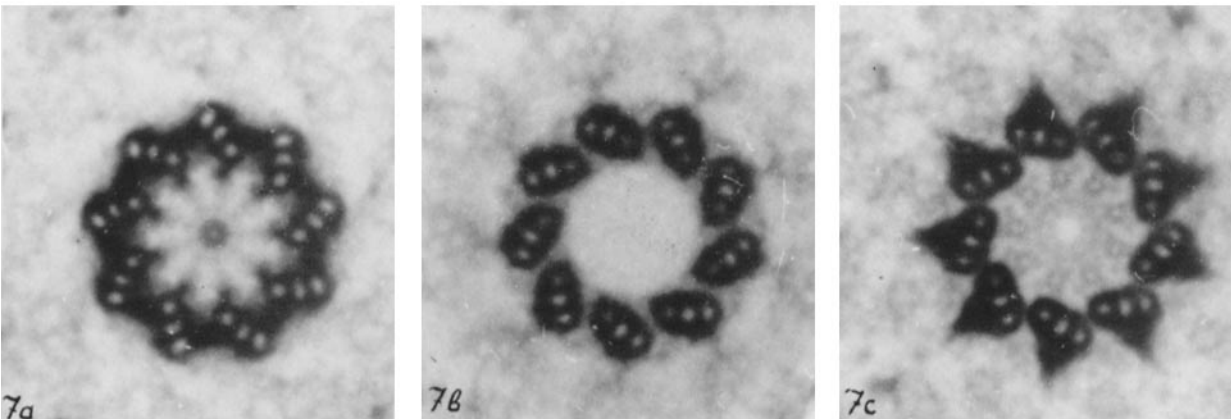
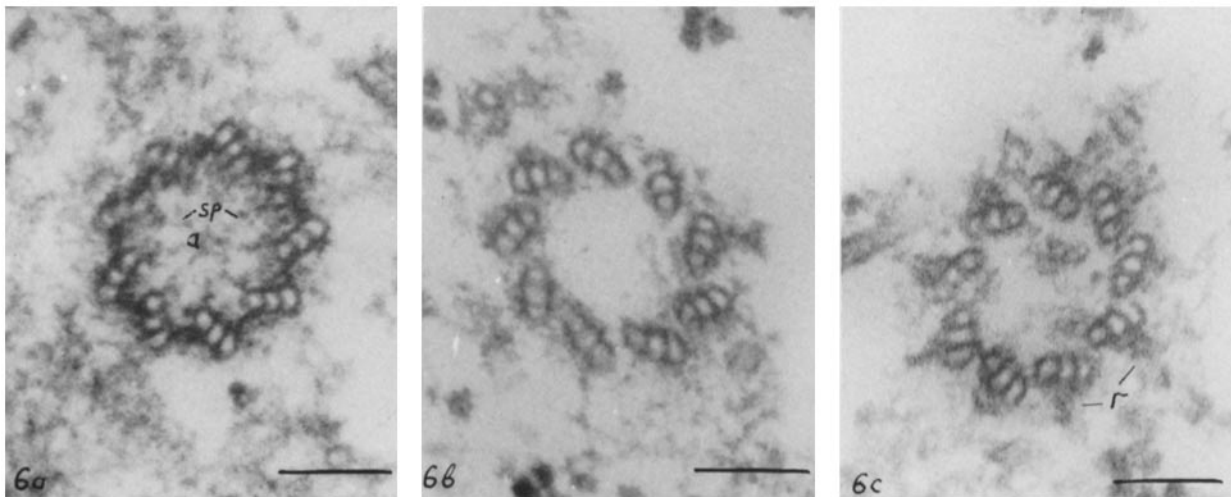
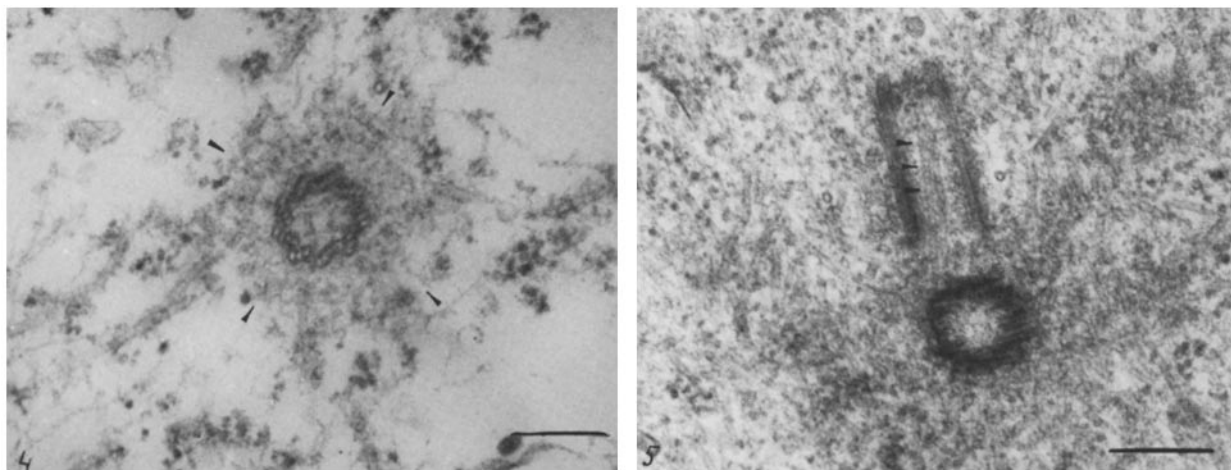


FIGURE 4 Mother centriole in the metaphase detergent treated cell. Arrowheads point to halo. Bar, 0.2 μm . $\times 60,000$.

FIGURE 5 Diplosome in metaphase cell. Note microtubules in the lumen of the daughter centriole (arrowheads). Bar, 0.2 μm . $\times 60,000$.

FIGURE 6 (a-c) Serial sections of the daughter centriole in the metaphase detergent-treated cell. Sections 1, 4, and 7 (numeration is the same as in Fig. 1). a, Axis; sp, spokes of a cartwheel; r, ribs. Note the absence of hub, and the ring of links in the middle part and appendages. Bar, 0.1 μm . $\times 160,000$.

FIGURE 7 (a-c) Rotation images of Fig. 6 a-c.

(the cartwheel). Its diameter is ~ 30 nm; the nine radiating spokes have a beadlike structure and end in expansions to which internal microtubules of the procentriole are attached.

During the S period, the size of procentrioles hardly changes,

their maximal diameter, as well as their maximal length, 0.2 μm . Their diameter probably increases due to the formation of complete microtubule triplets.

In G_2 , reconstitution of the two centrosomes occurs, which

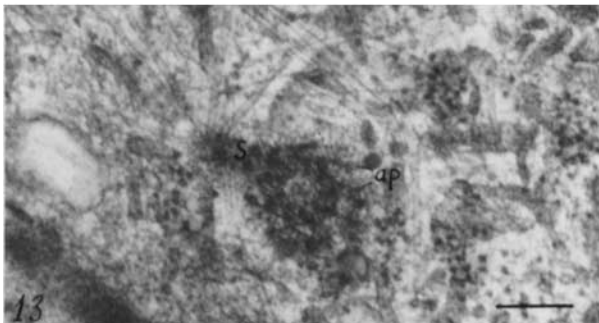
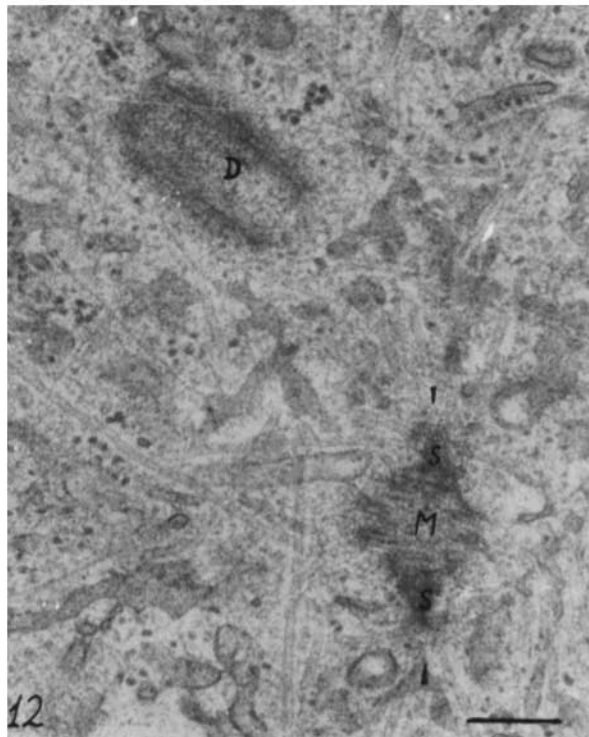
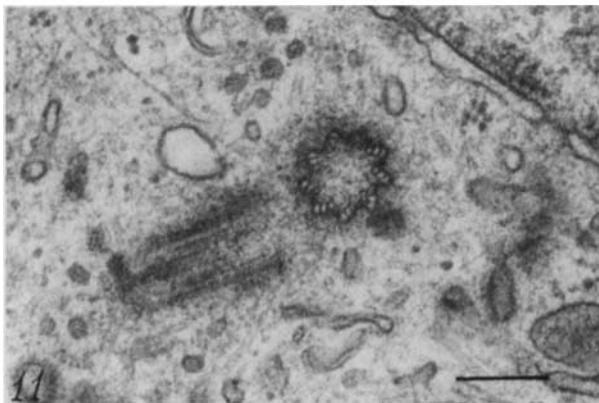
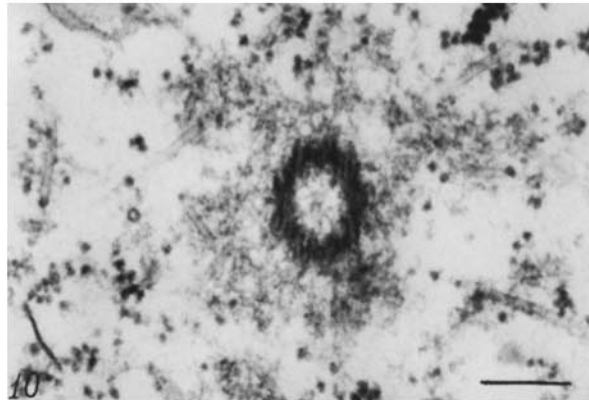
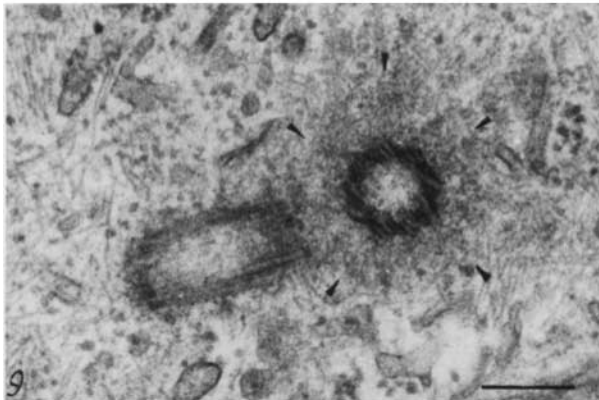
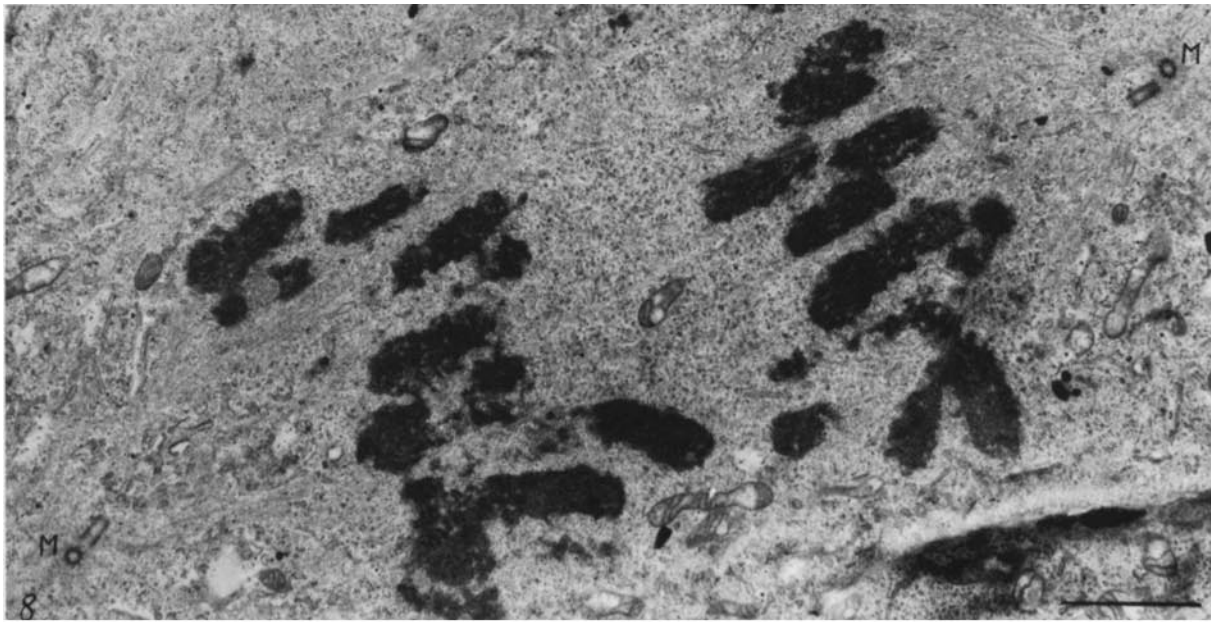


TABLE I
Orientation of Mother Centriole toward the Spindle Axis

Centriole tilt towards spindle axis	PE cells				Chinese hamster cells (metaphase)	
	Metaphase		Anaphase		No. of centrioles	
	No. of centrioles	%	No. of centrioles	%	No. of centrioles	%
90°-75°	23	45	13	82	14	48
74°-55°	7	13	0	0	6	21
54°-35°	7	13	1	6	5	17
34°-16°	9	18	1	6	3	10
15°-0°	6	11	1	6	1	3

The probability of random distribution, $P < 0.001$.

results in the formation of spindle poles (24). In all, we studied 16 cells at this stage. In five of the cells, one centriole had satellites; the remaining cells contained no centrioles with satellites. In the latter case, in the region of centrioles there were, as a rule, some microtubules which were in direct contact with the zone of the fibrillar material of both mother centrioles (Fig. 19a and b). The fibrillar zone surrounding the mother centrioles in the G_2 period is ~ 70 nm thick, just a little thicker than the centriole matrix in the rest of interphase and about half as thick as the mitotic halo. Thus, it appears that, in the G_2 period, pericentriolar satellites are lost and, then, after a certain period of "rest," a new microtubule-organizing structure, the halo, starts functioning around the centrioles. The halo develops fully only in mitosis. Despite the fact that in G_2 two mother centrioles become equivalent with respect to microtubules, the difference between them, i.e., the presence of appendages on one and their absence from the other, persists throughout the G_2 period and during the first half of mitosis.

Procentrioles grow during G_2 , reaching a maximal length of $0.35 \mu\text{m}$. The cartwheels in procentrioles grow in length together with the triplets which are completely formed. In the distal part of the centriolar cylinder, triangular ribs are attached to the triplets from outside (Figs. 20 and 21). The location of the triplets in the procentriole differs, at that time, from that in the mature centriole: they are tilted at an angle of 30° relative to the radius. There are connections between the triplets stretching from the external tubule of one triplet to the internal tubule of the adjacent one. The handles to which spokes are attached radiate from the internal tubules.

In 13 of the 16 cells examined during the G_2 period, one of the two mother centrioles was situated at an angle of $>75^\circ$ relative to the substrate surface (the probability of random orientation, $P < 0.0015$), i.e., an angle analogous to what we had observed at the beginning of interphase. The two diplosomes lie in the cell at a distance of $2-3 \mu\text{m}$ or more from each other. One diplosome almost always lies between the nucleus

and the cell surface oriented towards the substrate. The second diplosome is situated either in the same place or at the side of the nucleus.

In 3 of the 28 cells examined in the S period and in 6 of the 16 cells in G_2 , one of the mother centrioles formed a rudimentary cilium (Fig. 16). The cilium is not, as a rule, $>1 \mu\text{m}$ in length and is situated within the cytoplasm without being attached to the plasma membrane. The cilium forms only on the centriole with appendages. In mitosis, PE cells lack cilia.

Beginning with the G_2 period, around the centrosomes there is a great number of membrane-bounded vesicles of irregular form (24). They are usually $60-70$ nm in length. The vesicles become more numerous in prophase and disappear completely at the beginning of prometaphase. The appearance and increase in the number of membrane vesicles around centrioles coincides with the beginning of aster formation, and their disappearance coincides with formation of the spindle.

Even in early prophase, the diplosomes lie at a significant distance from each other. Their location is similar to that in the G_2 period. In late prophase, some invaginations of the nuclear envelope can be observed near the centrosomes (24, 36). Sometimes, the microtubules stretch into these invaginations. In prophase, each of the mother centrioles is surrounded by a halo consisting of fibrils 5 nm in diameter (Fig. 22). The width of the halo is $\sim 0.15 \mu\text{m}$, and it hardly changes throughout mitosis. All microtubules approaching centrioles terminate at the halo. The microtubules of the spindle were not found to have direct contacts with the centriolar tubules.

The daughter centriole keeps elongating during prophase but does not reach the length of the mother centriole. The structure of the daughter centriole changes in prophase; the cartwheel is about half the length of the centriolar cylinder, i.e., it hardly changes compared with the G_2 period; in the distal part, microtubule triplets are not linked. The tilt of the triplets relative to the radius along the entire daughter centriole is constant ($\sim 45^\circ$). In the distal part, electron-dense ribs are seen to stretch outside along triplets in two or three sections (Figs. 23 and 24). While elongating, the daughter centriole moves away somewhat from the mother, and the distance between them at the onset of mitosis is $0.1-0.15 \mu\text{m}$.

Throughout prophase the position of the diplosomes relative to the nucleus remains unchanged. Only in late prophase the diplosomes may locate in some cells on both sides of the nucleus. No bundle of microtubules or centrosomelike structures stretching from one diplosome to another was found in serial sections of the whole cell, as described previously (20).

From the beginning of prometaphase, i.e., with the first breakdown of the nuclear envelope, the membrane vesicles around the centrioles start to disappear. None of them is left by the onset of metaphase. While the general outline of the nucleus remains unchanged, both diplosomes are situated, as

FIGURE 8 Anaphase in PE cell. Mother centrioles (M) are oriented perpendicular to the spindle axis. Bar, $1 \mu\text{m}$. $\times 10,000$.

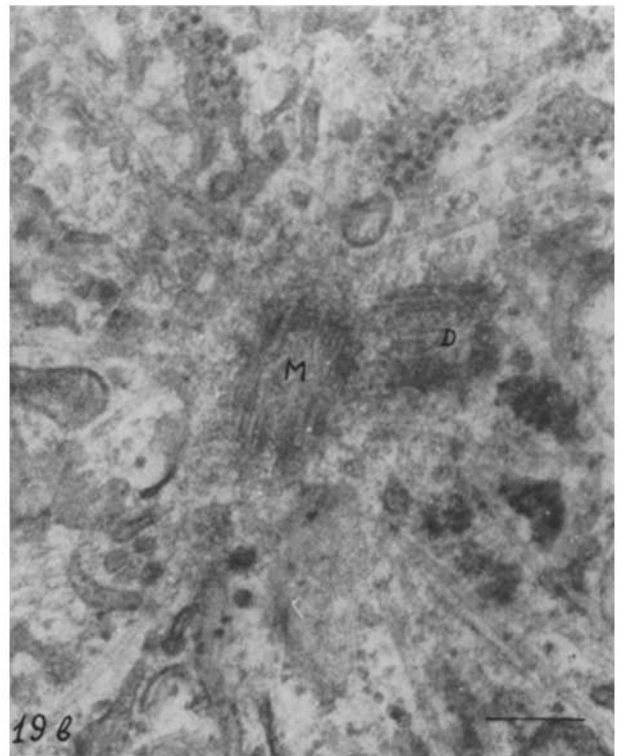
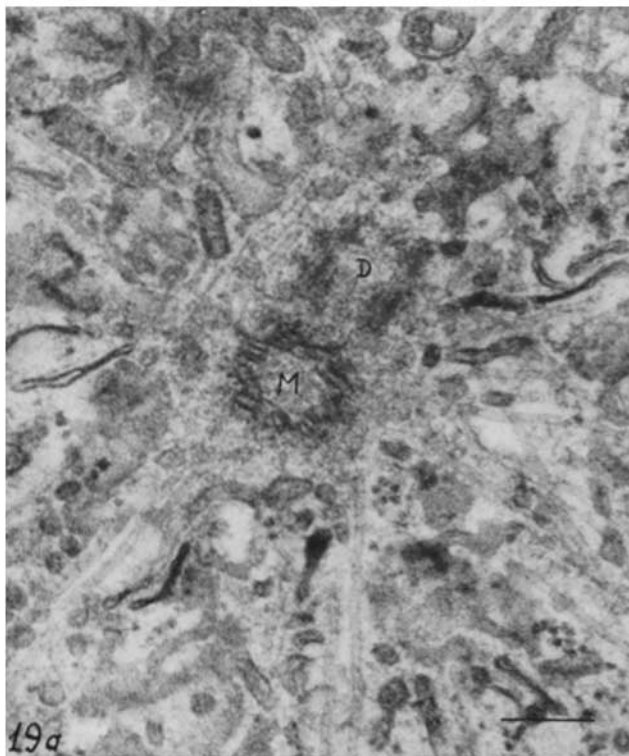
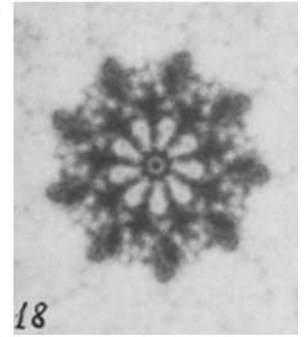
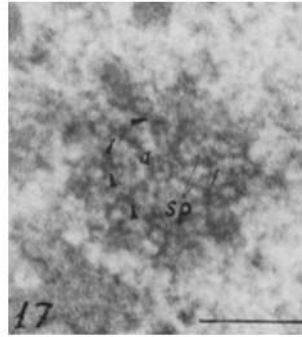
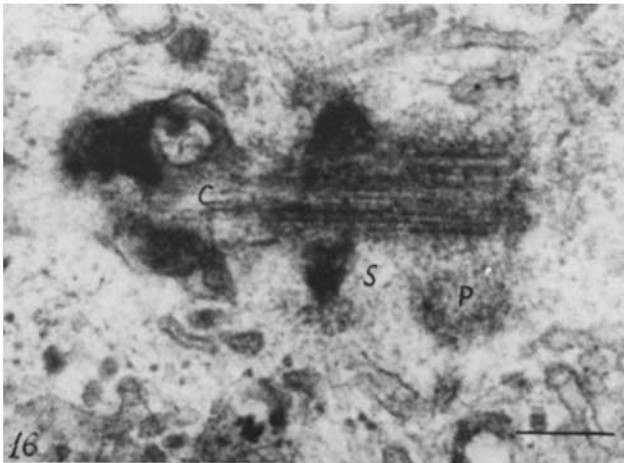
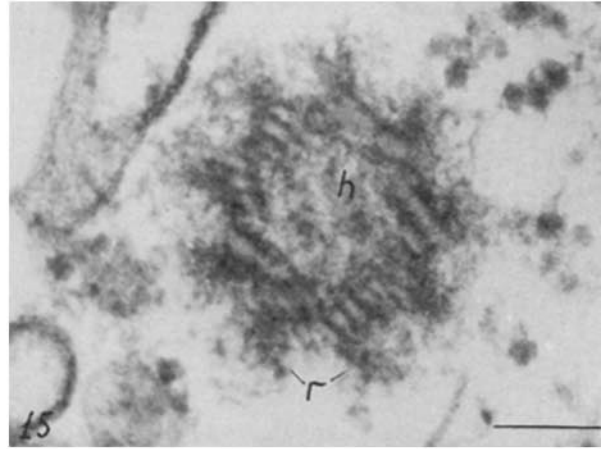
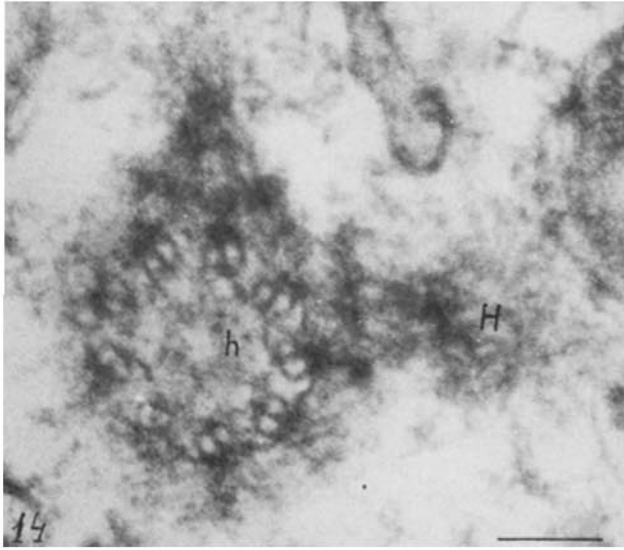
FIGURE 9 Diplosome in telophase cell. Arrowheads point to halo. Bar, $0.2 \mu\text{m}$. $\times 60,000$.

FIGURE 10 Mother centriole in the detergent-treated telophase cell. The fibrillar material of halo is clearly seen. Bar, $0.2 \mu\text{m}$. $\times 60,000$.

FIGURE 11 Diplosome at the beginning of G_1 . The halo is absent. Bar, $0.2 \mu\text{m}$. $\times 60,000$.

FIGURE 12 Centrioles in the G_1 period; M, mother; D, daughter centrioles; S, pericentriolar satellites. Microtubules come up to satellite heads (arrowheads). Bar, $0.2 \mu\text{m}$. $\times 60,000$.

FIGURE 13 Distal part of the mother centriole in the G_1 period; S, satellites; ap, appendages. Bar, $0.2 \mu\text{m}$. $\times 50,000$.



a rule, between the bulk of chromosomes and the substrate. Later, one of the diplosomes remains within the chromosomes up to metaphase, whereas the other lies apart from them. The distance between diplosomes in prometaphase is always shorter than in metaphase. The diplosome structure in prometaphase hardly changes except that daughter centrioles become as long as mother centrioles (Fig. 25), contrary to what takes place in Chinese hamster cells (8; also, our unpublished observations).

In the cells that had left the mitotic cycle (they constitute >90% of the PE cell populations on the sixth day of cultivation without medium change), we always found two centrioles of normal length that are not reciprocally perpendicular. The same was found in 3T3 cells (2, 32). None of the 62 cells examined by us at this stage contained procentrioles, i.e., in PE cells the centrioles do not replicate in the G_0 period. As a rule, the centrioles lie at a short distance from one another, as in the S period. In most cases, there are pericentriolar satellites on a centriole with appendages (Fig. 26). Their number is equal to that in the G_1 period, i.e., two to three per centriole. Ultrastructural differences between the two centrioles in G_0 are the same as in G_1 : one of them has appendages, the other has ribs in the distal part (Figs. 28 and 29).

The main distinction in the centriolar apparatus between the cells that have left the mitotic cycle and those in the G_1 period is the presence of cilia in the former (32); we found them in 25 of 62 cells. Unlike cilia formed in S and G_2 periods, the cilia of the G_0 period are, as a rule, 2–3 μm in length and come out to the surface of the cell (Fig. 26). They always begin from a centriole with appendages. The cilium ultrastructure is typical of abortive cilia of embryonic cells and of tissue culture cells, i.e., these cilia lack the central pair of microtubules. At the site where the centriole transforms into a cilium, there is no basal plate that is typical of kinocilia. The appendages locate strictly symmetrically, compared with the cilium-free centrioles the structure of which we described previously (34).

In the vicinity of centrioles, there are often striated rootlets (two to four) of a typical structure (Fig. 27). The same rootlets are sometimes seen in PE cells during the interphase of the mitotic cycle, but they are absent in mitosis.

In 24 of the 37 cilium-free cells we examined in the G_0 period, one of the centrioles located at an angle of $>75^\circ$ relative to the substrate plane (the probability of random orientation, $P < 0.001$). In ciliated cells, centrioles were found to have no fixed orientation towards the substrate.

The general scheme of centriole behavior in the cellular cycle of PE cells is given in Fig. 30.

DISCUSSION

It follows from the literature (9, 17) and from our previous (34)

and present observations that, in interphase, pericentriolar satellites are formed primarily on one of the two centrioles, always on the one with appendages. This allows us to state that in interphase both the satellites and the mitotic halo form on the mother centriole. Thus, it appears that the daughter centriole first becomes engaged in microtubule assembly only a whole cycle after its origination; in the subsequent S period, a new daughter centriole forms near it. After that, it plays the role of mother centriole in mitosis, and only after mitosis does it become capable of carrying pericentriolar satellites and forming a cilium.

Throughout the cell cycle, centrioles in PE cells are connected with microtubules by means of two structures, namely pericentriolar satellites in interphase and the halo in mitosis. The functions of the satellites are unknown, whereas the halo surrounding the mother centriole in mitosis has been intensely studied and is known (a) to induce polymerization of microtubules from exogenous tubulin (16, 27), (b) to withstand treatment with mitostatic doses of Colcemid, (c) to contain tubulin (19). Thus, it may be that the halo contains tubulin oligomers which are known (25) to withstand treatment with small doses of colchicine (inhibiting microtubule assembly) and may serve as nucleation sites in tubulin *in-vitro* polymerization (12). Then, at the beginning of mitosis, the halo may serve as a natural nucleation center for spindle-fiber polymerization. It is more difficult to understand the role it plays in the second half of mitosis when the microtubules surrounding the halo disintegrate while it remains intact (in anaphase and telophase). Moreover, in telophase, the halo is more detectable than in metaphase. Expansion of the halo was observed by McGill and Brinkley (16) when mitotic cells extracted with a detergent were supplemented with exogenous tubulin in the absence of EGTA; no microtubules were formed thereby, and the halo expanded. This indicates that tubulin is capable of being adsorbed on the halo without polymerization. Apparently, the same is observed in the living cell. When in the second half of mitosis some unknown factors induce spindle microtubule disassembly, it is in the halo that this tubulin may first be adsorbed. Disappearance of the halo is due to disappearance of some factors causing tubulin adsorption, which is regarded as the end of centriole mitotic "activity."

Poleward migration of centrioles occurs at different times in different cells within one population. It may start before, during, or after prophase and is always completed before metaphase (14, 20). Restoration of mitosis occurs in a similar way: centrioles first move towards the poles, then chromosomes line up to form a plate (8). But no investigators have yet succeeded in finding, between separating diplosomes, a bundle of microtubules to which the function of repulsion of poles has been hypothetically ascribed. Therefore, if the movement of

FIGURE 14 Mother centriole in the detergent-treated interphase cell. *H*, Head; *h*, centriole bush. Bar, 0.1 μm . $\times 60,000$.

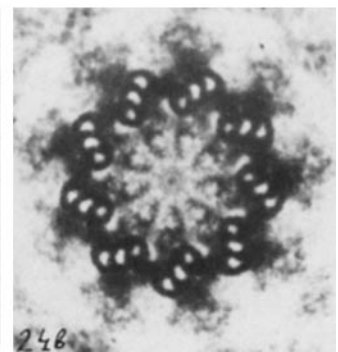
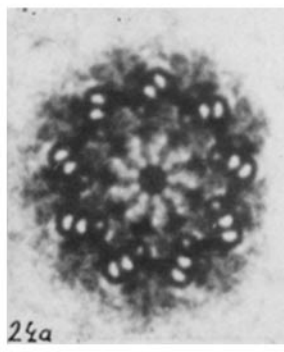
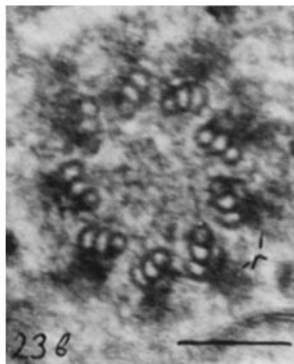
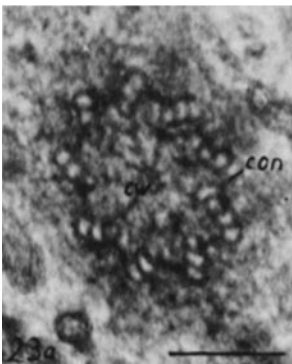
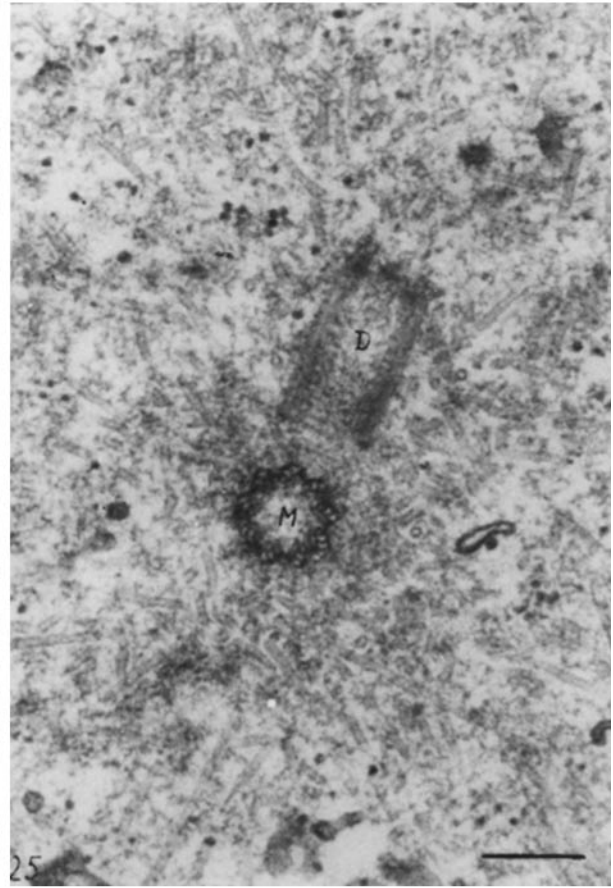
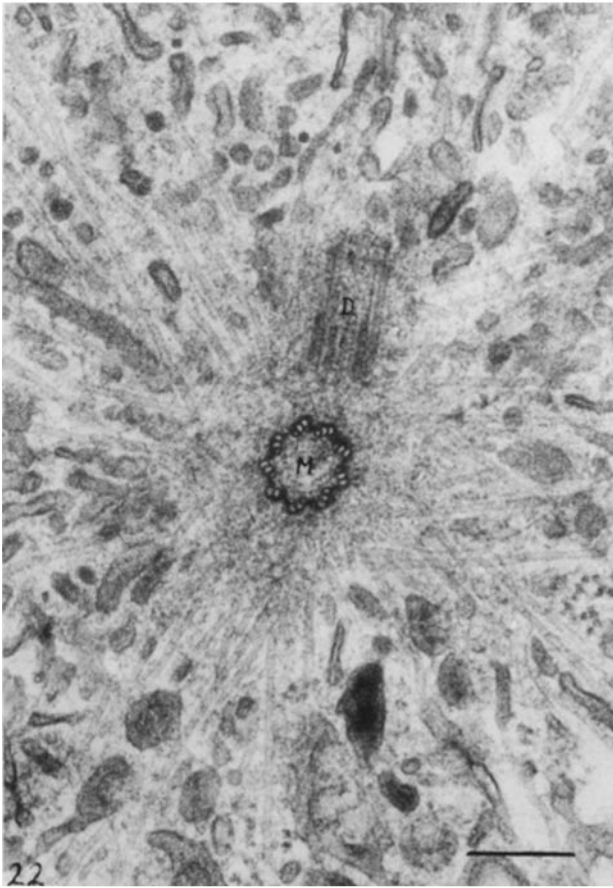
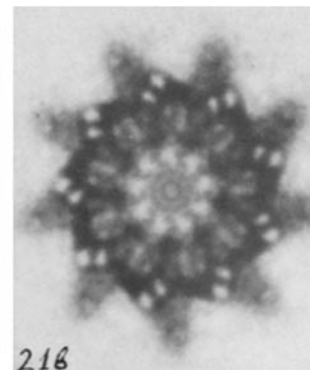
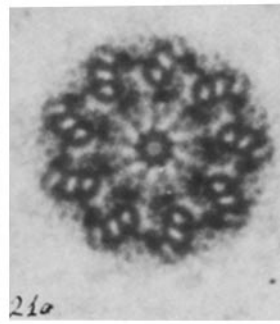
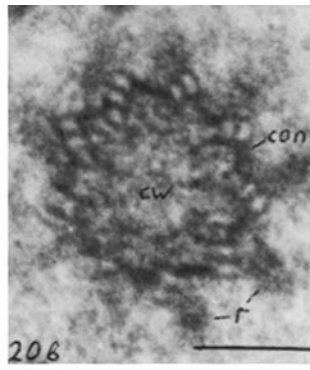
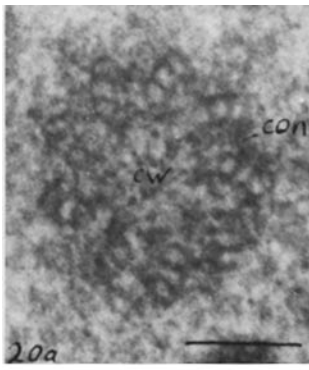
FIGURE 15 Daughter centriole in the detergent-treated interphase cell; *r*, ribs on the triplets; *h*, centriole bush. Bar, 0.1 μm . $\times 160,000$.

FIGURE 16 Replicating centriole in the S period; *P*, procentriole; *s*, satellites; *C*, cilia. Bar, 0.2 μm . $\times 80,000$.

FIGURE 17 The other procentriole in the same cell; *a*, axis; *sp*, spokes of a cartwheel. Note single and double microtubules attached to the ends of spokes (arrowheads). Bar, 0.1 μm . $\times 160,000$.

FIGURE 18 Rotation image of Fig. 17.

FIGURE 19 (*a* and *b*) Two diplosomes from one cell in the G_2 period. *M*, mother centrioles; *D*, daughter centrioles. Note microtubules radiating from the centriolar matrix of mother centrioles. Bar, 0.2 μm . $\times 75,000$.



centrioles is associated with microtubules, it is most likely that it is induced by the sliding of microtubules relative to each other or relative to other cytoskeletal structures. Recently, it was shown that centriolar separation may be inhibited without disruption of microtubules (4). So it seems unlikely now that poleward migration of centrioles occurs due to a microtubular system.

Of particular interest is the phenomenon of orientation of mother centrioles towards the spindle axis in metaphase and anaphase and towards the substrate plane at the beginning and at the end of interphase. Recently, Albrecht-Buehler and Bushnell (1) hypothesized that in the moving cell the centriole is oriented preferentially perpendicular to the substrate. According to our data, this orientation, when it exists, proves to be due to a certain state of the cell cytoskeleton rather than to cell movement along the substrate. During interphase, a centriole is oriented perpendicular to the substrates in the periods of complete rearrangement of the microtubule system (leaving mitosis and entering mitosis); during mitosis, centrioles are pole-oriented when chromosomes form a metaphase plate and are in the course of their separation. Thus, the orientation of centrioles may be associated with that of other intracellular structures. In this connection, Bornens' hypothesis (6) that the centriole may play the role of a gyroscope does not seem unfounded to us although the direct analogy suggested by this author is purely hypothetical.

Although centriolar replication in cells lacking cilia was described a comparatively long time ago (5), the ultrastructure of the growing procentriole has not been extensively described. In PE cells the procentriole is first found as a short cylinder whose walls already contain an incomplete set of microtubules. We failed to identify earlier stages of centriole formation, analogous to those of basal bodies (3, 14). Further development of the procentriole in PE cells is very similar to the growth of basal bodies in *Chlamydomonadidae* and in the epithelium of the respiratory tract (14, 28); it differs, however, from basal body development in the monkey's oviduct (3), i.e., the axis with spokes hardly elongates as the centriolar cylinder grows. (Sorokin [28] and Kalnins and Porter [15] were wrong to say that the axis disappears, since in both works the photos of longitudinal sections clearly show this axis, whereas the cross sections presented by them belong to the distal part of the cylinder.) The elongation of the daughter centriole is completed at different times in cells of different species: in Chinese hamster cells, both centrioles are of the same length at the beginning of interphase (10), whereas in PE cells this occurs in metaphase. After the centriole reaches normal length, it loses

the axis with spokes, and at the opposite end an amorphous spindle appears, which is not typical of basal bodies. Thus, in the next cycle, the new (ex-daughter) centriole differs from the former (mother) only by the absence of appendages. This difference, however, is functionally significant. This is probably why the second centriole is unable to form a cilium and pericentriolar satellites, and that would explain why there is only one cilium in each cell throughout interphase (2, 22, 31, 32). Appendages are probably needed for bridging the centriole to the membrane, which is also evidenced by the fact that when they become attached to the membrane their position and outward appearance are somewhat changed. At the same time, centriole appendages are probably also needed for the formation of pericentriolar satellites, i.e., for the "activity" of interphase centriole as a whole.

The centriole could influence microtubule formation in four ways (14): (a) by formation of the mitotic halo and spindle, (b) by formation of pericentriolar satellites with radiating cytoplasmic microtubules, (c) by centriole replication, and (d) by ciliation. For the PE cells we studied, we propose a regular distribution of these functions within the cell cycle (Fig. 31). In the cells of a given culture, it is only the first three functions that are necessary for the centriole since ciliation occurs only in a small portion of the cells, in contrast with 3T3 cells described recently by Tucker et al. (32). Therefore in all three cases, the centriole as such is not the center of microtubule formation; instead, microtubule-organizing centers are formed on it or in contact with it. It is noteworthy that two types of microtubule-organizing centers do not form on the centriole simultaneously, nor does one follow the other immediately, which is evidenced by the presence at the beginning and at the end of interphase of cells lacking both halo and satellites.

If we consider the centriole cycle as a whole, it appears that some of its phases, being synchronized to specific stages of the cell cycle, never fully coincide with them; rather, they are somewhat shifted in time. In other words, pericentriolar satellites exist from a certain moment in the G_1 period till a certain moment in the G_2 period; centriole replication starts (is first visible?) near the middle of the S period; elongation of the daughter centriole ends in prometaphase; the halo with microtubules forms at the end of the G_2 period and exists almost till the end of mitosis; the hub forms in the daughter centriole at a certain moment during the G_1 period; centriole appendages appear in the second half of mitosis.

Thus, during the cell cycle we can observe a regular change in relations between centrioles with microtubules and successive changes in the structure and position of centrioles. It takes

FIGURE 20 (a and b) Serial sections of daughter centriole in the G_2 period. a, Proximal end, b, Distal end. cw, Cartwheel; con, connectives; r, ribs. Bar, 0.1 μm . $\times 160,000$.

FIGURE 21 (a and b) Rotation images of Fig. 20 a and b.

FIGURE 22 Diplosome in the prophase cell. M, mother centriole; D, daughter centriole. Numerous membrane-bounded vesicles surround centrioles. Bar, 0.2 μm . $\times 70,000$.

FIGURE 23 (a and b) Serial sections of daughter centriole in the prophase cell. Sections 1 and 3. cw, Cartwheel; con, connectives; r, ribs. Bar, 0.1 μm . $\times 160,000$.

FIGURE 24 (a and b) Rotation images of Fig. 23 a and b.

FIGURE 25 Diplosome in the prometaphase cell. Note the absence of membrane-bounded vesicles around centrioles. M, mother; D, daughter centriole. Bar, 0.2 μm . $\times 75,000$.

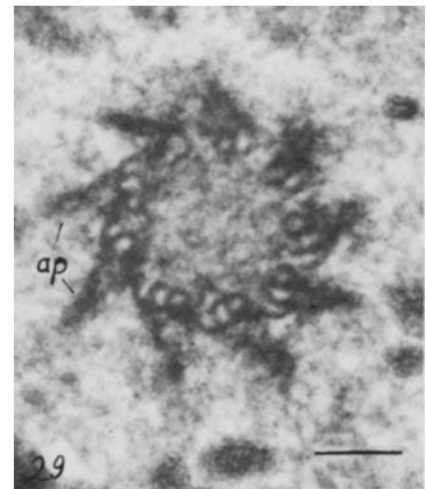
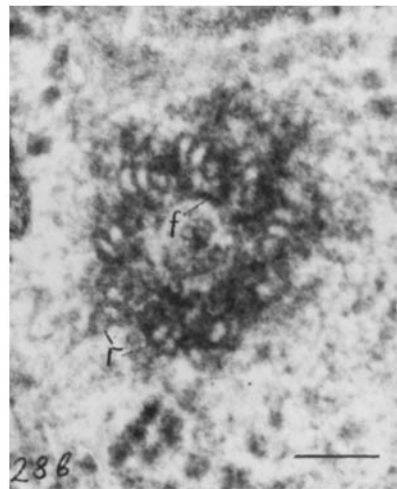
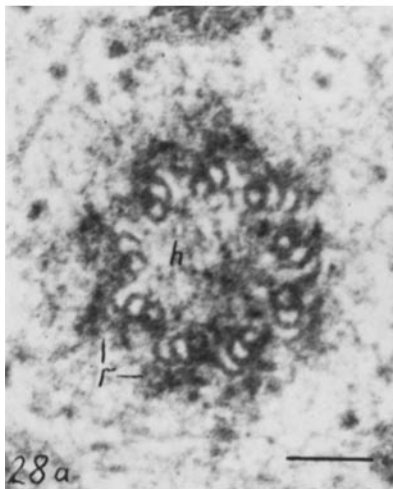
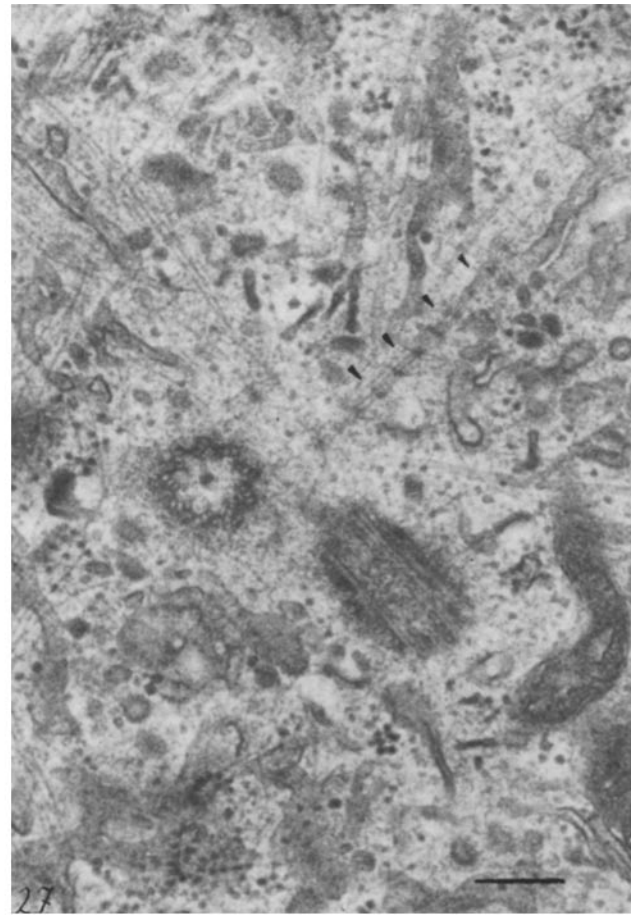


FIGURE 26 Centrioles in the G_0 period; *M*, mother centriole; *D*, daughter centriole; *c*, cilia; *s*, satellites. Bar, $0.2 \mu\text{m}$. $\times 75,000$.

FIGURE 27 Centrioles in the G_0 period. Arrowheads indicate striated rootlet. Bar, $0.2 \mu\text{m}$. $\times 75,000$.

FIGURE 28 (*a* and *b*) Serial sections of the distal part of the daughter centriole in the G_0 period. *h*, Bush; *f*, fiber; *r*, ribs. Note the absence of appendages. Bar, $0.1 \mu\text{m}$. $\times 140,000$.

FIGURE 29 Distal part of the mother centriole producing cilia in the G_0 period. Appendages (*ap*) are situated symmetrically. Bar, $0.1 \mu\text{m}$. $\times 140,000$.

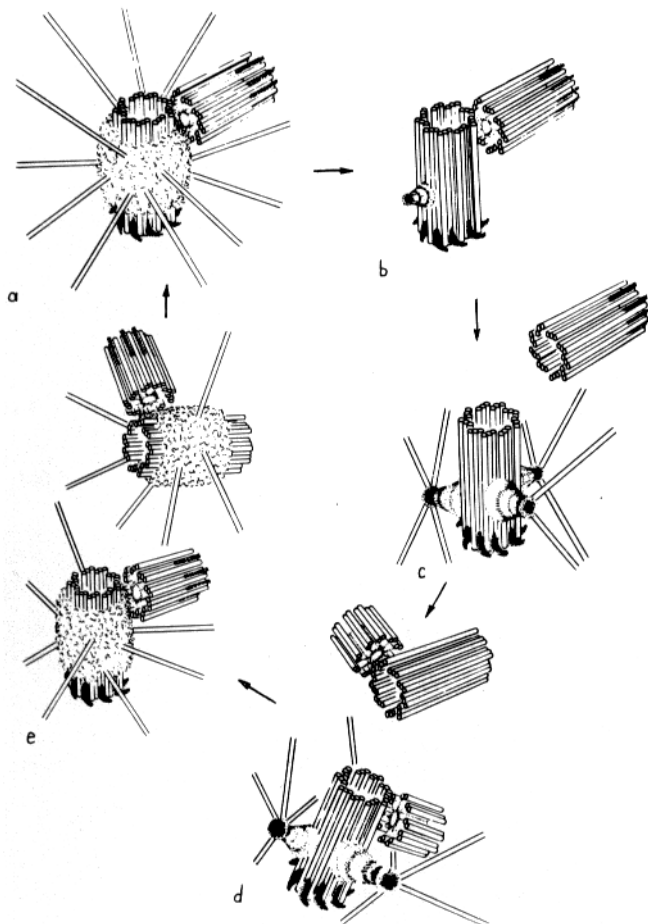


FIGURE 30 General scheme of the centriole cycle in PE cells. (a) Metatelo phase; (b) the beginning of interphase; (c) G_1 to the 1st half of the S period; (d) 2nd half of the S period to the 1st half of the G_2 period; (e) the end of the G_2 period to the prophase.

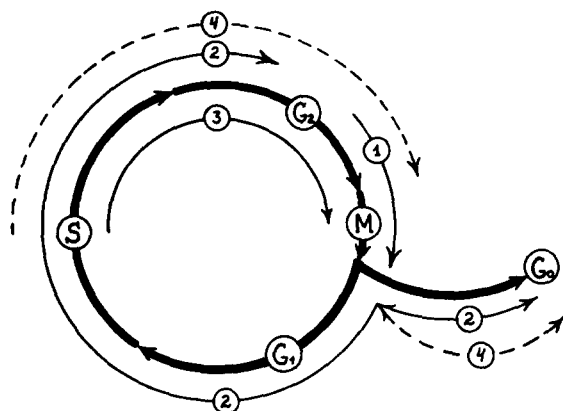


FIGURE 31 Different kinds of centriolar activity during the cell cycle in PE cells. (1) Formation of mitotic spindle; (2) Formation of interphase microtubules; (3) Centriole replication; (4) Formation of primary cilia.

the centriole a cycle and half to form completely, which explains the presence of two centrioles in the cell after mitosis.

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