ARCHITECTURE AND FUNCTION OF THE MAMMALIAN CENTRIOLE

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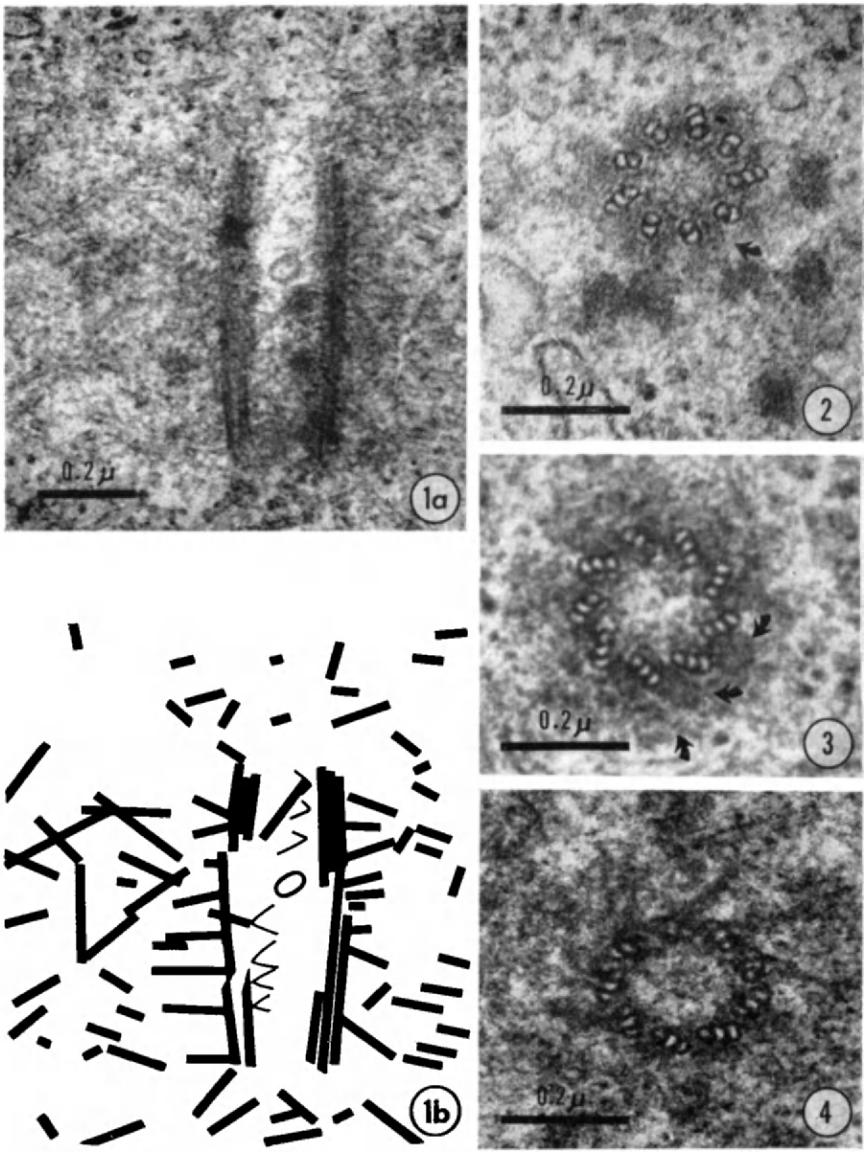
INTRODUCTION

For many years cytologists have known of the existence of the centriole from observations with the light microscope. Other than the fact that these tiny granules were associated with the process of mitosis in animal and some plant cells, very little progress was made in understanding either their structure or function until the electron microscope became available for biological studies. Light microscopists concluded that the centriole somehow organized the poles of the mitotic apparatus, but until recent years even the existence of the mitotic apparatus as a cell structure was doubted by many biologists. A second cellular organelle, the basal body, which has long been thought to be a structure derived from the centriole in ciliated cells [13, 21], has in recent years been clearly demonstrated to be the centriole in a second type of activity.

In 1956, DeHarven and Bernhard [5] were the first to publish ultrastructure studies of the centriole using electron microscopy. The ultrastructure of the basal body was quickly demonstrated to be identical with that of the centriole, and the homology of the two structures was later established by several groups of workers [9, 30, 36].

The activities of the centriole in both mitosis and ciliogenesis have recently been studied extensively in our laboratory [2, 3, 37]. Since both centriole functions are present in a fibroblast cell line *in vitro*, it has now become possible to study transitional stages in centriole behavior and to time centriole replication and ciliogenesis in terms of the cell reproductive cycle. In addition, new information on centriole ultrastructure is available as a result of improved preservation techniques for thin-sectioned material and photographic methods that reduce image noise in electron micrographs. The architecture of the centriole will be considered first, and experiments and discussions of centriole function will follow.



All material prepared for electron microscopy was fixed in 3% glutaraldehyde (pH = 7.4), postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Epon 812 (for more details see [2, 3, 37]).

FIG. 1. (a) Longitudinal section of Chinese hamster centriole in a mitotic cell. (b) Diagram showing the positions of the microtubules seen in (a) and also indicating the *central vesicle* and segments of the *internal helix* (see text).

ARCHITECTURE OF THE CENTRIOLE OF THE CHINESE HAMSTER

Almost all available information about centriole ultrastructure comes from electron microscopic studies. Only tentative biochemical studies on basal body composition have yet been made [14], simply because of the difficulty in isolating sufficient material; however, this deficiency will no doubt eventually be overcome. Cilia and the mitotic apparatus have been purified and analyzed biochemically [11, 23, 24, 39], so some chemical information is also available from the studies of these related structures.

One or more centrioles are visible in about 6% of the cell thin sections studied of the Chinese hamster cell strain Don-C [26]. Most sections through centrioles are oblique, but occasionally good longitudinal and cross sections are obtained as in Figs. 1–4. From these four micrographs alone we can extract much of what is known about centriole architecture. Basically, the centriole is a hollow cylinder. Although the overall shape is easy to demonstrate, a number of structures are contained within which are more difficult to see; these will be considered in sequence.

The Outer Wall

The outer wall of the centriole is composed of 27 microtubules that appear to run the length of the centriole parallel to its long axis. These microtubules are separated into 9 groups of 3, with the members of each triplet fused together to form a blade. The blades form an angle with the cylinder surface in such a way that when viewed in cross section (Figs. 2–4), they have a "pinwheel" arrangement. The microtubules of one triplet do not touch members of the adjacent triplets, but they appear to be embedded in a common osmiophilic matrix in the centriole wall. The microtubules are each about 200–250 Å in outer diameter; the overall centriole structure is about 0.25 μ in diameter

FIG. 2. Cross section of centriole at the proximal end showing the *cartwheel.* Note the microtubule (arrow) connecting the centriole wall and a *pericentriolar satellite;* several of the latter structures are present. Microtubule C is shorter than A or B in 6 of the triplets at this end of the centriole.

FIG. 3. Cross section of a centriole. One-third of a turn of the *internal helix* is visible at the left side of the centriole lumen. In 3 cases (arrows) microtubules are seen that attach to the centriole wall between triplets on the *triplet base* (see text).

FIG. 4. Cross section of a centriole at the distal end showing external fibrous appendages (*transition fibers*) and a dense structure in the centriole lumen which differs from the cartwheel. The section is oval in shape because of compression during sectioning (compare with Fig. 5).

and 0.5–0.7 μ in length. The microtubules of a triplet are designated by convention as A, B, and C, with A the innermost microtubule [12].

The Central Vesicle

Within the lumen of the centriole is a small vesicle about 600 Å across (Fig. 1). This structure is thought to be a vesicle rather than a ring, since it is always circular in profile when seen in cross, oblique, or longitudinal sections of centrioles. It has been observed in centrioles and basal bodies of other organisms [6, 29, 35] and may be present in all species. Its function is completely unknown.

The Internal Helix

A second component of the centriole lumen, and much more difficult to demonstrate, is a large helix which spirals just under the triplets for the full length of the centriole cylinder. In Fig. 1 the longitudinal sections of this helix are faintly visible as diagrammed below the figure. The element of the helix appears to be about 50–75 Å in diameter. About 8–10 turns can be counted with a spacing of about 750 Å per turn. In cross sections of the centriole only part of one turn of the helix is visible; in Fig. 3 it appears as a semicircle on the left side of the centriole lumen. Measurements indicate that the distance across the helix is about 1300 Å and the distance between turns about 750 Å; this would indicate a total stretched length of about 4–5 μ for this structure.

Perfect cross sections of centrioles provide a figure with 9-fold rotational symmetry. Such structures may be studied with a special photographic method devised by Markham *et al.* [22] for probing the structure of virus capsids. The method is based on the principle that an object viewed along an axis of n-fold symmetry may be seen with improved resolution (because of a decrease in "noise") if it is viewed in all its n different but equivalent rotational positions simultaneously. This effect can be achieved photographically, in the case of a centriole, for example, by making a 9-fold multiple exposure photograph with the centriole cross section rotated 40 degrees around its central axis between each exposure. The result is a kind of photographic "average," with enhancement of structure that repeats every 40 degrees and simultaneous reduction of the randomly distributed noise in the photograph. At the same time structures that repeat at other intervals (i. e., 60 degrees) will also be reduced in contrast.

Before the method could be applied to centriole cross sections, however, an additional problem had to be solved. We intuitively felt that

the centriole cross section should be symmetrical, i.e., circular, but it was always more or less oval in appearance. This was probably the result of compression of the structure as it was sectioned. If this was indeed the case, we reasoned, it should be possible to "recircularize" the image photographically. A photograph can be "stretched" in a given direction by projecting it on a slanting surface. That this process could be successfully applied to centrioles is seen in Figs. 4 and 5.

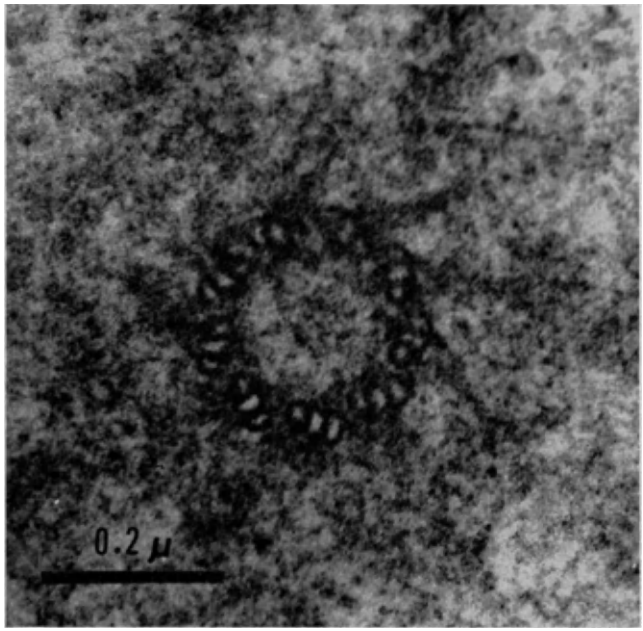


FIG. 5. Same section as Fig. 4 after "recircularizing" the image by printing the negative on a slanting surface to restore rotational symmetry.

Restoring overall circularity to the image also restored rotational symmetry, in that triplet angles and positions all became equivalent. The high-resolution photographs resulting from applying the Markham technique to the recircularized images of Figs. 2, 3, and 4 are presented in Figs. 6, 7, and 8, respectively.

The Triplet Base

With improved resolution, each triplet blade of the centriole was seen to rest on a faint double structure between it and the centriole lumen (Fig. 7). This *triplet base* appeared to be bounded peripherally by the dense matrix of the centriole wall and in the lumen of the centri-

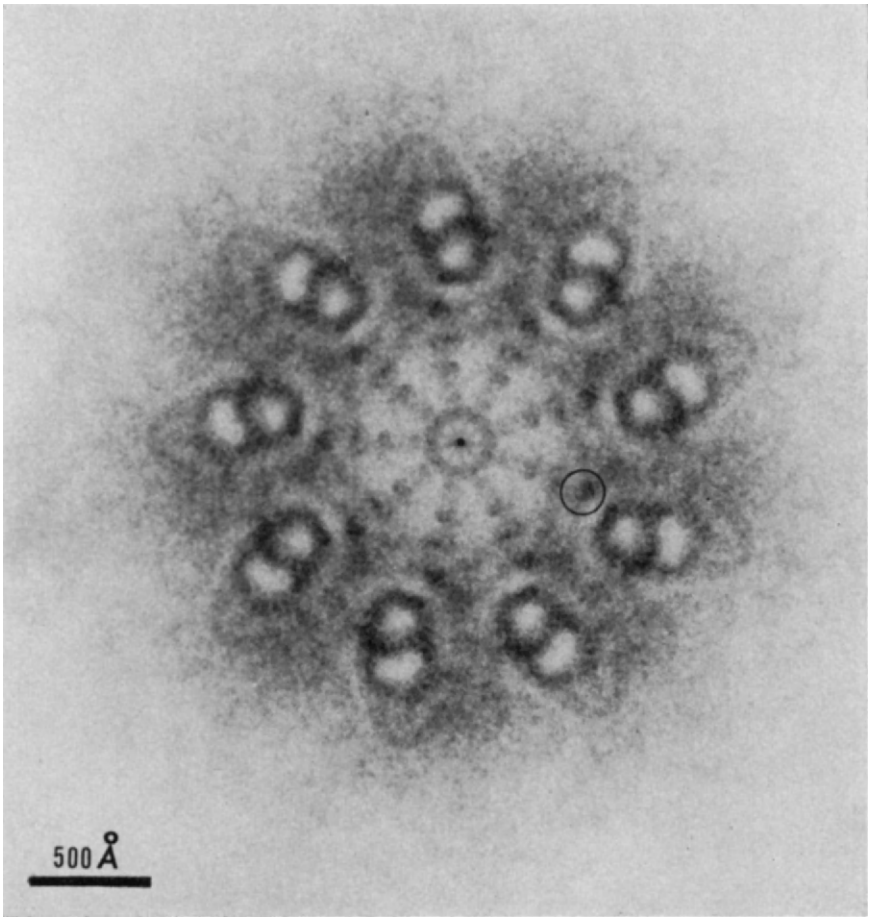


FIG. 6. High-resolution photograph produced with the technique of Markham *et al.* [22] using the recircularized image of Fig. 2. The *cartwheel* is now seen with greater clarity; note the segmentation of the spokes and the fine detail of the central hub. Microtubule C occurs but 3 times in this section and so does not reinforce well in this photograph. The dense particle of stain (at 1 o'clock in Fig. 2) is also eliminated by this procedure since it does not occur repeatedly at 40-degree intervals; all random stain (noise) is likewise reduced, while repeating detail is enhanced. Substructure is also now visible in the walls of the triplet microtubules, which indicates that the triplet architecture is rigidly related to the whole centriole structure. The dense structure in the circle is found in all cross sections (see Figs. 7–10) and is thought to contain RNA (compare with Fig. 15).

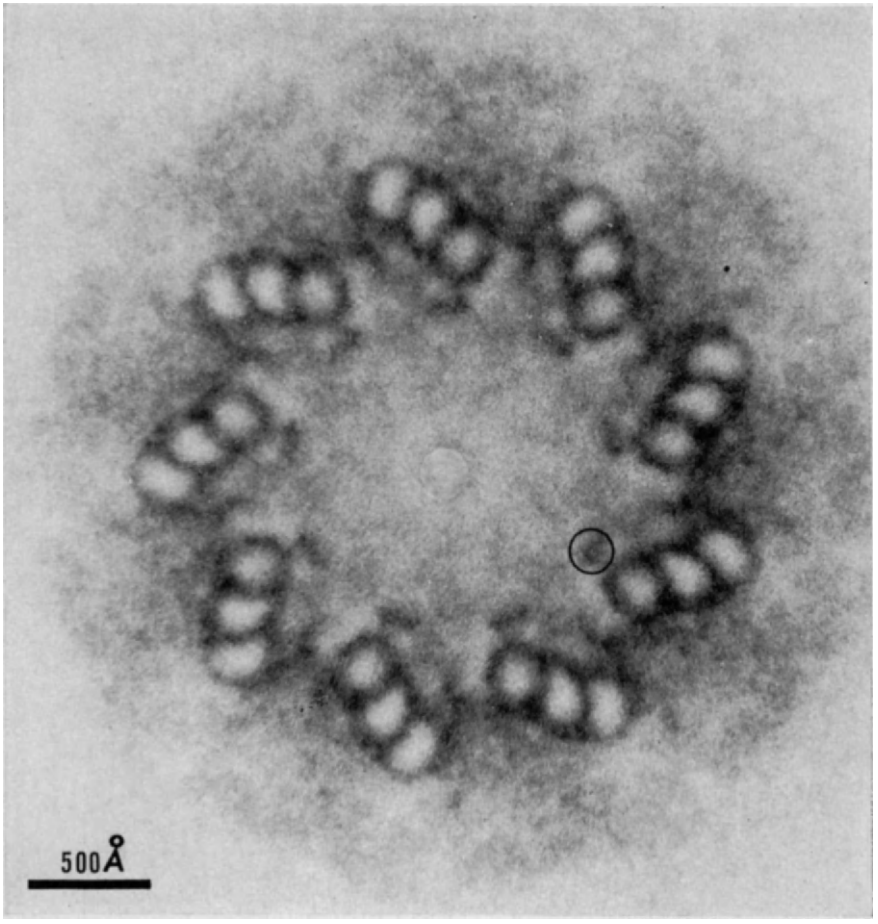


FIG. 7. High-resolution photograph of the image in Fig. 3. Each triplet is associated with a double structure, the *triplet base*. In the centriole lumen, each triplet base is bounded by a dense "foot" (circle). Peripherally, the triplet base attaches to microtubules oriented vertically to the long axis of the centriole, as can be seen in Figs. 2 and 3.

ole by a dense footlike appendage extending from microtubule A (circled in Figs. 6–8). All the "feet" appeared to rest on a circle about 1300 Å in diameter. The spiral structure described earlier also had a helical diameter of about 1300 Å, so we believed that the spiral was centered in the lumen on these feet. In Fig. 3 the spiral extends for only a short distance on one side of the centriole and did not reinforce adequately to appear in the high resolution photograph. However, in a thicker section a sufficient part of the spiral was present, and the rota-

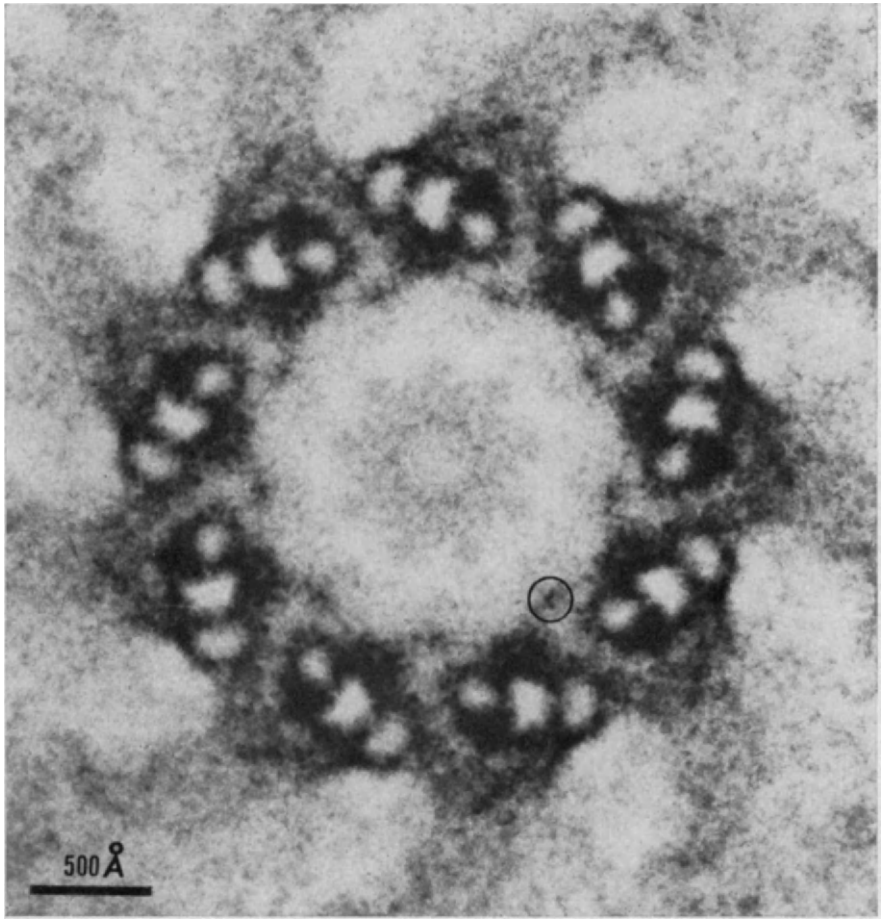


FIG. 8. High-resolution photograph of the image in Fig. 4. The *triplet base* is reduced to a single dense structure adjacent to microtubule B in this end of the centriole; the dense "foot" (circle) is still present, however. The central structure does not appear to have 9-fold symmetry since it does not reinforce well (compare with Fig. 13).

tional photograph shown in Fig. 9 demonstrates that the spiral does contact the feet projecting from microtubule A.

Peripherally, the triplet base sometimes appears to be continuous with microtubules extending away from the centriole. This is best demonstrated in cross sections, and several examples are shown in Figs. 2 and 3 (arrows). As in the case of Fig. 3, microtubules are present on only 2 or 3 triplet bases. This suggested the possibility that triplet bases may appear different in different positions around the centriole

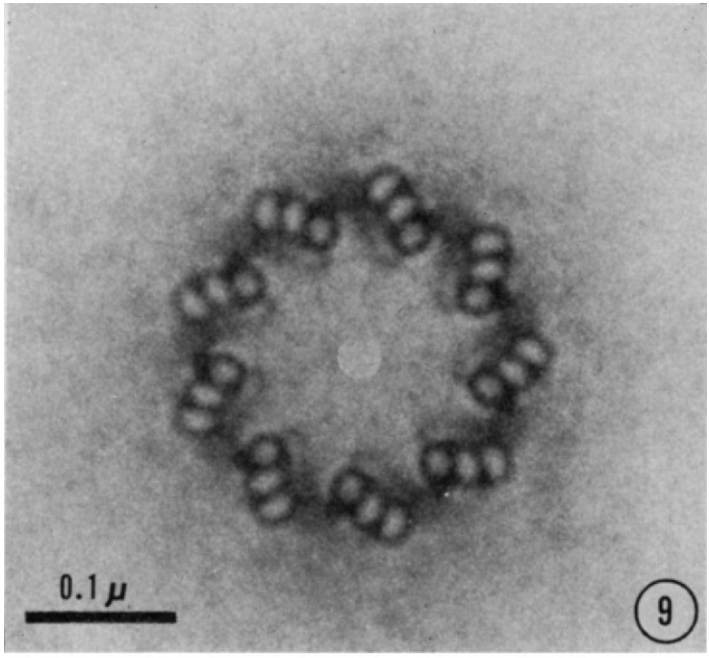
if they are arranged in some sort of spiral pattern. Accordingly, a 3-fold rotational photograph of the centriole cross section in Fig. 3 was prepared in which only 3 adjacent triplets were superimposed in each case (Fig. 10). The triplet bases looked much the same in each position; this was interpreted to indicate that they are linear structures running continuously from end to end parallel to the triplet microtubules. In Figs. 6 and 9 the triplet base is also present and appears to maintain a fixed relationship to the triplet plane. However, in Fig. 8, at the opposite end of the centriole from Fig. 6, the triplet base is apparently reduced to a single dense structure adjacent to microtubule B.

The concept of direct attachment of microtubules in the cell cytoplasm to the wall of the centriole has been suggested by other investigators [9, 34] but never clearly demonstrated. Although at first glance the microtubule populations in Figs. 1 and 11 (diagrammed in 1b) seems to bear no obvious relationship to the centriole, a more careful analysis reveals that many of the segments are roughly perpendicular to the long axis of the centriole. Those microtubules nearest to the centriole are more difficult to detect because of the increase in density of the surrounding material near the centriole wall. Two additional factors add to the confusion: (a) in mitosis some microtubules appear to be produced by the kinetochores of the chromosomes, and these probably do not actually attach to the centriole wall, and (b) in interphase there is more than one active centriole; thus many microtubules may be oriented with respect to a second unseen centriole and confuse the overall picture. Nevertheless, the only point of attachment seen consistently in mammalian centriole sections is to the triplet base as in Figs. 2 and 3. The attachment to several consecutive triplet bases suggests that the microtubules are attached in a spiral arrangement corresponding roughly to the helix spiral within the centriole lumen.

The Cartwheel

Cross sections at one end of the centriole reveal an additional structure (Fig. 2). Connecting the microtubules of the centriole wall to a central circle are 9 radial spokes. This "cartwheel" structure has been seen in both centrioles and basal bodies of several organisms [9, 12]; in some protozoan species it appears to be a multiple structure, occurring in successive cross sections for some distance through the length of the basal body [12]. However, in Chinese hamster centrioles it occurs in only the end section and only at one end.

In Fig. 6, the high resolution photograph of the end section shown in Fig. 2, the "cartwheel" structure can be seen with greater clarity.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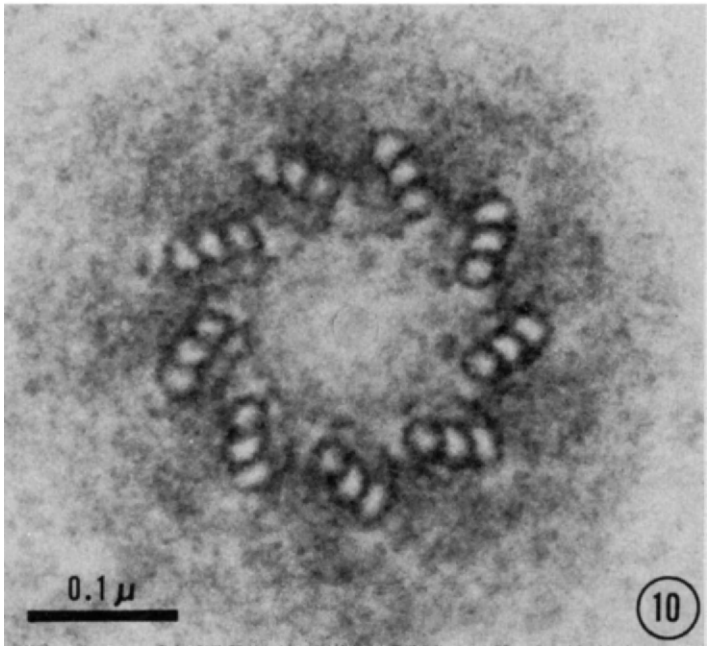
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TABLE I. *Relationship between Triplet Angle and Internal and External Centriole Diameters*[a]

Case	Triplet angle	Internal centriole diameter (Å)	External centriole diameter (Å)
Fig. 2 (end section)	10°	1600	2350
Fig. 9	33°	1860	2320
Fig. 3	38°	1840	2420
Fig. 14e (3rd section)	40°	1840	2360
Fig. 14d (2nd section)	46°	1880	2310
Fig. 14c (end section)	50°	1900	2320
Fig. 4 (end section)	57°	1950	2320

[a] In all cases the average distance within all triplets from the center of microtubule A to the center of microtubule is taken as 400 Å. The *triplet angle* is defined as the smaller angle at the intersection of a line through the triplet microtubule centers and the centriole radius through the center of microtubule C. The *internal centriole diameter* is defined as the average distance from the center of a microtubule A to the centers of the two microtubules A opposite it (across the lumen). The *external centriole diameter* is the analogous measurement between microtubules C. All measurements were made using high-resolution rotation photographs (see text).

The "spokes" are definitely segmented and appear to attach at one end of each triplet base. The microtubules in this centriole end-section were so well positioned that their subunits reinforced in Fig. 6, and the microtubule structure appears to be similar to that of other species as described elsewhere in this volume [20].

In Fig. 2 we also note that only 3 of the triplets are complete; in 6 cases the outer microtubule is missing. We interpret this to indicate that the outer microtubule is usually shorter than the other two in the end of the centriole containing the cartwheel. This was also the conclusion of Gibbons and Grimstone in their studies of flagellate basal bodies ([12], see their Fig. 13).

It is also of interest that in Fig. 2 the angle of the triplet plane with respect to the radius of the centriole is much smaller than in Fig. 3. Careful measurements show that the outer diameters of the centrioles in Figs. 2 and 3 are almost identical; the decrease in the triplet angle in Fig. 2 resulted in a decrease of the lumen diameter (Table I). It

FIG. 9. High-resolution photograph of a centriole cross section similar to Fig. 3. However, since the section was thicker, the *internal helix* was visible over an arc large enough to reinforce as a circle in the final photograph. The helix contacts the *triplet base* where the "foot" appears.

FIG. 10. Rotation photograph of Fig. 3 in which only 3 adjacent 40-degree sections were superimposed. The *triplet base* is visible at each position, suggesting that it is also a linear structure like the triplet.

thus appears that the triplet structures are hinged along microtubule C and that the triplet angle is decreased by moving microtubule A toward the central axis of the centriole.

The triplet angle does not appear to be the same at different points along the centriole axis. Data given in Table I for three consecutive sections at one end of a centriole indicate a change of 10 degrees in 3 sections. Complete serials are not yet available, however. At the point of transition from the basal body to the cilium such a twist in the triplet plane is evident in ciliates [12]. Figure 11 seems to indicate that the triplets are twisted from one end of the centriole to the other, but the serial sections of a centriole shown in Fig. 12 show all microtubules lying parallel with no evidence of any twist in the triplet plane. Therefore, we have concluded that the triplets are mounted in such a way that the triplet twist can be introduced during different centriole activity states. We will return to this point later.

External Fibrous Appendages

Figures 4 and 8 reveal another centriole structure, a series of fibrous appendages, which radiate away from the centriole near one end approximately vertical to each triplet plane. Similar structures occur near the junction of basal bodies to cilia, and these have been named "transition fibers" [12, 29]. That these fibrous structures also are found near one end in Chinese hamster centrioles is evident in the serial sections presented in Fig. 14. The fact that they do not reinforce well in the rotation photograph (Fig. 8) suggests that they are not rigidly fixed in space or are of a rather irregular composition.

Octagonal End Structure

A somewhat blurred structure is present in the lumen of the end section shown in Fig. 4. It does not appear to be the same as the cartwheel seen before (Figs. 2 and 6), since it is rather dense and does not possess the obvious 9-fold rotational symmetry of the cartwheel. In Fig. 8, the structure does not reinforce well, indicating a lack of 9-fold symmetry. The symmetry of this structure may have been partially destroyed by fixation and embedding, but we felt that a test of other possible symmetries was indicated. Accordingly, rotations of 120, 90, 72, 60, 51.4, and 45 degrees between exposures were tried (corresponding to tests for 3-, 4-, 5-, 6-, 7-, and 8-fold symmetry, respectively). The resulting rotation photographs are shown in Fig. 13. Surprisingly, the best reinforcement was obtained at 90- and 45-degree intervals, indi-

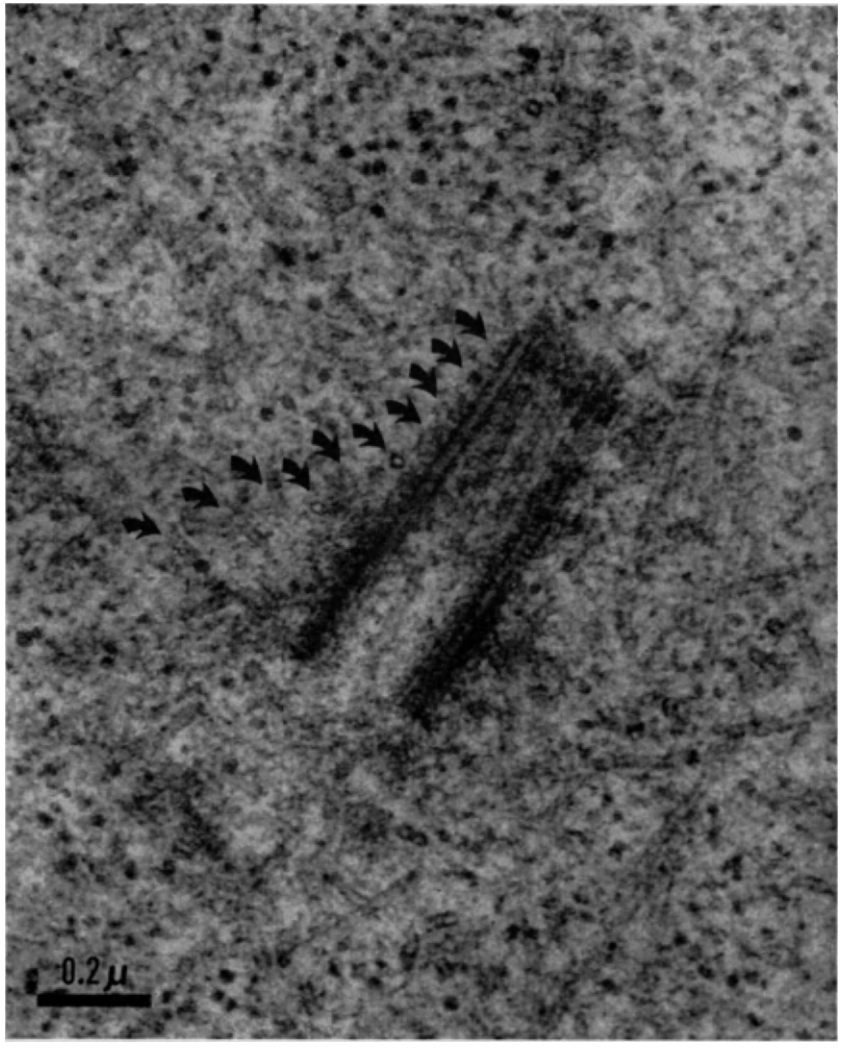
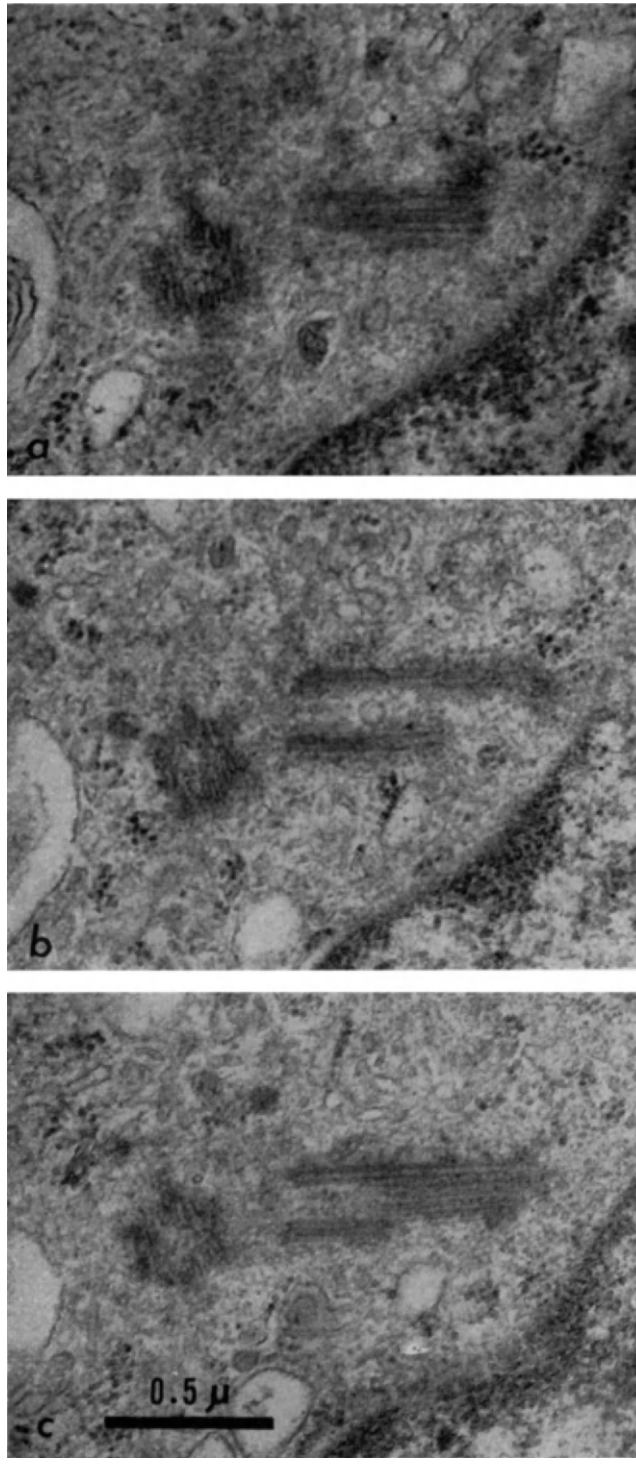


FIG. 11. Longitudinal section of a centriole in a mitotic cell. The twist of the triplets from one end to the other is apparent in the slant of the microtubules A in the lumen at the upper end. The arrows indicate the regular spacing of microtubules near the centriole wall; careful analysis reveals a similar spacing on the opposite side. Since the microtubules leave the surface of the centriole parallel to the triplet plane, instead of exactly vertical to the wall, only short segments are visible near the centriole. At the proximal end (bottom) the microtubules are more nearly vertical because of the triplet twist, thus longer segments are seen closer to the centriole surface.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cating that this end structure has 8-fold rotational symmetry. The implications of this discovery will be discussed later.

Pericentriolar Satellites

Many centriole sections reveal numbers of irregular dense masses near the centriole as in Fig. 2. These are termed *pericentriolar satellites* and their function remains unknown. Occasionally, they appear to be associated with microtubules near the centriole, and some authors have proposed that these are the point of microtubule attachment [5, 27]. This point will be raised again in a later section.

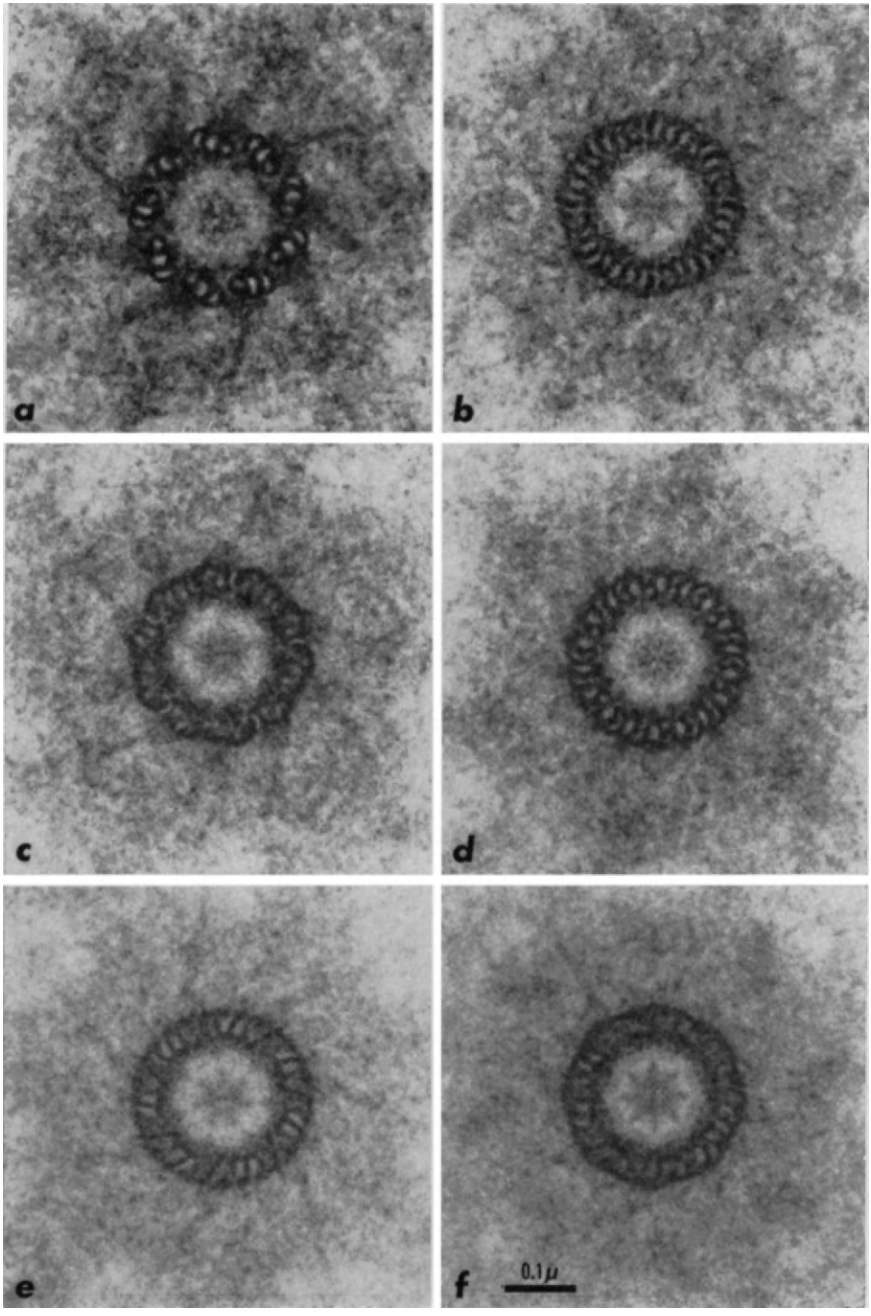
Structures Peculiar to Basal Bodies

In order to complete this section, we must include descriptions of several structures that were first described in basal bodies and therefore acquired different names, although some may be modifications of structures we have already considered. The *basal plate* [7, 12] is a flat structure across the end of the basal body nearest the cilium. It may be homologous with the octagonal end structure which we have already considered. The *basal foot* (see Fig. 23) may be a modified procentriole, although some find greater similarity to the pericentriolar satellites [6]. The basal foot always lies in the plane of ciliary beating [10], but its function is unknown. *Rootlets* appear at the end of the basal body opposite the cilium in some species [6, 7]. These are usually cross-striated and may extend several microns into the cytoplasm; they may serve in anchoring the basal body in the cytoplasm.

CHEMICAL COMPOSITION OF CENTRIOLE COMPONENTS

The biochemical composition of the architectural components of the free centriole remains open to investigation. Some information about the nucleic acids occurring in the basal body is available, however, and the composition of free centrioles will probably prove to be similar. Hoffman [14] found evidence for the presence of a small amount of RNA (about 2% of the total preparation) in isolated basal bodies

FIG. 12. Longitudinal serial sections through an immature procentriole. The parent centriole is obliquely sectioned on the left. In the procentriole, note that all the microtubules in the wall are parallel, i.e., there is no indication of a twist in the triplet plane (compare with Fig. 11). This suggests that the twist in the triplet plane is induced later as the centriole begins to function in microtubule production. The *central vesicle* is shown in (b).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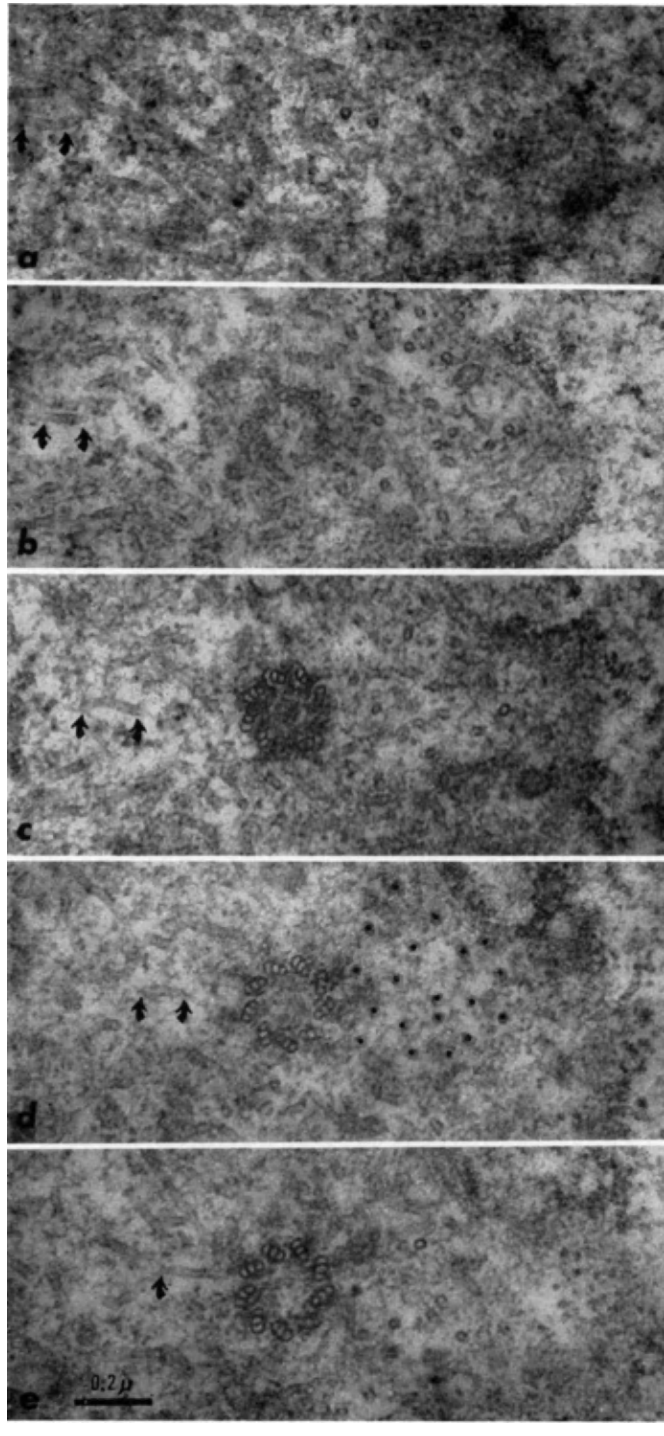
from *Tetrahymena*. DNA was less than 1% of the total preparation, and this amount was too low to be detected with certainty. Randall and Disbrey [28] found evidence for the presence of both DNA and RNA in the basal bodies of *Tetrahymena* using the fluorescent dye acridine orange. We can infer that the triplet microtubules of the centriole are protein (perhaps ribonucleoprotein), since this is the case for the microtubules of cilia, which are direct extensions of microtubules A and B of the basal body [11, 12].

We can now present indirect evidence concerning the chemical composition of a specific centriole component, the dense footlike appendage on the inner surface of the triplet base. Digestion of the glutaraldehyde-fixed cells with ribonuclease[1] before the osmium postfixing step resulted in the disappearance of this structure. This is demonstrated in Fig. 14, which shows a series of 5 consecutive sections through one end of a centriole in a ribonuclease-treated preparation. Ribosomes are now missing in the cytoplasm of this cell, so we can reasonably be sure that the enzyme was effective. The rotation photographs of the 3 centriole sections in Fig. 14 are presented in Fig. 15. Microtubules, fibrous appendages, and the octagonal end structure in the end section are all present. However, the dense foot normally attached to the triplet base and microtubule A is not visible. Fig. 8 demonstrates that the foot should be present at this end of the centriole, even though the triplet base is reduced in size. The presence or absence of the spiral structure after ribonuclease digestion is difficult to determine, but we believe it is still present in Fig. 14e as an irregular filament in the lumen of the centriole, as diagrammed in Fig. 16. This filament seems still to be attached to one triplet base (arrow) where the footlike structure normally appears.

The analogous treatments with deoxyribonuclease have been inconclusive, and the chemical composition of all other centriole components remains unknown.

[1] Ribonuclease digestion: RNase (Worthington) (2 mg/ml) dissolved in 0.1 *M* phosphate buffer pH 5.0; 60 minutes at 37°C. The enzyme solution was heated to 100°C for 5 minutes prior to use to destroy any contaminating enzymes.

FIG. 13. Symmetry tests of the image shown in Fig. 5. The lack of 9-fold rotational symmetry in the central structure in the distal end of the centriole (Fig. 8) prompted a search for other possible symmetries of this component. In (a) the triplets and external fibers reinforce well when tested for 3-fold symmetry (see text), but the central structure does not. In (b) through (f) are presented test images for 4-, 5-, 6-, 7-, and 8-fold rotational symmetry, respectively. The best reinforcement is seen in (b) and (f), where a central image with 8-fold symmetry is seen in both cases. We have termed this the *octagonal end structure*.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REPLICATION OF THE CENTRIOLE

In favorable cases where centrioles could be seen and counted by light microscopy [23], the centriole population in most animal cells numbers 2 at the start of a cell reproductive cycle (immediately after mitosis). Sometime before the cell divides again, the centrioles must be duplicated, just like all other cell components. When and how does this occur?

In living Chinese hamster fibroblasts the centrioles are not visible because of the multitude of small granules and vesicles around them in the cytoplasm of these cells. However, there are dilute alcohol solutions designed for direct isolation of the mitotic apparatus [17] that stabilize cell organelles containing microtubules while allowing most other cellular components to dissolve. For reasons unknown, the addition of digitonin to cells fixed briefly in Kane's fixative [17] brings out centrioles and other microtubular organelles in sharp definition when viewed with a phase contrast microscope [3]. Examples are given in Fig. 17. Since Chinese hamster fibroblasts can be easily synchronized by selection of mitotic cells [38], the question of when centriole reproduction occurs can be answered, in part, by direct counting in synchronous cultures.

Figure 18 shows a series of photographs of daughter cells prepared as described above at various intervals after mitosis. The generation time for cells is about 12 hours. Two centrioles are present in each cell during the first 8 hours of the cell cycle; by 10 hours almost all of the cells have 4 visible centrioles. This experiment does not tell us anything about the early stages of centriole replication; we learn only about the growth of the procentrioles into structures large enough to resolve with a light microscope. We must rely on electron microscopy to provide the details of the early events.

Centrioles reproduce by a *generative* mechanism, i.e., each centriole is the maturation product of a centriole *germ*, termed the *procentriole*, somehow produced by a parent centriole. This mechanism is in contrast to the process of fission, where a parent structure divides into two equiv-

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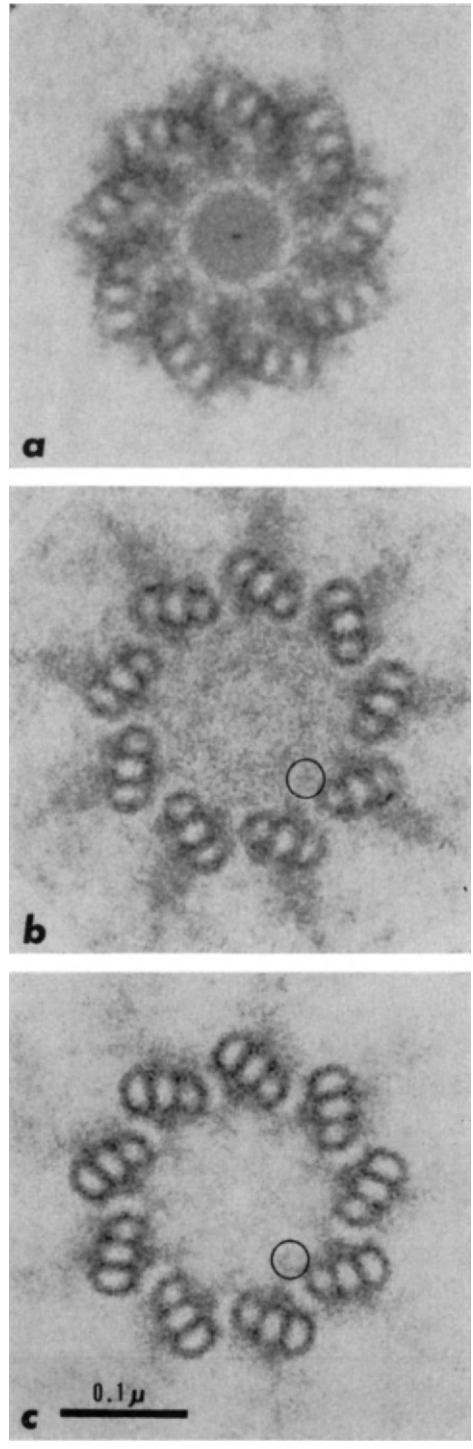
FIG. 14. Serial cross sections through the distal end of a centriole. After glutaraldehyde fixation the preparation was treated with RNase before osmium fixation and embedding. Ribosomes and nucleoli were effectively removed by this procedure. Most of the centriole structure was left undisturbed; however, the octagonal end structure in (c) is displaced to one side. There were other more subtle effects shown in Figs. 15 and 16. To the right of the centriole is seen a large bundle of microtubules in cross section; their spacing is indicated in (d). This bundle is probably directed toward the second unseen parent centriole nearby. The consecutive positions of a single microtubule in each section is indicated by arrows. The cell nucleus is at the extreme right.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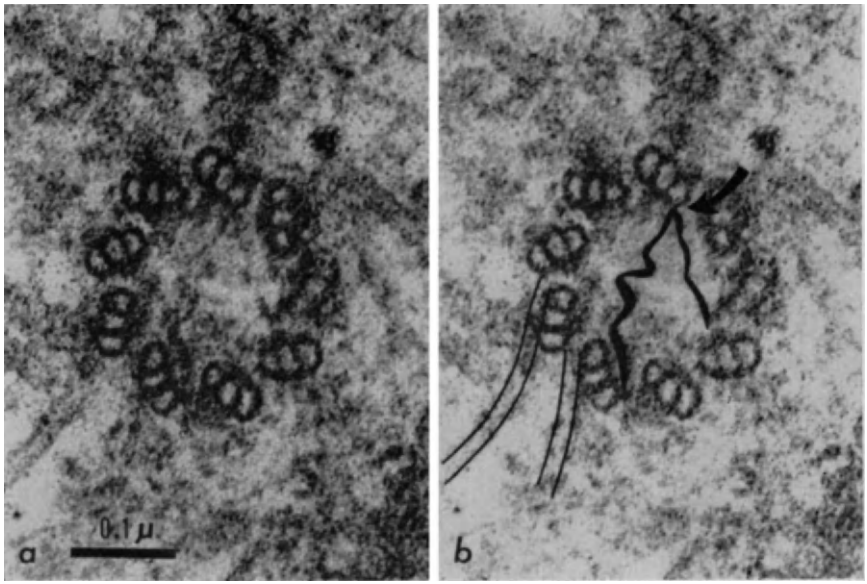


FIG. 16. (a) Enlargement of Fig. 14e. What is thought to represent a segment of the *internal helix* is seen in the lumen of the centriole, diagrammed in (b); at the point indicated by the arrow, the filament seems still attached where a "foot" structure usually appears.

alent daughter units [see Mazia, 23]. From our knowledge about the architecture of centrioles, considerable information can be inferred about the reproductive process.

The earliest event so far detected in centriole reproduction is the appearance of the procentriole near one end of the parent, as shown in Fig. 19. Viewed in its various aspects, the procentriole appears to be a very short cylinder of almost the parental diameter and possessing many of the cross-sectional features of the parent [9]. As it matures, the cylinder simply lengthens at right angles to the parent until it reaches full size. Figure 20a shows 2 parent centrioles in cross section, each with a maturing procentriole perpendicular to it. In the usual case each parent forms only one daughter, but potentially many procentioles can be produced simultaneously by a single parent. In the snail *Viviparus*, Gall [9] demonstrated that mulberry-like clusters of procentrioles around the parent are possible, each daughter roughly at right angles to the parent.

FIG. 15. High-resolution rotation photographs of the cross sections of the centriole in Fig. 14. Note the absence of a dense "foot" near microtubule A (circle) in (b) and (c) (compare with Figs. 6–8).

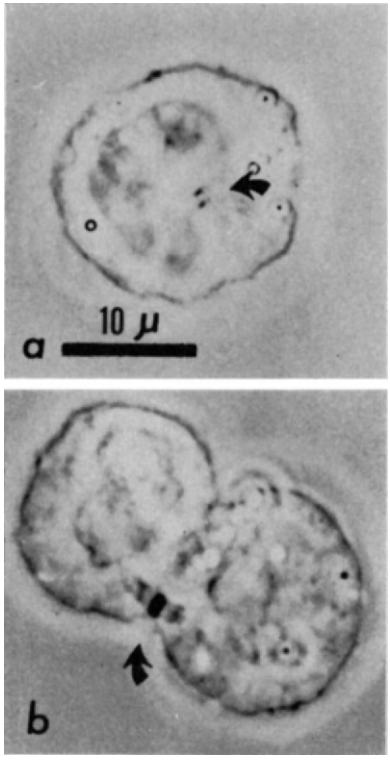


FIG. 17. Phase-contrast micrographs of centrioles (a) and Flemming body (b) (arrows) in cells treated with Kane's fixative and digitonin (see text).

Why does the procentriole develop perpendicular to the parent? A rather simple explanation is suggested by an understanding of the relationship of a mature centriole to the microtubules around it. As we demonstrated earlier, microtubules are attached to the triplet base and project approximately at right angles to the centriole wall. If the centriole actually functions as a producer of microtubules, which is almost certainly the case [23], then the microtubules projecting vertically around the centriole wall are the ones made by the centriole. If the procentriole relies on the parent as a source of microtubules for its outer wall, it will develop perpendicular to the parent, for its building blocks are already oriented that way. The observation that some of the microtubules of the developing procentriole actually terminate in the parent wall as in Fig. 20 (lower centriole pair) adds considerable support to this view.

What organizes the parent microtubules into the precise structure

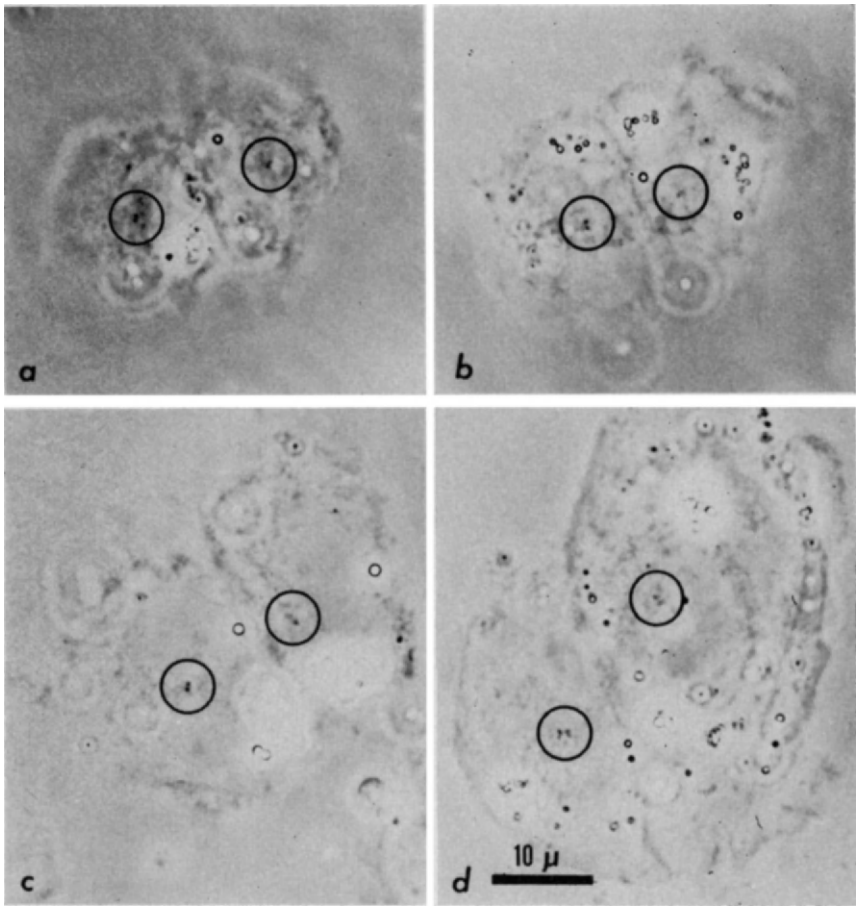


FIG. 18. Centriole replication in synchronized Chinese hamster fibroblasts (prepared as in Fig. 17). In each case the centrioles in the cell on the left are in focus; the other cell centrioles are somewhat out of focus. In (a) through (d) the cells were 3, 5, 8, and 10.5 hours old, respectively. The average generation time for these cells is 12 hours. Daughter centrioles become resolvable in the light microscope after 8 hours, although earlier replication stages are seen as early as 6 hours in electron micrographs (Fig. 19).

of 9-fold symmetry seen in the procentriole? Theoretically, the component best suited for this activity seems to be the cartwheel. It has several properties that are requisite for a procentriole organizer. (a) It has 9-fold symmetry and can thus serve as an appropriate attachment template for microtubules. (b) Whereas it seems to be a single structure in the centrioles of Chinese hamster fibroblasts, occurring only in one end section, it is clearly a multiple structure in basal bodies of

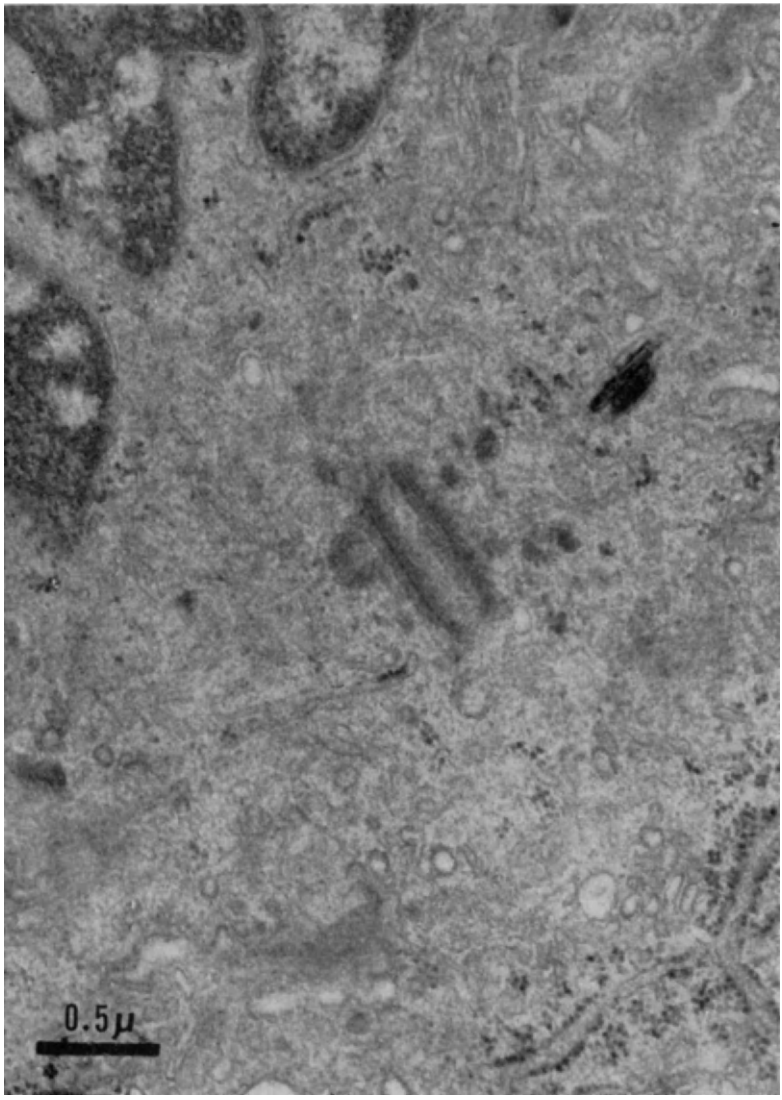
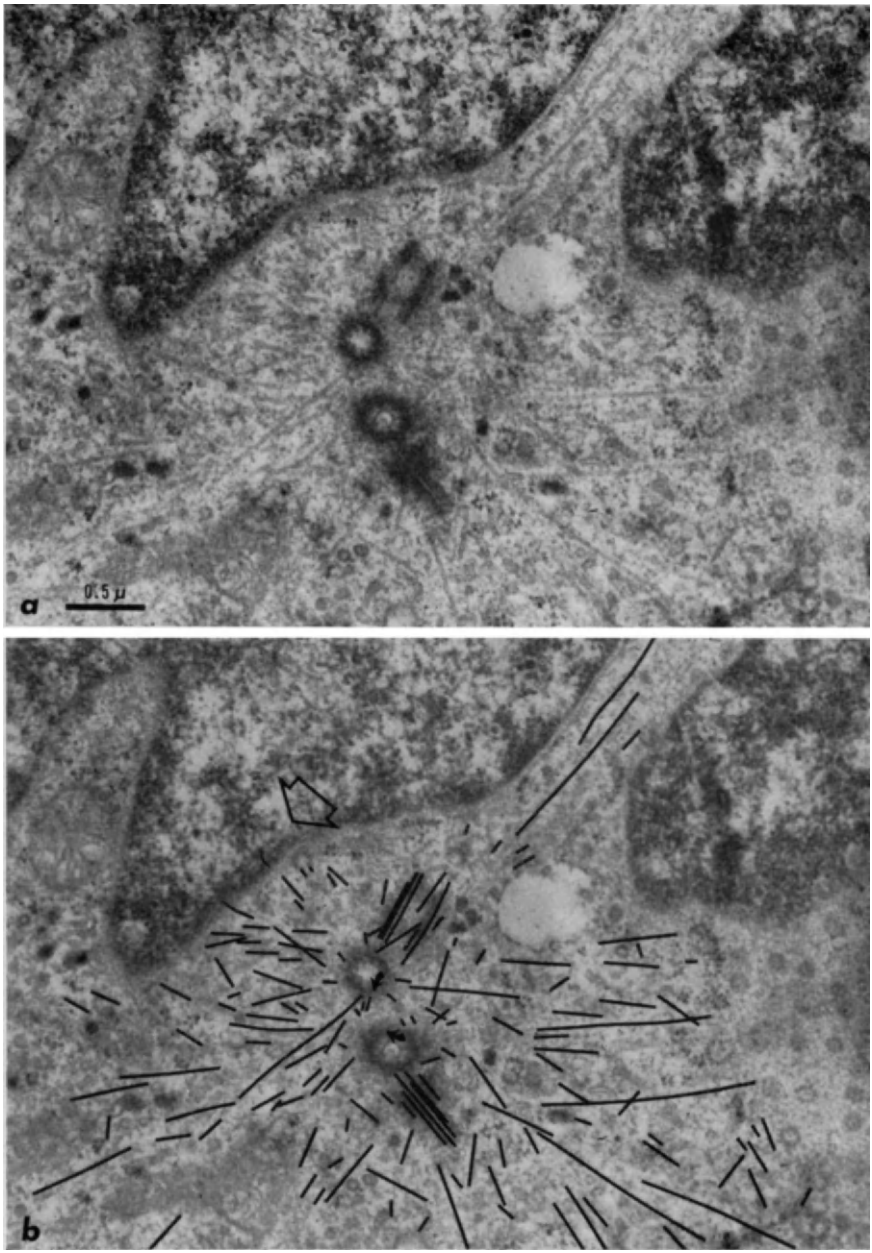


FIG. 19. Procentriole formation in a cell 6 hours old. The daughter forms at right angles to the parent as a short cylinder with most of the cross-sectional features of the parent.

FIG. 20. (a) Two parent centrioles (both in cross section) with immature daughter procentrioles sectioned longitudinally. The positions of all the microtubules in the photograph are emphasized in (b), and most of them are oriented toward the two parents, rather than the daughters. Note the apparent penetration of microtubules through the nuclear membrane (large arrow) and the numerous microtubules directed toward the field of nuclear pores at the extreme right. A fine fibril



connecting the two parent centrioles is indicated by the small arrows in (b). In the lower parent centriole, the oblique section has included a large part of a turn of the *internal helix*. Also note that two of the microtubules in the wall of the lower procentriole are still connected to the wall of the parent. See Fig. 12 for a later stage of procentriole maturation.

ciliates [12] occurring repeatedly throughout much of the length of the centriole lumen. This fact suggests that the cartwheel may be able to reproduce itself. (c) It is present in procentrioles [9].

Of course, the details of centriole reproduction are largely beyond our current understanding. To suggest that the cartwheel is the procentriole organizer only converts the question, "How do centrioles reproduce?" into "How do cartwheels reproduce?" However, in the cartwheel we at least have a structure of molecular dimensions where replication is theoretically simpler, if technically more difficult to study.

CILIOGENESIS

Recently we reported that Chinese hamster fibroblasts treated briefly during interphase with Colcemid were stimulated to generate cilia [37]. The details of ciliogenesis were already known from studies in other species [30, 32, 36], but the opportunity to study the process *in vitro* at timed intervals after stimulation was informative.

The first visible step in ciliogenesis is the approach of a small cytoplasmic vesicle (possibly of Golgi origin) to one end of a centriole. The vesicle flattens and invaginates to form a double-membraned cap over the end of the centriole (Fig. 21a). Microtubules A and B of each triplet extend into the cap and the ciliary bud is formed (Fig. 21b). At this stage the mechanism generating the axial doublet of the cilium is also formed. Ciliary bud formation is somehow promoted in Chinese hamster fibroblasts by treatment during interphase with Colcemid [37]. The mechanism is unknown. However, the growth of the ciliary bud into a cilium does not occur so long as the Colcemid is present. One hour of Colcemid treatment is long enough to complete bud formation. If the cells are then transferred to medium lacking Colcemid, the shaft of the cilium rapidly develops. In the span of 2 hours cilia up to 15 μ long can be produced. Each cell is potentially able to make 2 cilia, but actually only about half of the cells make cilia, and many of these are short and immature. However, some cilia seem to be structurally complete (Fig. 22) and in a few instances have been seen to beat erratically.

Whether or not cells that form cilia are capable of further division is not known. We have never seen any cilia in dividing Chinese hamster fibroblasts [37]. The experiment shown in Table II indicates that ciliogenesis occurs only during the latter half of the cell cycle, after centriole duplication has occurred. Since only the functioning parent centrioles participate in ciliogenesis [30, 37], the cells may retain the daughter centrioles for mitotic activity at a later time. The fate of either the cilia or the cells producing them has not yet been examined.

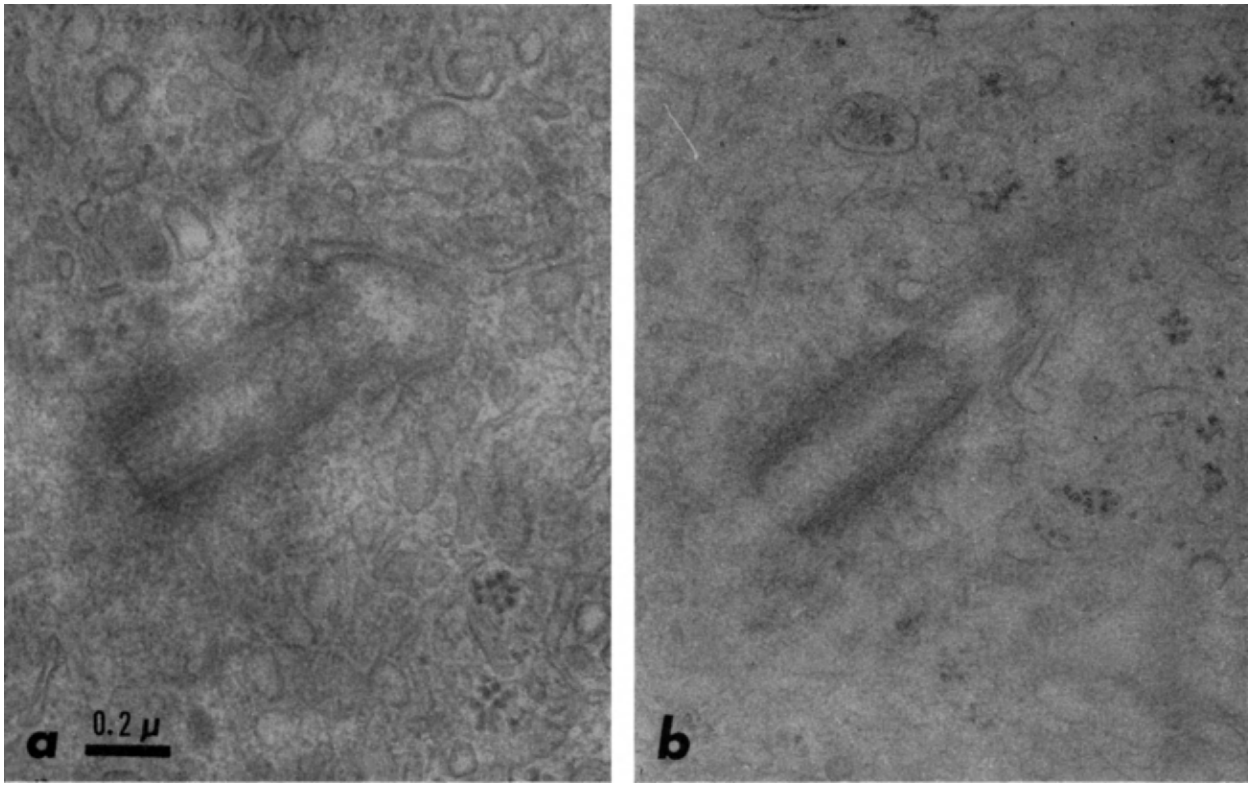


FIG. 21. Early stages of ciliogenesis in Chinese hamster centrioles. In (a) a vesicle has flattened across the distal end of the centriole to form a double membrane cap. In (b) centriole microtubules extend into the cap to complete the formation of a ciliary bud. Ciliary buds are formed in the presence of the drug Colcemid during the latter half of the cell reproductive cycle. After bud formation is complete, removal of the drug allows the cilium to develop as in Fig. 22.

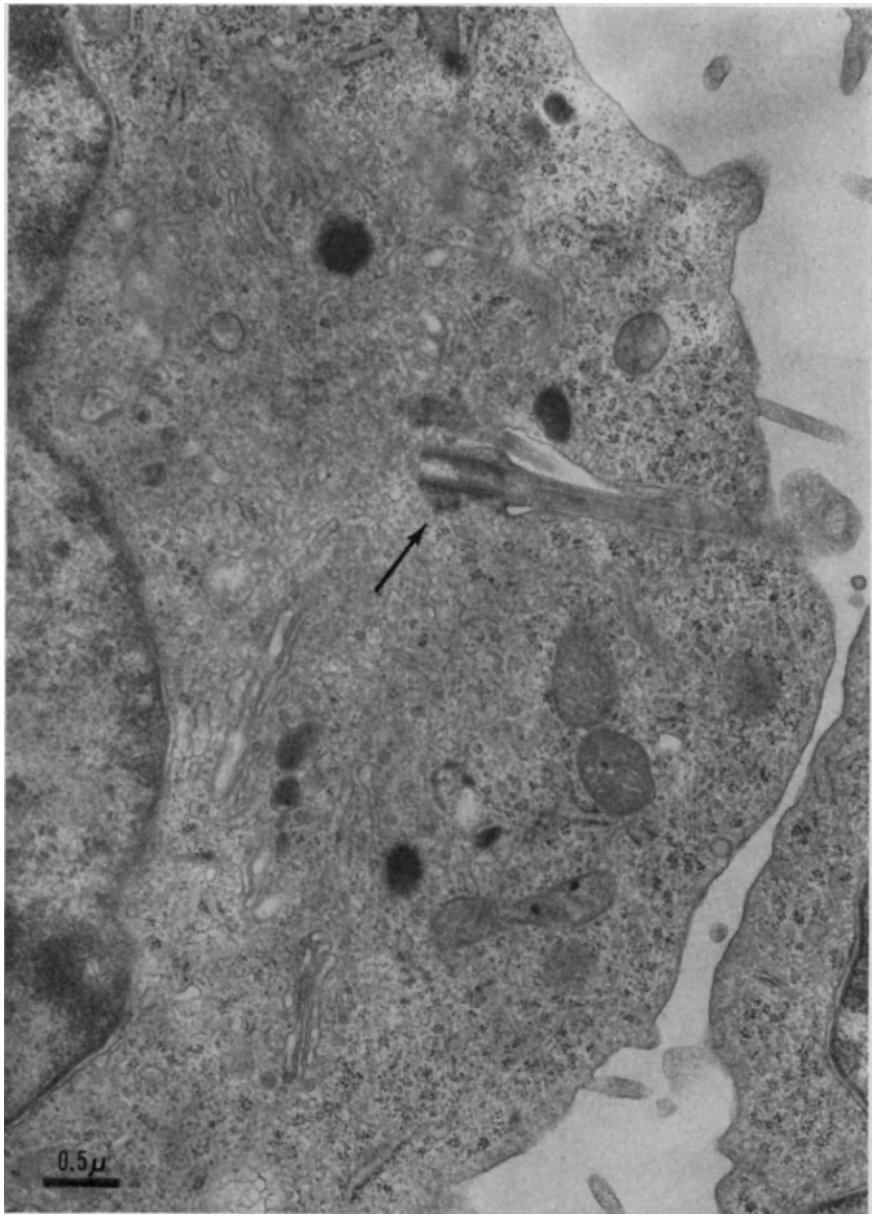


FIG. 22. Complete cilium formed in a Chinese hamster fibroblast by treatment with Colcemid (0.06 μg/ml) for 2 hours and further incubation for 1 hour in the absence of the drug. A second cilium is also present (obliquely sectioned). Arrow indicates the basal foot. Note the extensive development of the Golgi system in this cell.

TABLE II. *Colcemid Induction of Ciliogenesis in Synchronized Chinese Hamster Cells at Different Times in the Cell Cycle*[a]

Culture No.	Colcemid treatment for synchronization (hours)	Age of synchronized culture at beginning of 2nd Colcemid treatment for 1 hour (hours)	Post-Colcemid incubation (hours)	Percent of cells containing cilia
1	None	—	—	0
2	3	(Control)	(0 to 7)	16
3	3	4	5 to 6	10
4	3	6	7 to 8	11
5	3	7	8 to 9	46
6	3	8	9 to 10	35

[a] Colcemid-treated metaphase cells were incubated in medium lacking Colcemid to initiate the synchronous cultures [38]. At various times after mitosis the cells were again treated with Colcemid (0.06 μg/ml) for 1 hour to establish ciliary buds. This was followed by an additional hour of incubation in medium lacking Colcemid to allow the cilia to grow (post-Colcemid incubation). The cells (attached to coverslips) were then fixed and "stained" with digitonin as in Fig. 17 to reveal cilia, an example of which is shown in Fig. 23. Contaminating interphase cells in the original mitotic cell population produce cilia in response to the first Colcemid treatment (Culture No. 2), whereas untreated exponential cultures make no cilia (Culture No. 1).

Which end of the centriole is involved in ciliogenesis? By definition Gibbons and Grimstone [12] and Gall [9] termed the end attached to the cilium the *distal* end of the centriole and the opposite end the *proximal* end. Gibbons and Grimstone [12] also established that in ciliates the cartwheels are situated in the proximal end of the basal body. Although the centriole has radial symmetry, it does not possess bilateral symmetry. It necessarily follows that there are two possible centriole enantiomorphs, i.e., right- and left-handed forms. Ciliate basal bodies all appear to be of one type; possibly all species have centrioles of the same enantiomorph. When viewed along the major axis with the distal end (bearing the cilium) away from the viewer, the triplet planes slant away to the left at the top (counterclockwise), as in Figs. 2–10. Gall [9] cites an unpublished study indicating that parent and daughter centrioles are oriented with their proximal ends together in Lepidoptera, the distal ends later producing flagella without the pair first separating. We also showed that in the Chinese hamster centriole ciliogenesis and procentriole formation occur at opposite ends [37].

THE CENTRIOLE IN MITOSIS

The role of the centriole in mitosis was reviewed extensively by Mazia [23]. More recent results have in general supported some older

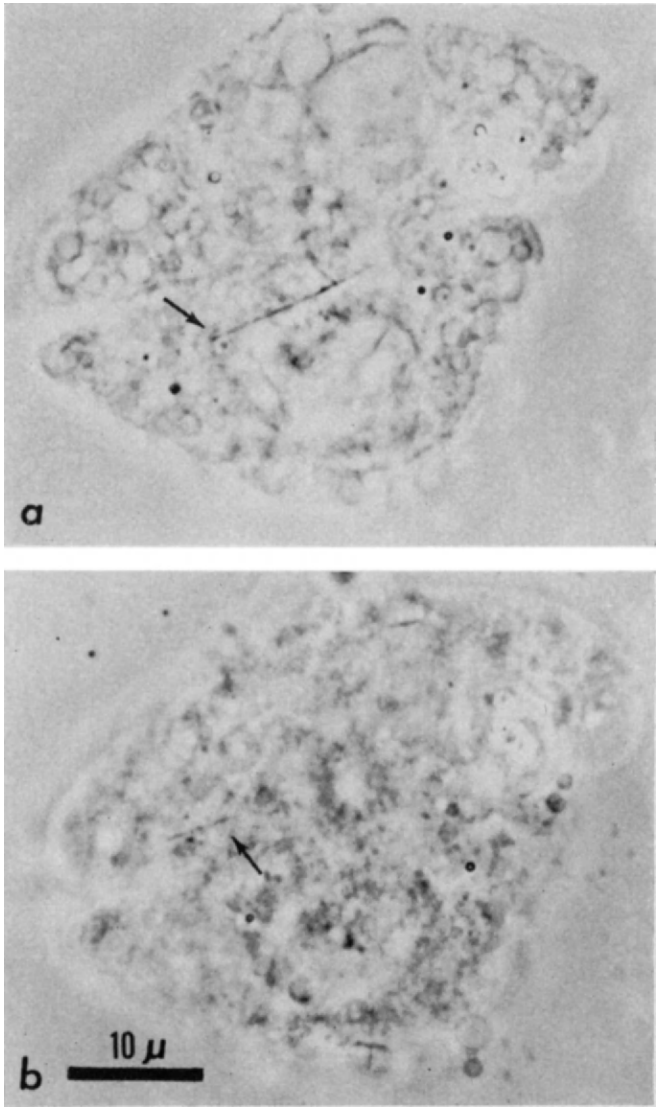


FIG. 23. Phase-contrast micrographs of cilia formed in synchronized Chinese hamster fibroblasts (treated as in Fig. 17). Each daughter contains a single cilium (arrows). Cells were 7 hours old when treated with Colcemid for 1 hour to initiate ciliary bud formation, and the culture was incubated an additional hour in the absence of the drug to allow the cilia to develop.

views of centriole activity in mitosis, and a simple updating of the information given by Mazia is all that is necessary in this section.

The mechanism which is responsible for the partitioning of daughter chromosomes into 2 daughter cells is called the mitotic apparatus. In animal cells it consists of 3 main components: the chromosomes, the centrioles, and a superstructure made of microtubules traditionally referred to as the spindle. Some higher plants, however, seem to lack centrioles entirely [23], so we may tentatively conclude that the centrioles are not an essential element of mitosis in certain cases. As a generalization, with certain special exceptions [33], we may state that centrioles are probably essential in cells which do not have a rigid wall.

Our recent ultrastructure studies of the effects of Colcemid on the mitotic process in Chinese hamster fibroblasts [2, 3] have shed some light on the role of centrioles in mitosis. Cells entering mitosis in the presence of Colcemid seem normal in all respects with one exception. Whereas normally the 2 centriole pairs separate to establish the poles of the mitotic spindle, in the presence of Colcemid the centrioles do not separate. Instead, the 4 remain clustered in the center of the cell, and chromosomes distribute peripherally around them. Careful examination revealed the presence of microtubules in the cytoplasm, but most of them were attached to chromosomal kinetochores, while near the centrioles most microtubules had disappeared. Furthermore, in each chromosome microtubules were attached to, and presumably made by, only one of the two daughter kinetochores. The one with microtubules was always oriented toward the cell center where the centrioles were.

Upon removal of Colcemid, the cells were able to divide normally in about 30 minutes. Within 5 minutes the centriole pairs began to separate, with an abundance of microtubules appearing all around them. Then the chromosomes began to move to the equator of the spindle between the centrioles, and microtubule attachment to both kinetochores on each chromosome became apparent. Within 20 minutes after removal of Colcemid most cells had established a metaphase configuration that was normal in all respects, so far as we were able to discern.

The structural defects observed in the Colcemid-blocked mitosis can be explained as the result of the effect of the drug on a single process, the formation of microtubules by the centriole. That Colcemid does not destroy all microtubules in the spindle, at least at the dosage which we employed, is seen in the fact that those microtubules attached to chromosomal kinetochores persisted. The studies of Inoué and coworkers [8, 16] very strongly suggest that the spindle is a structure in dynamic equilibrium, with simultaneous synthesis and degradation of its component microtubules. In plants, where mitotic spindles are produced in the absence of centrioles, there is good reason to believe that

the microtubules are somehow organized by the kinetochore region of each chromosome; at least they are attached there. The most coherent picture emerging from these observations is consistent with the assumption that the microtubules of the mitotic apparatus are made in two places in animal cells, i.e., at the ends where they attach either in the kinetochore or to the centriole. Whether the "making" of microtubules means synthesis from amino acids or assembly of preexisting proteins is not clear, but the latter seems more probable [23]. If microtubules are in equilibrium, they are probably being disorganized at their free end.

In the presence of Colcemid, if one kinetochore of each chromosome is able to make microtubules, why is its sister unable to do so? The sequential activation of kinetochores may be a phenomenon of normal mitosis (see discussion by Mazia [23]). The rationale is that if the parent (older sister) kinetochore orients toward one pole first, the daughter (younger sister) kinetochore will be "aimed" at the opposite pole when it become active. The impression gained from Fig. 20 is that there are microtubules penetrating the nuclear membrane (large arrow) during late interphase which would perhaps substantiate the idea of a prior orientation of one kinetochore (the older sister) to the centrioles.

It seems equally apparent, however, that both active parent centrioles in Fig. 20 have such connecting microtubules, and since the two parents normally separate at prophase, each accompanied by its immature daughter, we must discount the notion that the microtubule associations in interphase are simply those left over from the previous mitosis. Figure 24, which depicts an anaphase spindle at one pole, clearly demonstrates orientation of the microtubules toward only one member of the centriole pair at the pole. Figures 1 and 11, which are longitudinal sections of centrioles in mitosis, prove that microtubules are oriented along the full length of the centriole cylinder, so we cannot suppose that the second member of the centriole pair in Fig. 23 is simply sectioned at the wrong level to reveal the microtubules around it. That only one centriole at each pole is active was also concluded by Murray *et al.* [27]. The inactive daughter centriole matures early in the following interphase to become the second parent (as in Fig. 20), and subsequently it may also secure connection to some kinetochore sites.

The clearest demonstration that centrioles make part of the microtubules of the mitotic apparatus is seen in the phenomenon of centriole separation during prophase [23]. That this phenomenon requires microtubule formation has been inferred from observations using phase contrast microscopy [4]. Our own observation of the appearance of microtubules around the separating centriole pairs following removal of Colcemid inhibition [2, 3] also strengthens this conclusion.

How is it that the production of microtubules causes centrioles to

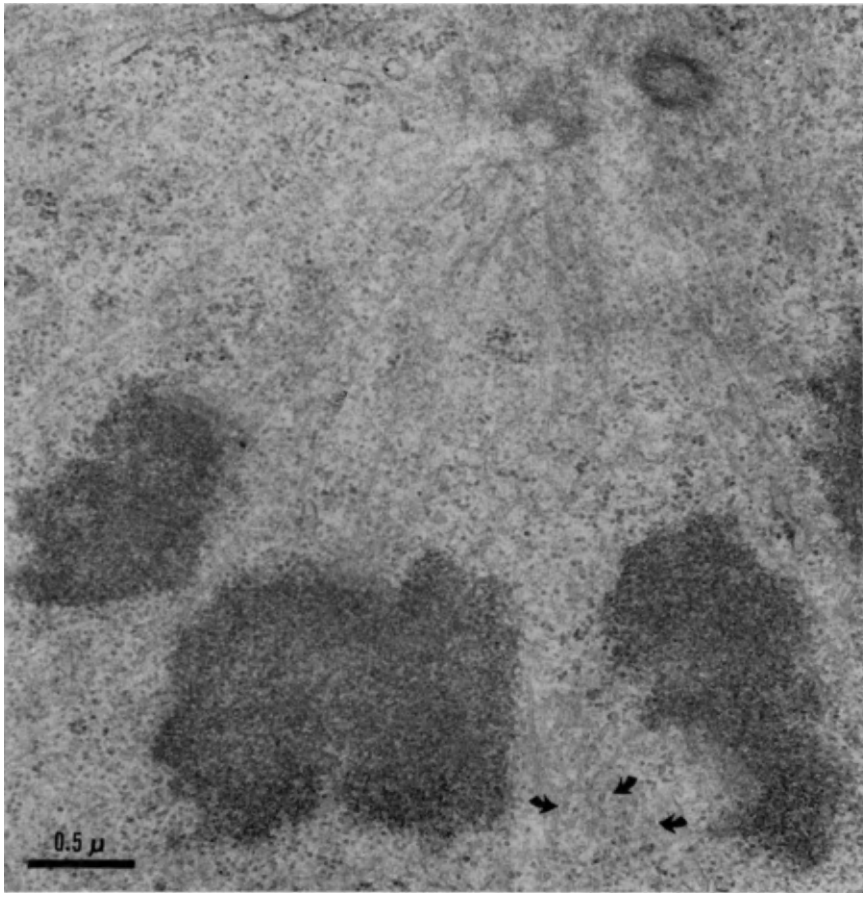


FIG. 24. Anaphase in a Chinese hamster fibroblast. The microtubules of the spindle are seen to converge on one centriole which was just grazed in this section; the second centriole, obliquely sectioned, is the immature daughter procentriole. The spindle microtubules are gathered into bundles; those reaching from pole to pole appear to be doublets in cross section (arrows).

separate? Individual microtubules appear to be relatively stiff structures, but they can be bent over short distances. In the mitotic spindle they occur most frequently in *bundles*, rather than single units, and such aggregate structures are probably much more rigid. The bundles are large enough to be seen with light microscopy as the spindle *fibers*. In cross section, spindle fibers are seen to contain about 10 microtubules (Fig. 25). Such bundles of microtubules are also seen in interphase (Figs. 14 and 20) in the region of the centrioles. If we think of the bundle of microtubules as the active unit of the spindle mechanism,

then it is possible to formulate some tentative explanations for centriole and chromosome movement during mitosis.

The reason why microtubules associate into bundles can readily be ascribed to their molecular structure. They are likely to be highly polarized structures, because of their great length and their attachment at one end to relatively massive structures, the centriole or the chromosome. Microtubules growing from one centriole would be expected to diverge in many directions, since the like charges at their free ends would repel each other. One such microtubule, upon encountering a microtubule from another centriole, would readily align with it if the two could align with opposite polarity. Charge forces would immediately draw the free ends of each microtubule toward the opposing centriole. In like fashion other microtubule pairs would align and join together to form bundles of microtubules connecting the two centrioles. As each centriole lengthens the microtubules attached to it, the two will effectively propel themselves to the opposite poles.

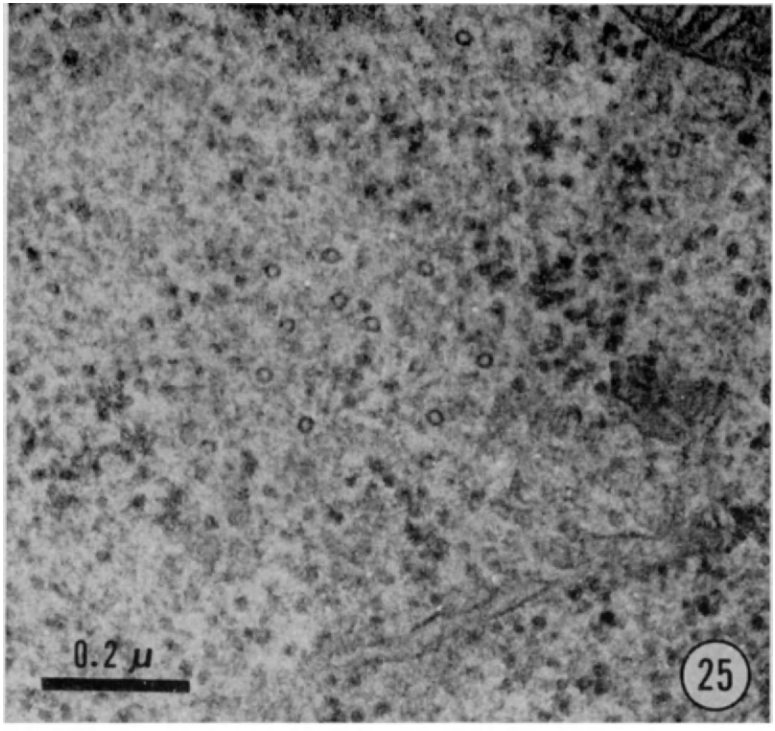
The microtubules of the mitotic spindle form two kinds of bundles [23], those which run from one parent centriole to the other (pole-to-pole fibers) and those which connect chromosomal kinetochores to the poles (chromosomal fibers). The former are not easily distinguished from the latter until anaphase, when the pole-to-pole fibers are seen at the spindle equator between the two sets of separating chromosomes. We may suppose that the same forces act to form bundles of microtubules between kinetochore and centriole as we have suggested for centriole-to-centriole fibers. From our earlier description we recall that the kinetochore microtubules appear to retain their association with the centrioles throughout interphase, although some kinetochores may shift their association to the newly active daughter centriole. Such permanent orientation to the centrioles should effectively prevent the mistake of forming kinetochore-to-kinetochore bundles. The additional feature of delayed activation of the "new" kinetochores, as discussed earlier, also will help to prevent misalignments.

Additionally, it may be supposed that the charge distribution is different in kinetochore and centriole microtubules. If the centriole microtubules are more highly polar than the kinetochore microtubules, so that one centriole microtubule could effectively align with several kinetochore microtubules, then centriole microtubules could perhaps disrupt any misaligned kinetochore-to-kinetochore bundles by competing more strongly for the kinetochore microtubules.

The idea of chromosomal fibers containing mostly kinetochore microtubules becomes even more probable when we take into account the number of microtubule attachment sites on the centriole. If microtubules attach to the triplet bases in a spiral pattern that follows the

large helix in the centriole lumen, and if the helix makes 10 turns in the total centriole length, then there are 90 attachment sites per centriole. Normally, there is only one active centriole at each pole, so we must construct the entire spindle structure using only 180 centriole-attached microtubules (90 at each pole) plus an undetermined number of kinetochore microtubules. If we allot half of the microtubules to make 9 pole-to-pole bundles of 10 microtubules each (5 from each pole in each bundle) then we are left with only 2 microtubules to form chromosomal bundles to each of the 22 kinetochores in the Chinese hamster cell. The problem is more acute in cells with higher chromosome numbers, where we must consider alternative ideas. The attachment sites on the centriole may bear no direct relationship to the internal helix, in which case there may be more sites. However, the upper limit is about 300 sites if the microtubules were packed tightly along the 9 triplet bases. On the other hand the centrioles may make microtubules on the triplet bases and then transfer them to an auxiliary attachment site, such as the pericentriolar satellite bodies, in order to make room for more microtubule production. The actual solution may be simpler than any of the foregoing. In species with many chromosomes, more microtubules may be provided by an earlier maturation of the daughter centriole, so that it also can function during mitosis. This would provide about 180 centriole-attached microtubules at each pole, which would be plenty for most species. In polyploid cells it might be anticipated that there is more than one centriole pair at each pole, since such cells frequently are multipolar. Such an assumption would account for the 500–600 pole-to-pole microtubules counted by Krishan and Buck [19] in L cells.

In connection with the foregoing speculation about microtubule bundle formation, it is interesting to note that there appear to be two types of bundles of microtubules in electron micrographs of dividing cells. These are shown in Figs. 25 and 26. In the one type (Fig. 25) all the microtubules are single, and there are usually 2 or 3 in the center of the bundle with 8 or 9 others peripherally distributed around them. In the second type (Fig. 26) some of the microtubules occur as *paired doublets*. Krishan and Buck [19] have already determined that the pole-to-pole fibers contain doublet microtubules, and this is in complete agreement with the concepts that we have presented here. The microtubules from one centriole, in forming pole-to-pole fibers, interact with the microtubules from a second centriole that have equal charge but opposite polarity (free ends point in opposite directions) to form doublets. The single fibers seen in such bundles may actually be double at a point nearer the spindle equator. In the case of the bundles running from the kinetochore to the poles, the microtubules of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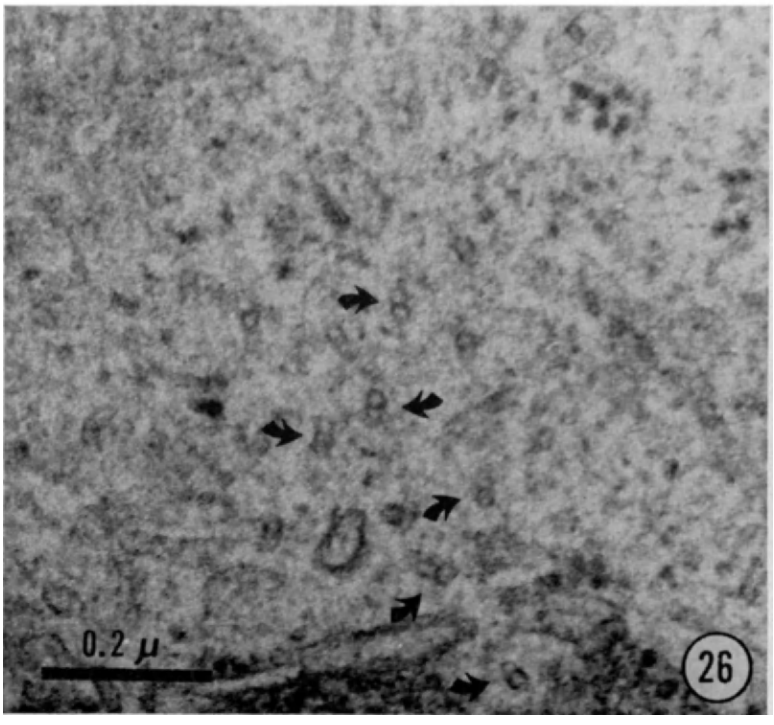
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opposite polarity do not form doublets, but instead, a distinct space is maintained between them. Such an arrangement would possibly allow the kinetochore microtubules to slide along the centriole microtubules, which is important to our concept of an anaphase mechanism.

During anaphase the chromosomal fibers are shortened, and this can be accomplished by any process, probably enzymatic, which degrades the free ends of the kinetochore microtubules in the vicinity of the centrioles. This is another possible function of the pericentriolar satellites. Actually the chromosomal microtubules are probably in an equilibrium state all the time, with simultaneous synthesis at the kinetochore and breakdown at the centrioles [8]. Anaphase could be initiated by either accelerating degradation or decreasing the rate of synthesis. If there are only one or two centriole microtubules in the chromosomal fibers, then we need not specify their equilibrium state. At anaphase the free ends of the centriole microtubules could pass directly through the metaphase chromosome with no ill effects, and such micrographs are occasionally seen (see Robbins and Gonatas [31], their Fig. 16). If the centriole microtubules are more polar, we might postulate that their free ends do not degrade as rapidly as those of the kinetochore microtubules. The proposed model would then account for the simultaneous decrease in chromosomal fiber length at anaphase, while the pole-to-pole fibers actually lengthen, if we speculate that production of centriole microtubules increases in anaphase, perhaps as a response to increased availability of precursors arising in the degradation of kinetochore microtubules.

The finding that plant cell walls have microtubules embedded in them [20] allows us to generalize our picture of a mitotic mechanism to include plant cells. All that is required is to specify that the end walls of a plant cell are able to produce microtubules, and the need for a centriole in plant cell mitosis is eliminated. The interaction of kinetochore microtubules with microtubules anchored in the end walls to make bundles of microtubules and the digestion of the free ends of the less polar kinetochore microtubules will account for metaphase alignment and anaphase movement analogous to the situation in animal cells. Although there are exceptions [33] mentioned earlier in this section, it appears that cells without rigid walls must manufacture a temporary scaffolding of some sort to anchor the poles toward which

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FIG. 25. Cross section of a bundle of microtubules probably connecting a kinetochore region of a chromosome with a centriole at one pole of a spindle.

FIG. 26. Bundle of pole-to-pole microtubules in cross section. Note that many appear to be doublets (arrows).

the chromosomes move in mitosis. This seems to be the major function of the centriole in mitosis.

DISCUSSION

Centrioles appear to be capable of activities associated with 3 major cellular events: mitosis, ciliogenesis, and centriole self-replication. Although each of these processes is distinctly different, all have certain aspects in common. We will attempt a correlation of what we know about centriole architecture with a hypothetical description of centriole activity in a molecular sense.

Basically, the centriole appears to be a specialized machine for microtubule production, since it produces microtubules for the mitotic apparatus, for the outer wall of the daughter centriole, and for the shaft of the cilium (or flagellum). However, the microtubules are made by two distinctly different processes. The microtubules of the mitotic apparatus are produced at right angles to the long axis of the centriole; these are attached to the structure we have termed the *triplet base.* On the other hand, the microtubules in the wall of the cilium are direct extensions of microtubules A and B in the centriole triplet [12], and are therefore produced parallel to the long axis of the centriole. A third type of microtubule, the central pair of the cilium, appear to arise from the basal plate and also extend parallel to the centriole long axis. Thus, although the microtubules all look alike, and may even be made of identical precursors, there are obviously several separate manufacturing processes, insofar as site on the centriole and direction of synthesis are concerned. In addition we can consider the microtubules which arise in the kinetochore and the plant cell wall as further examples of similar microtubles made in other sites and directions. Of these, perhaps the simplest in structure is the chromosomal kinetochore. Although we do not yet know the chemical makeup of this structure, we can make some reasonable conjectures about how it may function in microtubule production.

The kinetochore seems to be an integral part of the chromosome, and it has the same general "lampbrush" structure of the meiotic chromosome [2]. Thus it is composed of two major components, a pair of cohelical axial filaments and many fine filamentous loops extending vertically from the axial filaments to form the "brush." There are two such structures running parallel through the centromere region of each chromatid. Although the chemistry of the kinetochore is unknown, we may reasonably suppose that it is similar to the synaptinemal complex in meiosis, where some tentative conclusions have been drawn about the chemistry by Moses and Coleman [25]. In short, it is probably safe

to say that the kinetochore is composed of nucleoproteins, but it is not yet known which component contains DNA or RNA.

The very nature of the kinetochore suggests that it is an open, active segment of the metaphase chromosome, while the remainder of the chromosome appears to be compacted into an inert mass (except for nucleolus organizer sites, see Hsu *et al.* [15]). If the kinetochore is doing anything in mitosis, the most logical assumption is that it is making RNA. What has RNA synthesis to do with the formation of microtubules?

Chemical analysis of the mitotic apparatus from sea urchin eggs have consistently indicated that about 5% of the structure is RNA, and this suggests that the microtubules may actually be made of ribonucleoproteins [23, 39]. It is perhaps only a coincidence that there is a well-known case of a ribonucleoprotein structure that is a hollow microtubule of about the same diameter as spindle microtubules, and tobacco mosaic virus (TMV) also contains 5% RNA. The association of the RNA and protein in TMV is now well documented [18], with the protein subunits fitted in between the turns of the long RNA helix. The TMV structure is apparently self-assembled in the tobacco cell cytoplasm, and the RNA appears to determine the length of the TMV cylinder [1].

If the kinetochore produces a specific RNA capable of organizing microtubule subunits in a fashion analogous to TMV assembly, then we would expect the same process to occur at other sites of microtubule production, particularly in the centriole.

That the centriole contains RNA seems relatively certain; it probably also contains DNA. If there is DNA in the centriole, then we would anticipate its presence in the large helix in the centriole lumen. The analogous steps could then be postulated for microtubule formation in the centriole as in the kinetochore; i.e., the DNA helix would deposit RNA on the inner surface of the triplet base, which would then organize the microtubules extending vertically from the centriole wall.

The formation of the cilium microtubules is more difficult to imagine. Here we must consider the extension of existing microtubules A and B in the centriole wall, and a tentative explanation will require that we return briefly to the problem of daughter centriole formation.

The formation of *triplets* in the centriole wall can be understood on the same basis as the association of the pole-to-pole microtubules of the mitotic spindle into *doublets*. We recall that doublet formation would require only the contact of two microtubules aligned with *opposite* polarity; the doublets then form spontaneously. In the procentriole we would then logically expect triplet formation to proceed by several similar steps: (1) A microtubule of the parent (A) is first fused to a second microtubule (B) of opposite polarity (and thus from a separate

source, such as the kinetochore). (2) The end of the doublet would then be disconnected distally, but left attached to the parent centriole to reestablish overall polarity of the doublet. (3) The third microtubule (C), of "foreign" origin, would be added and stabilized in position to form the triplet. (4) The A and C microtubules would then be disconnected at each end to complete the formation of the short triplet of the procentriole. The fact that microtubule A appears slightly smaller than B and C ([12] and our Figs. 6–8) also supports its different origin and polarity.

The walls of the procentriole appear to lengthen distally as the procentriole matures [9]. We are thus faced with the problem of explaining how the triplet lengthens and how it stops growing when the centriole attains the proper length.

A related question, which must be considered first, is whether a short microtubule segment is structurally alike at either end. Since it contains protein subunits, which are asymmetric, we might anticipate that the ends are asymmetrical; however, if the basic building block is actually a symmetrical protein dimer, then the ends could be alike. If the microtubule has an RNA component, however, then the two ends of the nucleic acid would be different, unless the RNA were double-stranded, which is improbable.

Returning to the TMV analogy, we would postulate the splicing of RNA molecules of a specific length into the distal ends of the procentriole triplet microtubules and the subsequent self-extension of the microtubules until the length of RNA is used up. However, the distal end of microtubule A may differ from those of B and C, if the polarity is opposite. The analogous process, with much longer RNA strands spliced into microtubules A and B, would account for the formation of the doublets of the cilium. In such a process, however, the growth point of the microtubules would always be at their distal tips; subunits would have to be shipped out to the end of the cilium before they became incorporated into the microtubule. We thus have reasons for specifying two different types of microtubule formation: in one case (spindle microtubules) the microtubule moves away from its fixed point of synthesis, whereas in the other case (cilium doublets) the point of synthesis moves away from the site of primary attachment on the centriole (basal body). The central pair of microtubules in the cilium may be of the former type, i.e., synthesized at the basal plate.

There are several features about the architecture of the centriole as a whole that suggest that it contains a rotation mechanism. Upon building a centriole model, we discovered that a few assumptions about the kinetic relationships between the parts turned the model into a very clever machine. The model is shown in Fig. 27 and is constructed

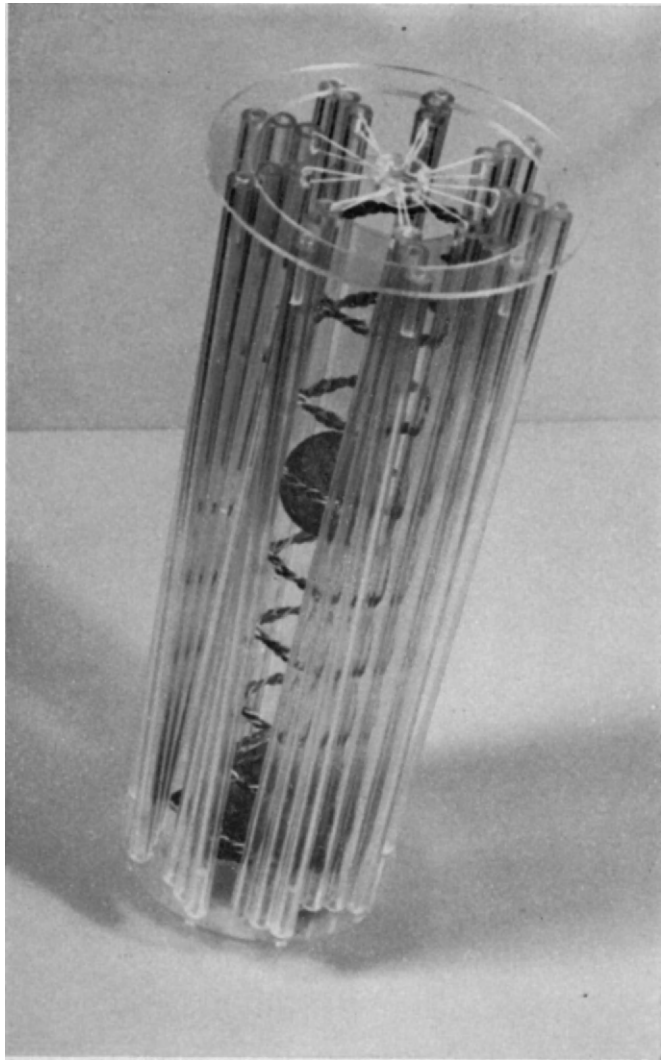

FIG. 27. Centriole model constructed to demonstrate possible kinetic relationship of its parts (for details see discussion in text).

with the triplets hinged along microtubule C. The cartwheel, in the proximal end, attaches to microtubule A and the triplet base of each triplet. The octagonal end structure in the distal end is attached to the spiral in the central lumen of the centriole. Assuming the cartwheel to be a contractile structure that pulls the triplets inward, one after the other in a counterclockwise sequence (viewed from the proximal end),

the octagonal end structure becomes a ratchet that is driven clockwise and can thus be used to turn the central helix. If the helix were DNA, then this would be a very ingenious mechanism for moving the information source (template) past the fixed attachment sites of microtubule formation in the triplet bases.

The fact that the cartwheel is a multiple structure in basal bodies suggests that some such motion in the triplet blades may be related to ciliary beating; the added mass of the cilium would require the extra cartwheels to twist the doublets. We are currently studying this possibility.

Admittedly, much of what we have presented in this report is speculative and rather abstract. However tenuous the arguments may be, they have been presented with the aim of stimulating more investigation into the nature and function of a very fascinating, though complex, cell organelle, the centriole.

SUMMARY

Ultrastructure studies presented in this report have indicated that the centriole of the Chinese hamster is a complex structure made of a number of separate components:

1. The *outer wall* is a hollow cylinder composed of 27 microtubules separated into 9 groups of 3, with the members of each triplet fused together to form a blade. The blades form an angle with the cylinder surface that varies continuously from one end of the centriole to the other in some centrioles, while no such twist is found in other cases. The twisting of the triplet blades may be characteristic of certain centriole functions.

2. A small spherical vesicle, termed the *central vesicle*, is found in the centriole lumen.

3. A filamentous structure spirals just inside the outer wall of the centriole; this *internal helix* makes about 8–10 turns through the entire length of the cylinder.

4. Each triplet blade rests on a diffuse *triplet base*, located inside the centriole wall and running parallel to the microtubules of the wall. The triplet base appears to be the site of assembly and attachment of *spindle* microtubules oriented perpendicular to the long axis of the centriole.

5. In one end of the mammalian centriole a *cartwheel* structure is found. Radial spokes connect the triplets of the centriole wall with a small central circle. The cartwheel is a multiple structure in basal bodies. We propose that it is a contractile structure capable of twisting the triplet blades in the centriole wall.

6. *External fibrous appendages*, projecting vertically from each triplet blade, are found near one end of both centrioles and basal bodies; in the latter case they are termed "transition fibers."

7. In the end opposite the cartwheel is a flat stellate structure with 8-fold symmetry, which we have called the *octagonal end structure*. It may be homologous to the basal plate of basal bodies.

8. *Pericentriolar satellites*, dense masses of irregular composition, are frequently found near the centriole; their function remains unknown.

9. A *procentriole* is frequently seen near a mature centriole. It possesses most, if not all, of the cross-sectional features of the mature centriole, but it is much shorter. It matures into a centriole by lengthening at right angles to the parent centriole to which it is attached.

These components, along with certain specialized structures found in basal bodies, such as the *basal foot* and *rootlets*, are the known parts of the centriole.

Chemically, the microtubules of the centriole wall are probably ribonucleoprotein, and RNA is also present on the inner surface of the triplet base. The chemical composition of the other centriole components remains undetermined.

The probable functions of the centriole in mitosis, ciliogenesis, and procentriole formation are discussed in detail, and a hypothetical model for microtubule formation is presented.

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