THE FORMATION OF BASAL BODIES (CENTRIOLES) IN THE RHESUS MONKEY OVIDUCT

RICHARD G. W. ANDERSON and ROBERT M. BRENNER

From the Oregon Regional Primate Research Center, Beaverton, Oregon 97005

ABSTRACT

Basal body replication during estrogen-driven ciliogenesis in the rhesus monkey (*Macaca mulatta*) oviduct has been studied by stereomicroscopy, rotation photography, and serial section analysis. Two pathways for basal body production are described: acentriolar basal body formation (major pathway) where procentrioles are generated from a spherical aggregate of fibers; and centriolar basal body formation, where procentrioles are generated by the diplosomal centrioles. In both pathways, the first step in procentriole formation is the arrangement of a fibrous granule precursor into an annulus. A cartwheel structure, present within the lumen of the annulus, is composed of a central cylinder with a core, spoke components, and anchor filaments. Tubule formation consists of an initiation and a growth phase. The A tubule of each triplet set first forms within the wall material of the annulus in juxtaposition to a spoke of the cartwheel. After all nine A tubules are initiated, B and C tubules begin to form. The initiation of all three tubules occurs sequentially around the procentriole. Simultaneous with tubule initiation is a nonsequential growth of each tubule. The tubules lengthen and the procentriole is complete when it is about 200 mμ long. The procentriole increases in length and diameter during its maturation into a basal body. The addition of a basal foot, nine alar sheets, and a rootlet completes the maturation process. Fibrous granules are also closely associated with the formation of these basal body accessory structures.

INTRODUCTION

Ovariectomy in rhesus monkeys is followed by atrophy, deciliation, and loss of basal bodies in the ciliated cells of the oviductal epithelium. Estrogen treatment of spayed monkeys leads to complete restitution of these epithelial cells. During the estrogen-driven differentiation, a new group of basal bodies is synthesized. This experimental system permits convenient sampling of basal bodies at various stages of their development.

The complete formation of basal bodies in the oviduct of the rhesus monkey during ciliated cell differentiation takes about 4 days and is preceded by several well-defined morphological events. Two pathways of basal body genesis occur in each ciliogenic cell. In one, the basal bodies develop in contact with the diplosomal centriole; in the other they develop in contact with structures that bear no resemblance to either centrioles or basal bodies. We have named these two processes centriolar and acentriolar basal body formation, respectively. The major steps in the acentriolar pathway have been described by Brenner (11–13).

The centriolar pathway has been observed only in tracheal epithelia of the rat, where it plays a minor role in basal body formation (35). We now report on the presence of this pathway in the oviduct of the rhesus monkey. Approximately 5% of the basal bodies are produced by this mechanism.

This investigation of basal body formation in the monkey oviduct is oriented toward understanding

the gross morphologic relationships of the two pathways, as well as the structural details of the intermediate organelles and early procentriole stages. Serial sectioning, stereomicroscopy, and image-enhanced photography (rotation analysis) are the techniques employed. Several conclusions are drawn concerning the formation of the triplet tubules, the structure and function of the cartwheel, and the significance of two pathways that synthesize the same organelle.

MATERIALS AND METHODS

Rhesus monkeys, *Macaca mulatta* (Woodard Asiatic Corp.), weighing 4–8 kg each, were ovariectomized at least 6 wk before use to insure complete atrophy of the epithelium of the oviduct. The animals were then injected intramuscularly with estradiol benzoate in sesame oil (15 μg twice daily, morning and evening, for 3 days).

Biopsies of the fimbriated end of the oviduct were taken by laparotomy daily for 6 days and fixed for 30 min at room temperature in either cacodylate-buffered 0.75% glutaraldehyde or a cacodylate-buffered 0.75% glutaraldehyde–3% formaldehyde mixture (11, 12). Tissues were washed overnight in cacodylate buffer at 37°C, postfixed in cacodylate-buffered 1% OsO_4 for 2 hr at room temperature, and embedded in Araldite.

Sectioning was done with a Porter-Blum MT-2 ultramicrotome and a du Pont diamond knife (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.). Serial sections were prepared by the methods of Anderson and Brenner (4).

Electron micrographs were taken with a Philips 200. Stereophotographs were obtained by photographing the specimen after tilting the stage forward and backward 6°. The image-enhanced photographs were prepared by the methods of Markham et al. (24).

RESULTS

Centriolar Basal Body Formation

On the third day of estrogen stimulation, the epithelial cells of the oviduct are hypertrophied, with well-developed Golgi complex, rough endoplasmic reticulum, polysomes, and numerous mitochondria—evidence that the cells are actively synthesizing the molecules necessary for differentiation. Frequently, these cells have two centrioles (diplosomal pair) in the apical region, and the members of the pair are usually oriented at right angles to each other, although various other orientations have been seen. A rudimentary cilium (Figs. 1, 2) often develops from one of the centrioles.

The rudimentrary cilium is similar to the primary cilium described by Sorokin (35). Only one cilium per cell occurs in secretory cells as well as in future ciliated cells. These cilia appear on the third day of estrogen treatment, 3 days before the main cilia are formed. Rudimentary cilia are never seen after the fifth day of estrogen stimulation and probably do not become a part of the main complement of cilia.

The cell membrane is always modified in the region where the rudimentary cilium arises. Commonly the membrane is invaginated to form a cup from which the cilium emerges (Fig. 2). The cilia in these cups are short and wavy with an incomplete tubule system. The peripheral doublets are imperfect and a degenerate central pair is sometimes seen; the tips are often bulb-shaped. The irregular structure seems to be the result of abnormal tubule organization. In some cases, the rudimentary cilium extends from a finger-like projection of the cell surface (Fig. 1). These latter cilia are tall, very straight, and have a more complete tubule system. These more orderly rudimentary cilia, with their accompanying basal bodies, are similar to the main complement formed later, although their tips are more sharply pointed.

The centrioles associated with the well-formed rudimentary cilia are never involved in the production of procentrioles. However, the basal bodies of the imperfect rudimentary cilia usually have procentrioles associated with their walls. Since procentrioles form after the rudimentary cilia, the differences in cilia structure may reflect the age and, therefore, the state of maintenance of the cilium.

The structure of the centriole is similar to that of other mammalian cells (5, 6, 8, 16, 27, 33, 39). The basic geometric form is cylindrical and measures 500 mμ in length and 250 mμ in diameter (Fig. 1). The walls of the cylinder contain 27 tubules arranged into nine evenly spaced groups of three. The centriole does not have any accessory structures; however, when it acts as a basal body for a rudimentary cilium, sometimes one or two pyramidal basal feet project at right angles from the midregion of the cylinder and a rootlet occasionally extends from the proximal end deep into the cytoplasm. In addition, a filament radiates from each triplet set near the basal body–cilium junction. These nine filaments are analogous to the transitional fibers of *Pseudotrichonympha* basal bodies

(20), or the alar sheets found in the basal body of the monkey oviduct (2, 3).

Many of the centrioles, particularly those involved in procentriole formation, do not have normal morphology. Commonly seen modifications include a lengthening of the structure with a concomitant decrease in diameter, degeneration of the tubule system, and distortion of the basic cylindrical geometry. The significance of these structural changes is not understood.

Late in the third day of estrogen stimulation, procentrioles (probasal bodies) begin to form at right angles to the walls of both centrioles (Figs. 2, 3). Electron-opaque granules (fibrous granules) appear near the centrioles either shortly before or concurrently with the procentrioles (Figs. 2, 3). The number of procentrioles generated by each centriole varies from 1 to 10. The fact that all of the procentrioles associated with any one parent are in the same stage of development indicates that all are initiated at the same time and that the differences in the number of procentrioles per parent reflect differences in the ability of each centriole to initiate procentriole formation.

Centrioles engaged in procentriole production have a 40–50 mμ thick coat of flocculent material around their outer walls (Fig. 3). This material varies from amorphous to filamentous and is not uniformly thick around the centriole. The base of the forming procentriole is enmeshed in the outer region of this corona so that 30–40 mμ separate the parent from the daughter during the early stages of induction. Several filaments extend into the base of the procentriole from the corona, one of which is aligned with the longitudinal axis of the daughter structure (Fig. 3). These filaments are part of the cartwheel (20).

Generally the procentrioles form at right angles to the centriole and are distributed along the centriolar axis from midregion to base (Figs. 3, 4). Serial sections often show one set of three or four procentrioles encircling the midregion and another set around the basal region of the same centriole. Sometimes the procentrioles spiral up the centriole from base to midregion. A basal foot or an anomaly in the centriole structure distorts the normal perpendicular orientation of daughter to parent. Characteristically, these procentrioles point away from the centriole apex, an indication that they have been pushed toward the basal end of the parent. In extreme cases, procentrioles project from the basal opening of the centriole lumen.

The procentrioles rapidly develop into mature basal bodies by elongation and expansion, during which the characteristic daughter-to-parent orientation is maintained (Fig. 4). At maturity, the basal bodies break away from the parent and join the population of basal bodies made via the major pathway. They are indistinguishable from acentriolar-generated basal bodies.

Acentriolar Basal Body Formation: the Major Pathway

FIBROUS GRANULES: Late on the third day of the differentiation cycle, granules 40–60 mμ in diameter, irregularly shaped and differing in electron capacity, appear in the apex of the future ciliated cells (Fig. 7). Stereomicroscope and high magnification studies reveal that the darkest granules are composed of 40–75-A long fibers embedded in amorphous material adhering in diminishing amounts towards the periphery (Fig. 7). The fibers thus appear to taper as they extend from the granule center, and this gives the granule a stellate appearance.The lighter granules seem to be amorphous material without fibers or with smaller diameter fibers (Fig. 7). The fibrous substructure of these granules has suggested the name "fibrous granule," which we prefer as an apparently unique morphological description of these elements. Other terms—proliferative elements (16), procentriole precursors (36), and axonemal precursors (36)—which have been applied to a similar organelle, have functional connotations that are premature at this time.

Fibrous granules are sometimes seen within the folds of the nuclear envelope (Figs. 5, 6), usually in cells that have not begun to make procentrioles. Similar granules are located in the nucleoplasm in juxtaposition to those in the cytoplasm, and some appear to be in transit across the nuclear envelope (Fig. 6).

Individual granules are sometimes seen scattered throughout the apical cytoplasm, but usually they are aggregated into groups that form sheets or spheres, the boundaries of which are delimited by subtle differences in electron opacity and texture from the surrounding cytoplasm (Fig. 7). The ground plasm within the groups of granules contains numerous fibers of various lengths and from 40 A to 80 A thick. The fibers, similar to those in the granules, are sometimes grouped into bundles. Occasionally microtubules are present.

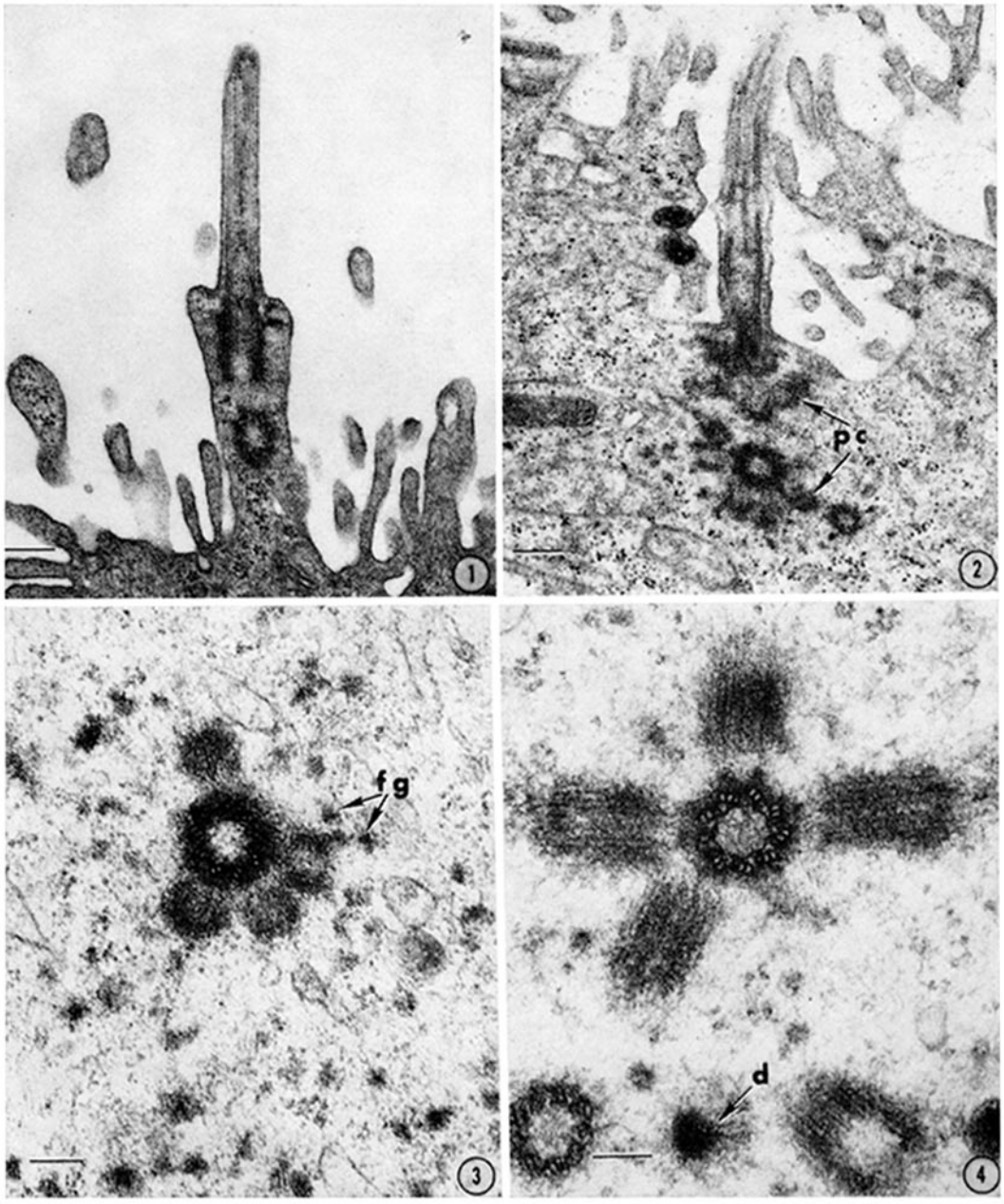


FIGURE 1 On the third day of estrogen stimulation, the diplosomal centrioles have migrated to the luminal surface. One centriole has produced a rudimentary cilium. Scale 2500 A. × 28,000.

FIGURE 2 A rudimentary cilium with abnormal axonemes. Procentrioles (*pc*) are being generated from the free centriole as well as from the basal body. The presence of procentrioles indicates that the cilium is older than the one in Fig. 1. Scale 2500 A. × 28,000.

FIGURE 3 Diplosomal centriole generating four procentrioles. The procentrioles are enmeshed in a corona of flocculent material that encircles the parent. Fibrous granules (*fg*) are being deposited at the apical end of a procentriole (arrows). Scale 1250 A. × 61,000.

FIGURE 4 Nearly mature basal bodies associated with a diplosomal centriole. The angle each triplet set makes with a tangent to the luminal circumference is less in this centriole than in the one in Fig. 3. This establishes that these basal bodies are being generated from the midregion of the parent whereas those in Fig. 3 are being generated from the base region (2, 3). Maturing basal bodies associated with a deuterosome (*d*) indicate that the acentriolar pathway is at the same stage of basal body production. Scale 1250 A. × 64,000.

Fibrous granules appear just before the formation of procentrioles and disappear when the basal bodies are completed (Fig. 28). They reappear after the basal bodies have migrated to the surface of the cell and have begun to form cilia and rootlets. At these two stages, a fibrogranular sphere measuring up to 800 mμ in diameter (Figs. 8, 29) is sometimes seen. This structure is composed of numerous, ill-defined patches of dense material embedded in a lighter matrix. These dense areas sometimes appear to be strung together into whorls within the sphere, and near the periphery they closely resemble fibrous granules; true fibrous granules usually surround the sphere. In rat trachea (35), a similar structure (the "fibrogranular aggregate") is a precursor to procentriole formation. In the monkey oviduct, however, the sphere occurs so seldom that we cannot determine whether it is a precursor or just a fortuitous arrangement of fibrous granules. Its infrequent occurrence indicates that either it does not occur in every ciliogenic cell or its lifetime is very short.

Procentrioles develop in association with aggregates of fibrous granules (Figs. 9, 10). Initially, these granules fuse to form the wall of the procentriole (Figs. 13, 14, 15); later they become symmetrically arranged around the distal end of the growing procentriole and merge with the existing wall material (Figs. 3, 10, 14). By the time the triplet tubules of the basal body are completed, only a few granules remain which become attached to the wall of the basal body to form the basal foot (Fig. 11). During rootlet formation, fibrous granules reappear and are deposited at the proximal end of the basal body (Fig. 29). These kinetic and morphologic patterns suggest that the granules are consumed during the synthesis of basal bodies.

DEUTEROSOMES: The procentrioles begin to form shortly after or simultaneously with the appearance of fibrous granules, and each procentriole usually develops in contact with an electron-opaque sphere (Figs. 9, 10, 14) that measures 90–110 mμ in diameter at maturity (Fig. 27). Most commonly, one or two procentrioles project from each sphere, but as many as four procentrioles per sphere do occur (Fig. 10). Occasionally procentrioles develop independently of the sphere. A similar structure, which appears in the rat trachea during ciliogenesis, has been called a "deuterosome" by Sorokin (35). Other investigators have used the terms "condensation form" (16) and "procentriole organizer" (36) to denote a similar organelle; however, we prefer "deuterosome" because the sphere is the second morphological entity in the ciliogenic sequence and because other terms have functional connotations best avoided.

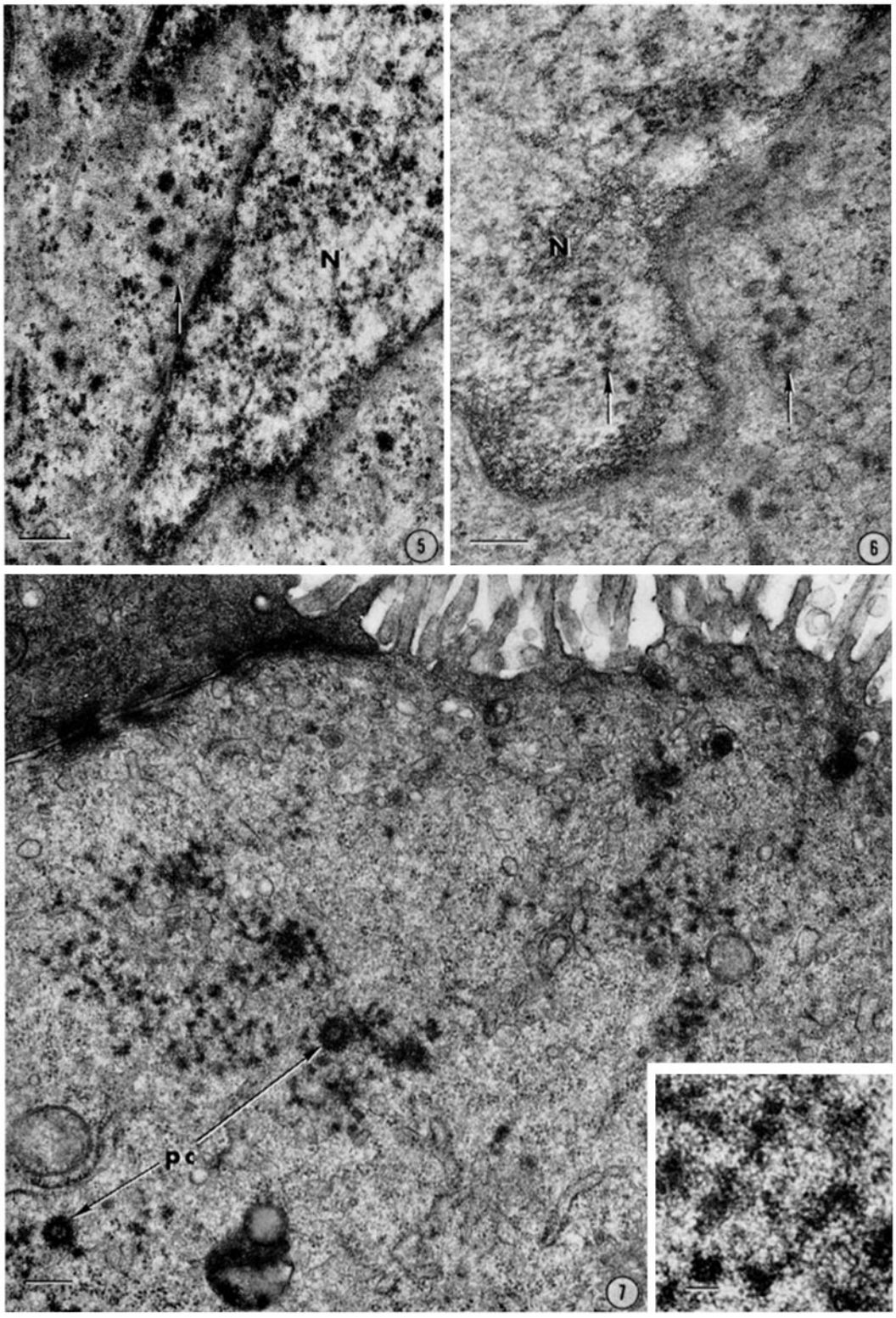
The oviduct deuterosome is 10–20 mμ smaller in diameter in the early stages of procentriole development than in maturity and is irregularly shaped, resembling an enlarged fibrous granule (Fig. 9). Thus, deuterosomes originate within aggregates of fibrous granules which they initially resemble in structure; this suggests that fibrous granules can form deuterosomes as well as procentrioles. Other investigators have suggested a similar origin for deuterosomes (16, 35).

The mature deuterosome is a spherical mass of fibers organized into an inner dense region and an outer, more delicate, corona. The inner dense region is composed of tightly interwoven fibers (each 50 A in diameter) in a surrounding matrix. At the junction between the inner dense mass and the corona, the amorphous matrix becomes thinner and the fibers radiate centrifugally in a random fashion. At the periphery of the corona the matrix diminishes further and the fibers become less numerous. Hollow deuterosomes, like

FIGURE 5 Fibrous granules (arrow) located within the fold of the nuclear envelope. Fibrous granule–like structures are located in the nucleus (*N*) in juxtaposition to those in the cytoplasm. Scale 2500 A. × 33,000.

FIGURE 6 Fibrous granules (arrows) seem to be located on both sides of the nuclear envelope (*N*). In some regions, granules appear to be in transit out of the nucleus. Scale 2500 A. × 37,000.

FIGURE 7 Aggregates of fibrous granules appear on the fourth day of estrogen stimulation. Notice that there are few ribosomes within the aggregate. Numerous fibers are grouped together in the aggregate on the left. Procentrioles (*pc*) have begun to form. Scale 2500 A. × 28,000. The *inset* shows that each granule is composed of a fiber surrounded by amorphous material. Scale 500 A. × 98,000.


N
5
N
6
Pc
7

those in the trachea of the rat (16, 35), are never seen in the oviduct of the monkey.

INITIATION OF PROCENTRIOLE FORMATION: A procentriole is first recognizable as an annulus of amorphous material with an irregular outside diameter (100–195 mμ), a very uniform internal diameter (85 mμ), and an approximate length of 125 mμ (Fig. 14). The variation in the outside diameter is created by the unequal distribution of material around a uniformly circular band that demarcates the circumference of the lumen (the luminal band). This wall material is either solid or fibrous and does not contain microtubules or subfibers.

The annulus is formed within groups of fibrous granules almost immediately after the granules appear. Because of this intimacy, it is very difficult to interpret the first phases of annulus formation. Favorable sections indicate that the luminal band is formed from fibers or thin sheets of material similar to those associated with the granules (Fig. 13). Fibrous granule–like material simultaneously gathers around this band, and the individual granules become incorporated into the wall (Figs. 12, 14). A filament system that is analogous to the "cartwheel" (20) begins to form at this stage (Figs. 13–15).

This primitive cartwheel is composed of nine filaments radiating symmetrically from a central filamentous ring to nine equidistant points on the procentriole luminal band (Fig. 14). The number nine here is partly conjecture because not all the filaments are clearly seen; however, evidence from rotation photographs indicates that the structure has ninefold symmetry. Longitudinal sections show the central filament component to be a thin-walled (~40 A) cylinder extending from the base to the apex of the procentriole (Figs. 14, 17) and the elements extending from the central cylinder to the luminal circumference to be rod-shaped filaments rather than sheets. The rods originate at right angles to the cylinder and seem to be more numerous near the base of the procentriole.

There is structural continuity between the corona of the deuterosome and this primitive cartwheel. The filaments of the cartwheel are similar to the fibers of the deuterosome, and longitudinal sections show the central cylinder in contact with the deuterosome (Fig. 17). Similar continuity exists between the corona of the diplosomal centriole and the cartwheel of the daughter during centriolar procentriole formation.

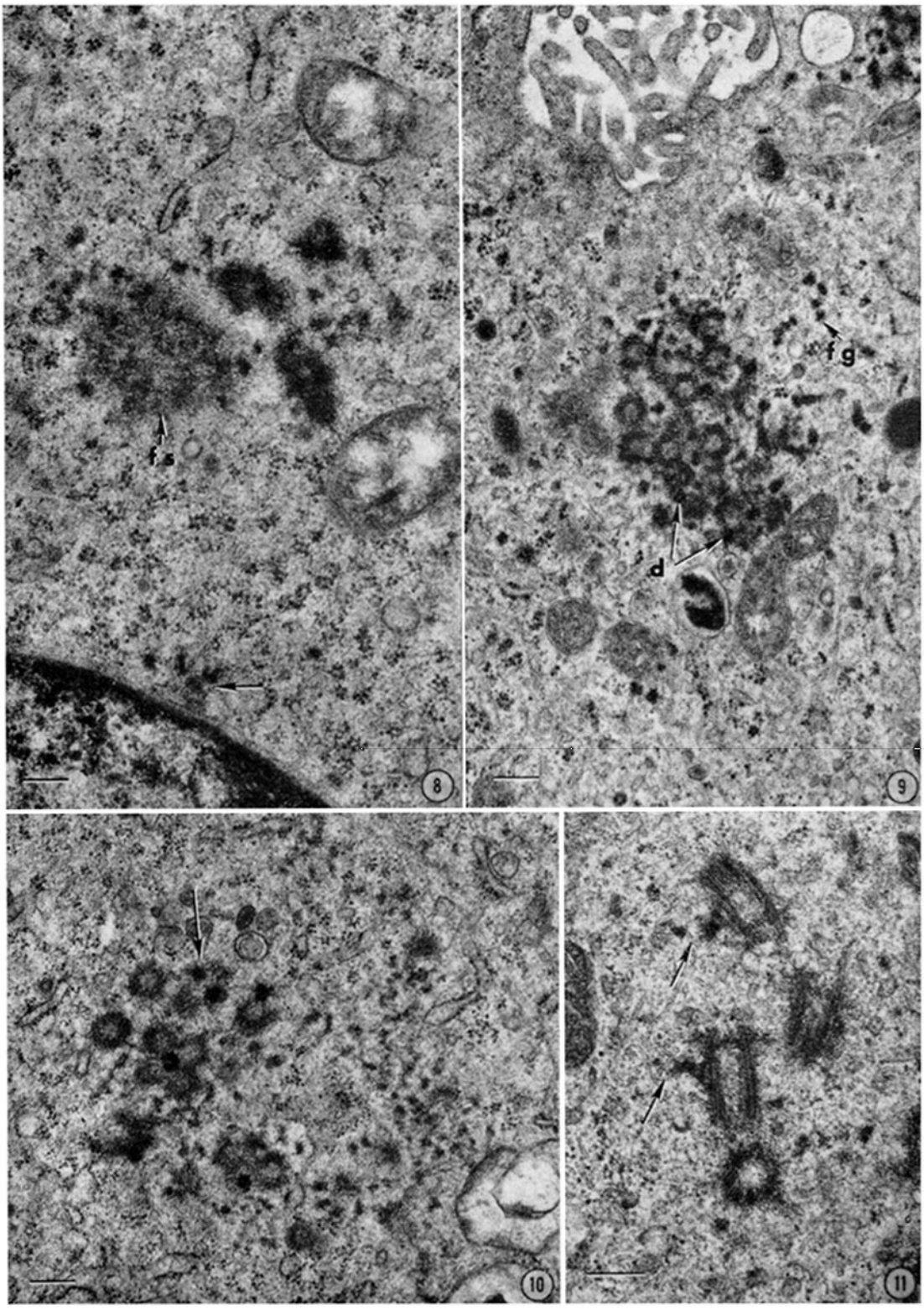
More information about the radial arrangement of the cartwheel can be obtained by studying rotation photographs (24). Each of the nine radial filaments seen in such photographs represents the superposition of all nine cartwheel filaments, i.e., each filament is the average of nine photographs of nine different filaments. If one or two individual filaments were incomplete or absent, the superposition of the other eight would fill in the void. Therefore, this reinforcement process can effectively increase the resolution. However, one must interpret these photographs conservatively because a particularly electron-opaque structure can reinforce with any chosen symmetry. We feel that these photographs show actual cartwheel structure because rotation photographs of several different cartwheels have all disclosed identical structural patterns and because the photographs are similar to those from two other studies on cartwheel structure (29, 39).

FIGURE 8 A fibrogranular sphere (*fs*) surrounded by fibrous granules. Procentriole synthesis is in progress to the right of the sphere. The arrow points to fibrous granules that appear to be coming from the nucleus. Scale 2500 A. × 28,000.

FIGURE 9 A tightly arranged group of procentrioles that have formed within an aggregate of fibrous granules. Notice that the deuterosomes (*d*) have a structure similar to that of the fibrous granules (*fg*) at this stage of development. Scale 2500 A. × 28,000.

FIGURE 10 Forming procentriole surrounded by fibrous granules. One deuterosome is inducing four procentrioles to form. The arrow points to two deuterosomes that are common to the same procentrioles. This type of deuterosome-procentriole relationship is frequently seen (see Fig. 27). Scale 2500 A. × 28,000.

FIGURE 11 Basal feet (arrows) forming on the walls of nearly complete basal bodies. The upper arrow shows an arrangement between a fibrous granule and a basal foot that suggests a precursor-product relationship. Scale 2500 A. × 35,000.


fs
8
fg
d
9
10
11

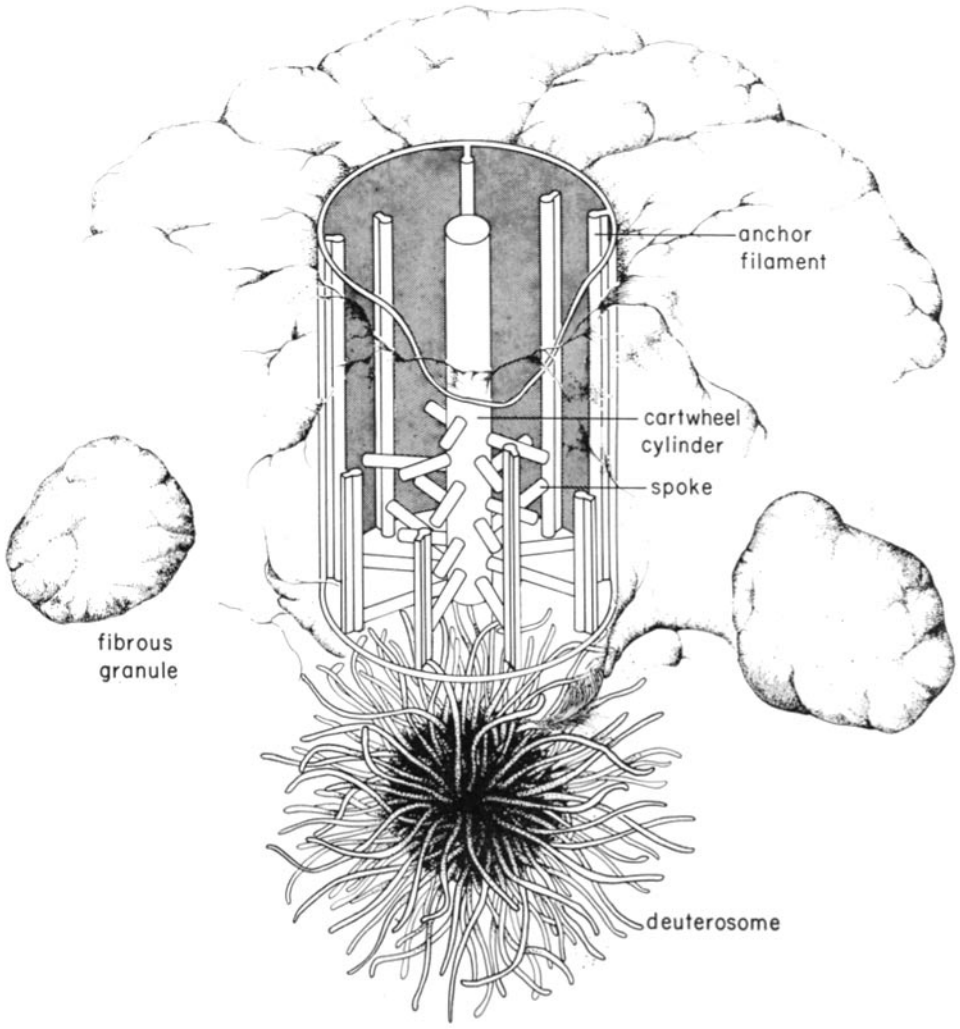


FIGURE 12 A diagrammatic interpretation of the earliest stage of procentriole formation. At this stage, neither the annulus nor the cartwheel is completed.

Fig. 21 is a processed photograph of Fig. 14, a very early procentriole. The triplet tubules have not begun to form, but the basic pattern of the cartwheel structure has been established. The band of material that delimits the procentriolar lumen (luminal band) is segmented into nine electron-opaque regions. Nine corresponding "anchor" filaments (100 A long, 30 A thick) are arranged parallel to the circumference within the procentriolar lumen. Each anchor filament is separated by 100 A from the dense regions in the procentriolar wall, and there is a suggestion that a connection exists between the two. From each anchor filament a slightly curved 30 A thick filament radiates toward the center of the lumen for a distance of 165 A (outer spoke component). This component terminates just subjacent to another filament (40 A thick), the inner spoke component. Each of these latter filaments originates from the central cylinder, projects outwardly for 140 A,

TABLE I

Dimensional Changes during Basal Body Formation

	Procentriole without tubules	Procentriole with completed doublet tubules	Procentriole with completed triplet tubules	New basal body with cartwheel	Mature basal body
Cartwheel cylinder diameter, A	160	250	280	350	
Inner spoke component, A	140	80	80	60	
Outer spoke component*, A	180	160	200	250	
Anchor filament–luminal band connector, A	110	125	150	125	
Total internal diameter, A	1000	1000	1150	1250	1500
Angle of triplet to luminal band		60°65°	60°–65°	50°	40°
Total outside diameter, A	1600	2000	2500	2650	2500

* Measurements on outer spoke component and anchor filament–luminal band connector include the anchor filament and the attachment site dimensions.

and terminates just clockwise to the endings of each outer spoke component. The central cylinder is 160 A in diameter. As the procentriole develops, this three-part pattern of cartwheel structure (central cylinder, spokes, and attachment sites) becomes reinforced and strengthened (Figs. 20, 22). All of the cartwheel components persist during the maturation of the procentriole although their dimensions change. (Table I).

Shortly after the annulus is formed, single 200-A in diameter tubules start to form within the thicker regions of the wall (Fig. 15). In transverse sections, they resemble semicircular 50-A fibers. The open half of each incomplete tubule is randomly oriented to the procentriolar lumen (Fig. 15). As more material is added, the wall of the tubule thickens and the semicircle gradually closes. The final tubule has a uniformly thick wall, from which two 100-A long fibers project at right angles (Figs. 16, 20). The fibers are positioned 180° apart on the tubules, and one of them eventually attaches to the luminal band.

A three-dimensional interpretation of tubule initiation is depicted in Fig. 19, which shows that tubule formation begins with a half cylinder that closes gradually from base to apex. The 100-A long fibers originating from the tubule wall are sheets of material extending the length of the tubule. The luminal band is also a thin-walled cylinder, and one of the two 100-A broad sheets of material connects its tubule to this cylinder. This is referred to as the A tubule attachment sheet.

The first tubule to form is the A (20) or inner tubule of the eventual triplet set; this is also the first tubule to form in *Paramecium* (15) and in chicken tracheal cells (23). As in these other species, the A tubules seem to form in sequence around the annulus. In a section where the cartwheel structure is well developed (basal procentriolar regions), the tubules are spaced 40° from each other around the annulus (Fig. 16). In sections where the cartwheel is incomplete (apical procentriolar regions), gaps in the sequence of tubules sometimes exist so that two well-formed tubules may be separated by 80°. This indicates that in the basal procentriolar regions the tubules first develop in sequential order, clockwise (looking from apex to base) around the annulus (Fig. 20). The nonsequential appearance of the tubules in the procentriolar apex indicates that tubule growth is a nonuniform process. Tubules of various lengths are therefore randomly arranged around the procentriole. Serial sections of more mature procentrioles substantiate these interpretations (Figs. 26, 27). Therefore, tubule formation consists of an initiation and a growth phase.

During the initiation of the A tubules, a new tubule may form before its nearest neighbor is

complete. The new tubule is always 40° of arc distant from this neighbor (Fig. 15). The final orientation of the two 100-A broad sheets usually occurs after the tubule is formed, and occasionally there appear to be more than two sheets per tubule (Fig. 16). One of the sheets eventually attaches to the luminal band, probably to orient and maintain the position of the tubule in the wall. The initiation of the A tubules is complete by the time B and C tubule initiation begins.

Fig. 20 shows various stages of B and C tubule initiation. In the upper left corner of the procentriole, a single A tubule is aligned so that the A tubule attachment sheet and the outer 100 A sheet are in a plane that intersects the circumference of the lumen at a 90° angle. The next A tubule counterclockwise seems to have rotated on its axis, simultaneously displacing the outer sheet counterclockwise and changing the angle of the attachment sheet–lumen intersection to 70°. B tubule formation begins by the perpendicular attachment of a new sheet to the outer 100 A sheet of the A tubule. The newly added wall material projects clockwise from the 100 A sheet. The right angle smooths out into a curve and more wall material is added at the open edge. Eventually the wall curves around through another 90° and attaches to the clockwise side of the A tubule. This completes the initial formation of the B tubule. The C tubule forms in a similar way; however, the initial sheet may develop on either the clockwise or counterclockwise side of the B tubule. The B and the C tubules share a wall with the A and B tubules, respectively. Thus, the B and C tubules are kidney-shaped rather than circular.

Like the A tubules, B and C tubules are initiated in sequence around the procentriole. The C tubules begin to form before all of the B tubules are completed. When all three of the tubules are formed, a line drawn through their transverse axis intersects a tangent to the luminal circumference at about 65°. The transverse axis of each triplet set is slightly curved in the clockwise direction of the 65° angle. This curvature is created during triplet formation by a slight clockwise displacement of each succeeding tubule.

The cartwheel has undergone some changes by the time B and C tubule initiation takes place

---

FIGURE 13 Forming annulus. The arrows outline the lumen of the annulus, and the upper two arrows are at each end of a segment of the luminal band. Fibrous granules are condensing around the band to form the wall material within which the triplet sets will form. A weakly established cartwheel is present in the lumen (see cartwheel in Fig. 15 for orientation). Scale 1000 A. × 84,000.

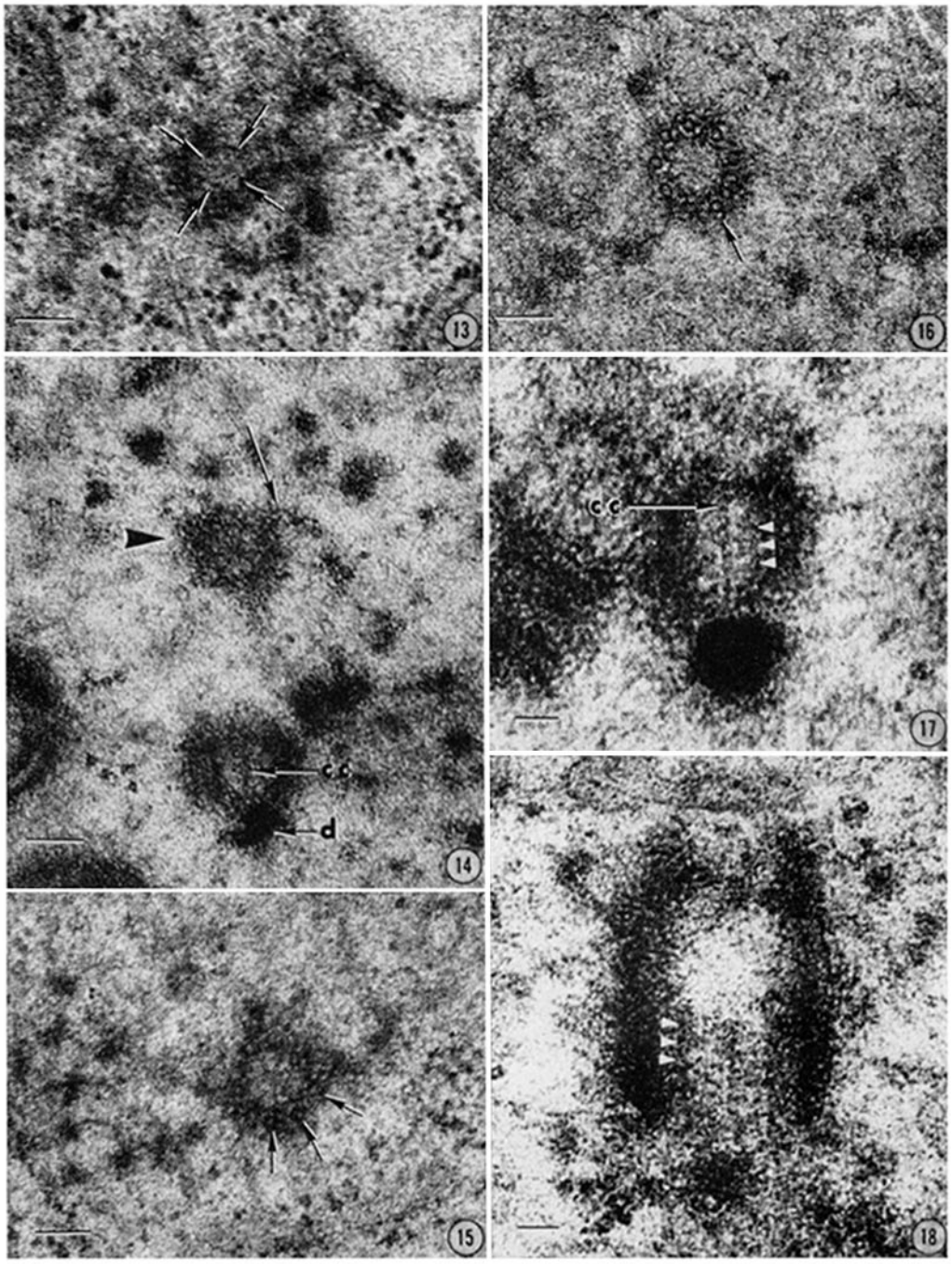
FIGURE 14 A transverse (arrowhead) and longitudinal view of a complete annulus. The tubules have not begun to form but the cartwheel is well established. Fibrous granule material is being added to the wall material (arrow). The longitudinal view shows the central cylinder (*cc*) in contact with the corona of the deuterosome (*d*). Scale 1000 A. × 84,000.

FIGURE 15 A tubule formation is in progress. The bottom arrow and the one counterclockwise to it point to completed A tubules; the third arrow points to the open half of a semicircular band that represents a partially complete A tubule (see Fig. 19). The cartwheel is seen in the lumen. Scale 1000 A. × 84,000.

FIGURE 16 A procentriole with nine A tubules formed. At the arrow, an A tubule is seen with two fibers extending at right angles to the wall of the tubule. The fibers are positioned 180° from one another on the wall. The fiber on the luminal side is the A tubule attachment sheet whereas the fiber on the opposite side (tip of arrow) is the outer sheet from which the B tubule will begin to form (see Fig. 25). Scale 1000 A. × 84,000.

FIGURE 17 A longitudinal view of a nearly complete procentriole showing the cartwheel. The central cylinder (*cc*) extends from the deuterosome to the apex of the procentriole. The spokes arise at right angles to the central cylinder and run laterally to the anchor filament, which is outlined by the white arrowheads. The spokes are less numerous in the apical region of the procentriole. Scale 500 A. × 120,000.

FIGURE 18 Longitudinal section of a newly formed basal body showing the cartwheel. Notice that it has not lengthened with the growth of the procentriole into a basal body. The white arrowheads mark an anchor filament. Spokes and central cylinder are well formed. Notice the light and dark areas of the central cylinder (see text). Scale 500 A. × 120,000.


13
16
c
c
14
d
17
15
18

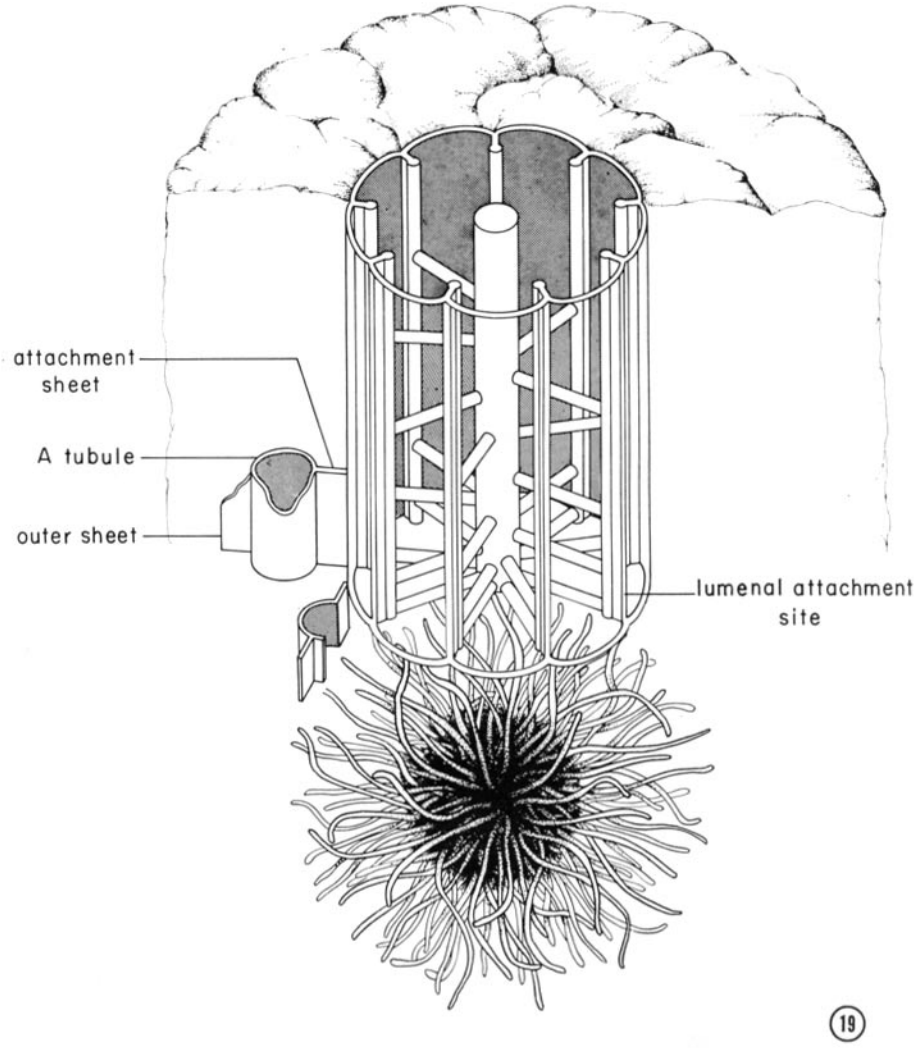


FIGURE 19 Diagram showing A tubule initiation. The cartwheel is complete, and the first A tubule is completely initiated. Counterclockwise from the first tubule, a second A tubule is forming.

(Figs. 20, 22). The diameter of the lumen is unchanged, but the central cylinder has expanded to 250 A in diameter and the inner spoke components have decreased to 80 A in length. In addition, a core material is present in the center of the cylinder that was not apparent in the early cartwheel. The outer spoke components and the anchor filaments have become more prominent. The dense regions on the luminal band have bulged toward the lumen and formed concavities within which the A-B tubule complexes lie. The anchor filament connectors and the A tubule attachment sheets now meet from opposing sides at the same site on the luminal band.

A longitudinal section of a procentriole at this stage of development (Fig. 17) shows a well-developed cartwheel extending from base to apex. The spoke components project from the central cylinder to the circumference of the lumen and are longitudinally spaced every 150 A. At the circumference they attach to a filament running parallel to the luminal wall through the entire length of the procentriole (Figs. 17, 18). This is the anchor filament. The central cylinder appears

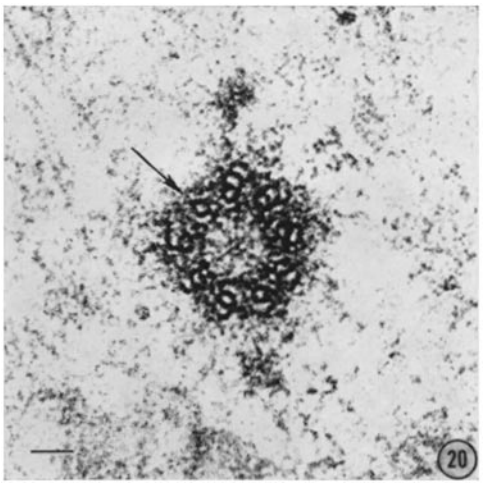


FIGURE 20 Transverse section through a procentriole engaged in B and C tubule initiation. The arrow points to a complete A tubule with its attachment sheet and outer sheet present. The initiation of the B and C tubule is shown in progressive stages counterclockwise around the procentriole (see text). The angle of the completed triplet to the luminal circumference establishes that we are looking from apex to base of the procentriole (2, 3). Therefore, tubule initiation occurs clockwise in this orientation. Scale 500 A. × 112,000.

to be segmented into light and dark regions in the more mature cartwheel, and the dark regions correspond to the positions of the spoke component.

The lengths of the newly initiated tubules cannot be determined because they begin to grow immediately after they are initiated. Thus, the A tubule grows longer while the B and C tubules are still being initiated. When all the triplets are completed at the basal end of the procentriole, the outside diameter at the basal end measures about 200 mμ. The diameter of the lumen is unchanged, and the length is about 200 mμ.

LONGITUDINAL GROWTH OF THE PROCENTRIOLES: The A tubule is the first to appear at the base and to complete its procentriole growth phase. Growth occurs by the uniform deposition of material at the apical end, as if the initial tubule acts as a template for the laying down of new tubing. One side of the tube may grow faster than the other so that in one section the tube looks like a semicircular band (Figs. 15, 19) and in the serial section below like a complete circle.

The longitudinal growth of the B and C tubules occurs in two ways. It usually begins with the elongation of the outer 100-A sheets. Thus, complete B or C tubules occur in one transverse section through the procentriole whereas in the section above only the outer 100 A sheet is present (Figs. 26, 27). The outer sheet, therefore, lengthens rapidly whereas its lateral growth involving formation into a tube progresses more slowly from base to apex. This results in a spiral arrangement of the open edge from base to apex (Fig. 25). In the other mechanism the tubule becomes complete from base to apex except for the final attachment of its open edge to the base tubule (that tubule from which the outer 100 A sheet initially projected). The final attachment probably occurs simultaneously throughout the length of the new tubule.

Like the A tubules, the B and C tubules grow in length and breadth at different rates even within the same forming triplet. Tubule growth is orderly only in the sense that A tubules are completed first, B next, then C within each triplet set. Occasionally, however, incomplete A, B, and C tubules within one triplet set have been seen (Fig. 27). Interpretive drawings which summarize these characteristics of longitudinal tubule growth are presented in Figs. 19 and 25.

The nine triplet sets are completely formed when the procentrioles attain a length of 250 mμ and a basal diameter of 210 mμ. Many procentrioles are found with these dimensions, an indication that there is a lag in the growth process at this stage. The basic pattern of the cartwheel structure has not changed (Figs. 23, 26), although the diameter of the lumen and the dimensions of the various parts of the filament system have slightly increased. The angle of the triplet set to the lumen circumference is still 60°–65°, and serial sections establish that this angle decreases slightly from base to apex (Figs. 26, 27). Also, the A and C tubule linking sheet is beginning to form (2, 3).

THE TRANSITION FROM PROCENTRIOLE TO BASAL BODY: The mature basal body is formed by the further lengthening of the procentriole and the addition of several accessory structures. The triplet sets extend apically in a rapid and disorderly manner. Often the triplets do not completely develop until ciliogenesis has begun. As the tubules grow, the angle of the triplet sets to the lumen circumference (3) decreases from base to apex. After the cylinder has almost reached its mature length, one or two basal feet (usually one) begin to form in the midregion

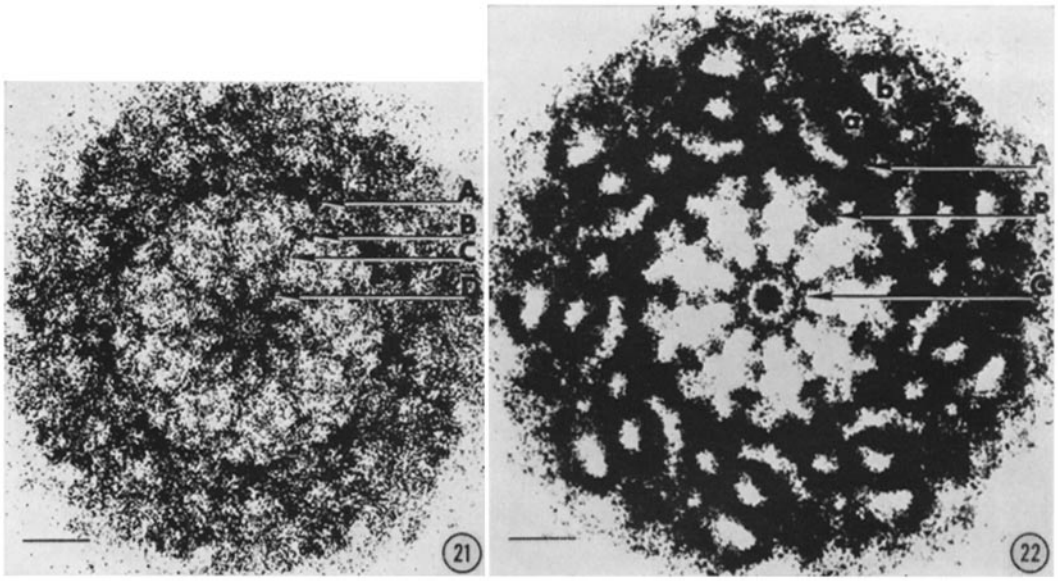


FIGURE 21 An image-enhanced photograph of Fig. 14. No tubules have formed, but the basic architecture of the cartwheel is established. Attachment site on luminal band, *A*; anchor filament, *B*; outer spoke component, *C*; inner spoke component, *D*, radiating from the central cylinder. Scale 250 A. × 350,000.

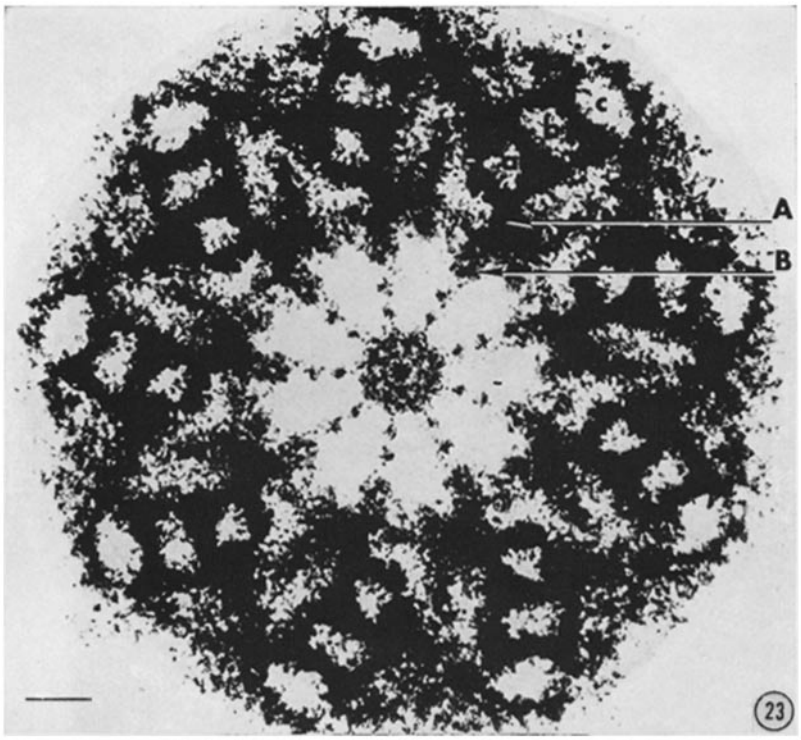
FIGURE 22 An image-enhanced photograph of Fig. 20. Two tubules, *a* and *b*, are complete. The A tubule attachment sheet (*A*) connects the tubule to the luminal band attachment site. The anchor filament (*B*) also attaches to this site. The inner and outer spoke components are the darker and lighter segments of the spoke, respectively. Core material is seen within the central cylinder (*C*). Scale 250 A. × 350,000.

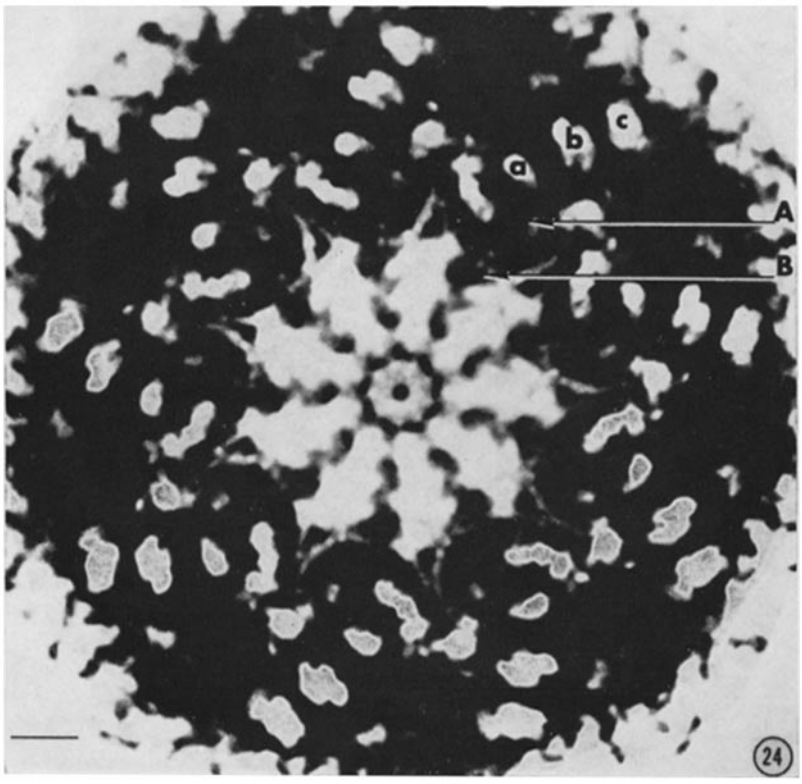
at right angles to the wall. These pyramidal structures appear to be formed by the deposition of fibrous granule–like material onto the wall of the basal body (Fig. 11). The "alar sheets" (2, 3) begin to form as extensions from the C tubules in the apical region but do not assume their final arrangement until the basal body-cilium relationship is established.

The cartwheel seems to be best developed in the recently completed basal body. A rotation photograph shows that the various components are more prominent at this stage than in any of the previous stages (Fig. 24). In addition, the central cylinder is now segmented into nine equal regions corresponding to each inner spoke component, and the centrally located core material is very prominent.

FIGURE 23 An image-enhanced photograph of a completed procentriole, similar to the one in Fig. 26. The triplets are complete (*a*, *b*, *c*), and the attachment sheet (*A*) connects the triplet to the concave-shaped attachment site. The anchor filament (*B*), inner and outer spoke components, central cylinder, and core material are present. Notice the dense material connecting the C tubule of each triplet set with the A tubule of the triplet clockwise to it. These are the forming linker sheets (2, 3). Scale 250 A. × 350,000.

FIGURE 24 An image-enhanced photograph of a fully formed basal body just before the cartwheel disappears. Attachment sheet (*A*) and anchor filament (*B*) connect to the elliptically shaped attachment site. The central cylinder is segmented into nine regions in this maximally developed cartwheel, whereas the core material is diminished in size. The inner spoke material is quite light compared with the outer component. Triplets, *a*, *b*, and *c*. Scale 250 A. × 350,000.


c
A
B
23


a
b
c
A
B
24

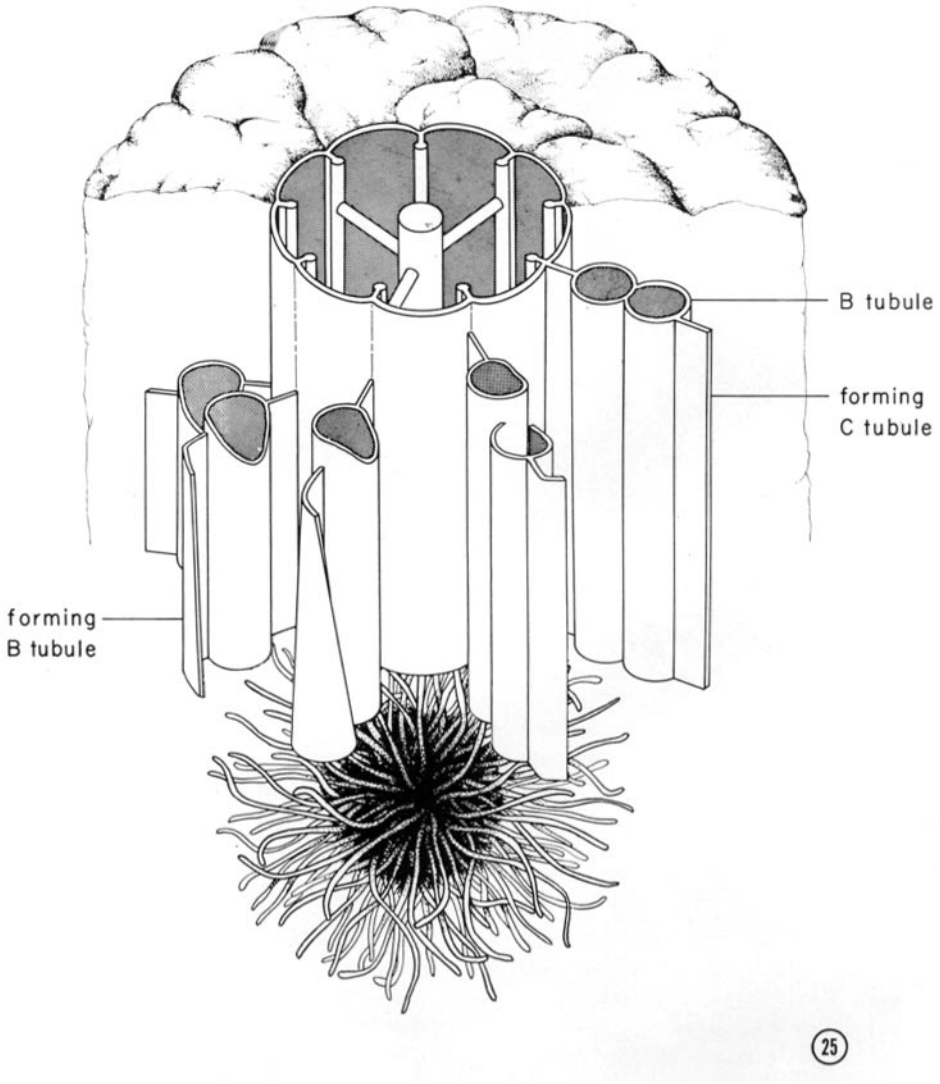


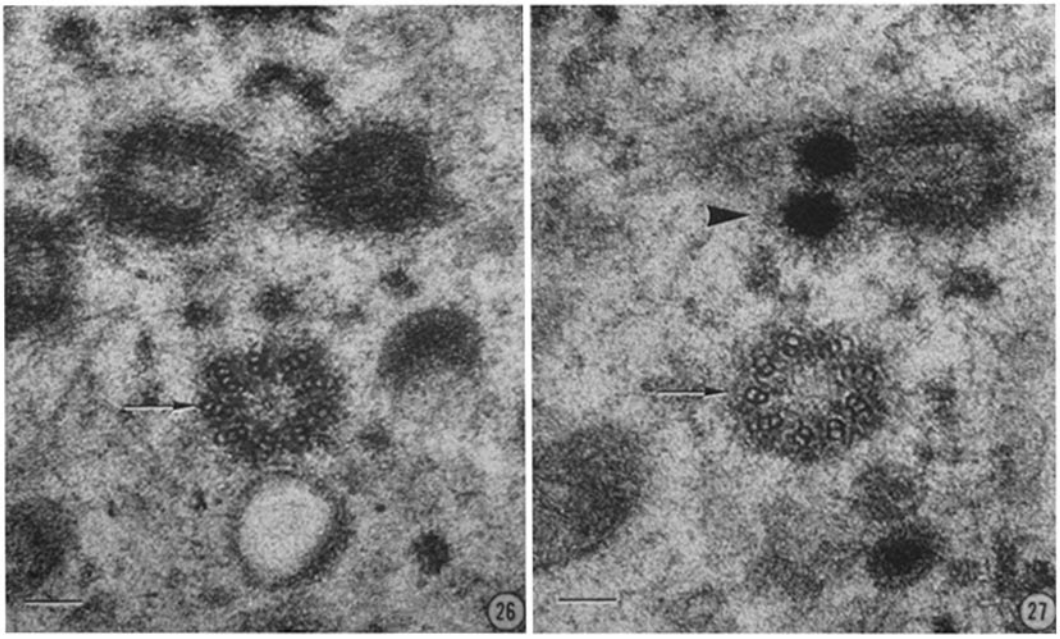
FIGURE 25 A diagram showing triplet tubule growth. The tubules tend to be progressively less complete from base to apex.

The attachment sites for the triplet sets have become maximally concave.

As the procentriole grows to the length of the mature structure, the cartwheel remains a constant length and becomes a robust network of filaments resembling a scaffolding to establish basal body morphology (Fig. 18). The relationships between the anchor filament, spoke components, and central cylinder are well developed at this stage.

When the completed basal body begins to migrate to the cell surface (Fig. 28), or while cilium formation is taking place, the cartwheel structure breaks down. As it does, the central cylinder expands in diameter and the spoke system disappears. Simultaneously, the luminal band attachment sites disappear. When the cartwheel is gone, a vesicle representing the expanded cylinder remains; a similar vesicle is commonly seen within the lumen of centrioles and basal bodies in other cell systems (35, 39). The vesicle eventually disappears.

The various components of the cartwheel as well as of the procentriole undergo dimensional



FIGURES 26 and 27 Serial sections through a partially completed procentriole. The triplet tubules are in the growth phase, and the arrow points to the same triplet in each micrograph. Whereas the C tubule is complete in the basal section (Fig. 26), this tubule is absent in the more apical section (Fig. 27). The third triplet set clockwise from the arrow shows a complete triplet basally, but only a partially formed C tubule apically. These micrographs demonstrate the nonsequential aspect of the growth phase as well as the nature of the tubule growth (see Fig. 25). The mature deuterosome (arrowhead) in Fig. 27 has a well-developed inner dense region and outer corona. Scale 1000 A. × 74,000.

changes during basal body development (Table I). The central cylinder consistently increases in diameter and the inner spoke component becomes shorter. The outer spoke component lengthens, but the anchor filament–luminal band connector does not change much. These dimensional changes accompany an increase in the luminal diameter of the procentriole as measured from attachment site to attachment site. Since the mature basal body does not have attachment sites, the luminal diameter is measured from A tubule to A tubule; therefore, the increased luminal diameter between new and mature basal bodies is due to the loss of the attachment sites. When the triplets are forming, the angle of the triplet set to the luminal circumference measures 60°–65°. In the corresponding region of the mature basal body, this angle is 40° (2, 3). The angle change is gradual and incomplete until after the cartwheel disappears.

The completed basal bodies produced by both pathways are now randomly arranged within the apical cytoplasm of the cell (Fig. 28). Eventually they migrate to the cell surface. Their random orientation is lost as the apex of each structure comes in contact with the cell surface and cilia formation begins. At the same time, fibrous granules reappear near the basal bodies, and rootlets start to form. The rootlets, like the basal feet, seem to form by aggregation of fibrous granules (Fig. 29).

DISCUSSION

Introduction

The results of the present study show that acentriolar basal body formation in the oviduct of the rhesus monkey is similar to that in rat and mouse trachea (9, 16, 17, 35, 37), chicken trachea (23), *Xenopus laevis* embryos (36), rat choroid plexus (25), and centriole replication in the fern

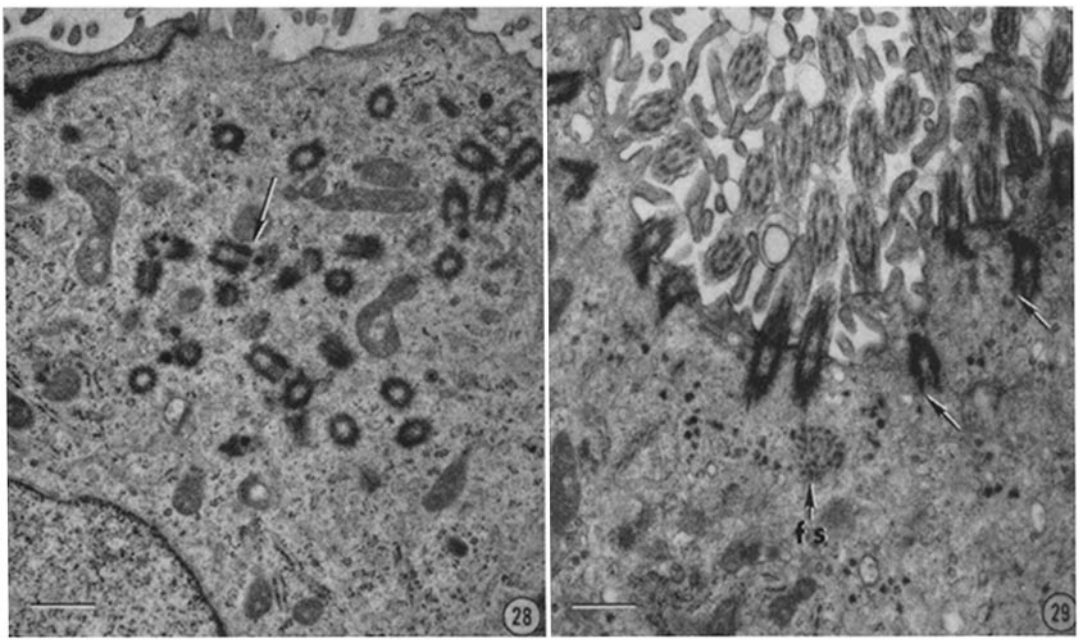


FIGURE 28 Completed basal bodies randomly arranged in the apical cytoplasm of a ciliogenic cell. The basal bodies are disassociating from the deuterosome (arrow); however, the deuterosome usually remains attached to one basal body until cilia formation begins. Scale 5000 A. × 17,000.

FIGURE 29 Basal bodies forming cilia. Fibrous granules reappear, and occasionally a fibrogranular sphere is seen (*fs*). The arrows point to the basal region of two basal bodies where fibrous granules seem to be depositing for rootlet formation. Scale 5000 A. × 17,000.

Marsilea (26). The centriolar pathway resembles kinetosome formation in unicellular ciliates (1, 10, 15, 19, 22, 31), centriole replication during spermatogenesis in the snail *Viviparus* (18), and diplosomal centriole replication (6, 21, 27, 32, 33, 35, 38). The purpose of the following discussion is to present, from all available data, some new hypotheses concerning the mechanism of basal body formation.

The Origin of the Fibrous Granules

Various investigators have suggested that the fibrous granules, or analogous structures (procentriole precursor bodies [36], dense granules [23], proliferative element components [16], fibrogranular aggregate components [35]), are synthesized by the diplosomal centriole. The best morphologic evidence for this hypothesis was obtained by Kalnins and Porter (23) in their study of ciliogenesis in chicken trachea. In these epithelial cells, the fibrous granule–like material always surrounds the diplosomal centriole during procentriole formation. The juxtaposition of pericentriolar satellites (6) and other fibrous granule–like material (27, 33, 38, 39) to procentrioles during diplosomal centriole replication also supports the "centriole origin" hypothesis. In rat trachea and in *Xenopus* embryos (16, 35, 36), however, fibrous granules are only occasionally associated with the parent centriole. Another possibility, proposed by Sorokin (35), is that the Golgi complex and annulate lamellae also provide a component of the procentriole precursor material.

In oviduct cells, the fibrous granules usually appear in the apical cytoplasm and are not at first associated with any particular organelle. Endoplasmic reticulum, Golgi complex, and mitochondria are frequently found in the same cell region, but it is impossible to decide on morphological grounds whether the proximity of these organelles to fibrous granules is significant or

fortuitous. When fibrous granules are seen near the diplosomal centriole, procentriole induction is in progress. In our studies, two or three quiescent centrioles associated with fibrous granules have been seen, but it seems more likely that these granules were near the centriole because of their future involvement in centriolar basal body formation than because of being produced by the parent for use elsewhere in the cell. This could be the case in rat and chicken trachea (16, 23, 35) and in diplosomal centriole replication (6, 27, 33, 38, 39).

We have noted several examples of fibrous granules within pockets of the nuclear envelope, an indication that fibrous granules may originate in the cell nucleus. The granules are somewhat atypical since the fibrous substructure is not easily distinguishable (Figs. 5, 6), but no other cytoplasmic structure, including polyribosomes (9), resembles these organelles. In addition, the nucleoplasm contains similar granules juxtaposed to those in the cytoplasm, and there are morphological indications that the granules are transported through the nuclear envelope (Fig. 6). Granules in transit have not actually been seen, but transport may occur rapidly across many different sites on the nuclear envelope, and the form of the granules may change during transit. Whether the nucleus is indeed a source of fibrous granules remains an important question that must be considered in any hypothetical scheme concerning basal body replication.

The Fate of the Fibrous Granules

The ubiquitous occurrence of fibrous granule–like material during procentriole formation suggests this organelle as either a source of precursor material or a direct precursor to the procentriole. Except for studies on the cells in the rat choroid plexus (25), every study on ciliated epithelium has revealed a fibrous granule component nearby forming procentrioles (16, 17, 23, 35, 36, 37). In addition, most of the studies on diplosomal centriole replication reveal that such granules surround the parent centriole during procentriole induction (6, 7, 27, 33, 39).

Several investigators have proposed that fibrous granules are arranged to form the procentriole wall. Bernhard and de Harven (6) term the granules associated with centriole replication "pericentriolar bodies" and suggest that they are transformed into procentrioles. Stockinger and Cireli report that in the respiratory ciliated cells of rats nine granules are formed into a ring, each granule representing a future triplet (37). Studies on ciliated epithelial cells in chicken trachea and *Xenopus* embryos indicate that these granules are consumed during the production of the basal bodies (23, 36).

In rhesus monkey oviduct, the spatial and temporal relationships of fibrous granules to developing procentrioles indicate a direct precursor-product relationship. During annulus formation and the apical growth of the procentriole, the granules are incorporated into the wall material. The various bands and tubules formed during procentriole development seem to be made of granule-derived material.

Fibrous granules may also form other structures. Dirksen and Crocker (16) and Sorokin (35) suggest that the deuterosome (or condensation form) is a product of granule condensation. In monkey oviduct, early deuterosomes are similar in size and shape to fibrous granules and are usually first recognized within granule aggregates. These relationships indicate that deuterosomes develop from fibrous granules. The granules have also been implicated in rootlet formation in other cell systems (23, 35); Steinman hypothesizes that they provide a precursor for developing cilia (36). In monkey oviduct, shortly after the basal body reaches its mature length, fibrous granules are deposited on the wall in conjunction with basal foot formation. In addition, during cilium formation the basal body rootlet is generated, and new fibrous granules appear, many of which are in close proximity to the basal end of the basal body. Either the granules act as initiators that stimulate rootlet and basal foot development, or they are transformed into these appendages.

The Organizer Hypothesis

With few exceptions (14, 28, 29, 34), procentrioles appear first as a bud or protuberance from some other organelle. During diplosomal centriole and kinetosome replication, the procentriole forms at right angles to the parent (1, 6, 15, 18, 19, 22, 27, 31, 33, 35, 38), and in ciliogenic cells, solid spheres (36), hollow spheres (16, 35), trellis-shaped membranes (25), and cylinders (23) function in a similar capacity. These morphologic arrangements suggest that the inducing organelle contains a factor that is

responsible for procentriole organization (15, 35, 36).

The diversity in the morphology of the various inducer organelles suggests that the structure of the inducer cannot itself determine that of the procentriole (38). Several observations in the oviduct cells support this hypothesis. In centriolar procentriole development, the daughter is initiated at different regions of the parent wall; and in situations where the perpendicular orientation of daughter to the parent wall becomes distorted, the daughter loses its intimacy with the architecture of the parent. Deuterosomes may not even be essential to procentriole formation, since serial sectioning has disclosed that not all procentrioles formed in the acentriolar pathway have a deuterosome. Also, deuterosomes are not fully formed until after the procentriole is fairly well established.

It seems more likely that some organizer substance (molecule or groups of molecules) is common to all of these procentriole-inducing structures (organizer organelles). The organizer organelle would produce the proper environment for procentriole formation, and the organizer substance would regulate the behavior of the granules.

The diplosomal centrioles may be the initial site of synthesis of the organizer substance. These organelles normally have procentriole-inducing capacity, and numerous investigators have noted that basal bodies often form near the diplosomal centrioles. If some of this substance diffused away from the diplosomal centriole, and some remained behind, then the site of procentriole formation would depend on where and when the fibrous granules came into contact with organizer substance. Perhaps, in the trachea of the chick, where procentrioles develop in the immediate neighborhood of the diplosomal centriole, the fibrous granules are synthesized early, and migrate to the site of organizer synthesis. In those cases where the basal bodies develop randomly in the cytoplasm (the oviduct of monkey, trachea of the rat, epidermis of *Xenopus* embryo) the fibrous granules may be synthesized late and interact with organizer substances already present in the cytoplasm. If the interaction occurred at a distance from the centriolar pair, then a secondary parent structure (deuterosome, cylinder) would form first, perhaps because the information for assembly of a procentriole can be expressed only when the surface of some kind of parent structure is available. In monkey oviduct a few procentrioles do form in the cytoplasm without any parent structure, but these are exceptions.

The centriolar and acentriolar mechanisms for basal body production may be two different expressions of the same fundamental process—an interaction between organizer substance and fibrous granules. The acentriolar process is the major pathway for basal body production, perhaps because large amounts of organizer substance diffuse away from the diplosomal centrioles. The centriolar mechanism may be an expression of an incomplete dissociation of the organizer substance from its synthesis site. The procentriole-inducing potential of the parent centriole would then be a function of the amount of organizer substance associated with the organelle at the time the latter comes in contact with the fibrous granules. For example, anywhere from 1 to 10 procentrioles can be initiated by a parent centriole.

In summary, fibrous granules are direct procentriole precursors, whereas the deuterosome and diplosomal centriole may possess a common organizer material that acts like a catalyst to initiate procentriole formation and to determine the structure of this organelle (Fig. 30). The different precursor-product patterns seen in various ciliogenic cells could result from (*a*) the relative times of precursor and organizer synthesis, and (*b*) the spatial relationships of the sources for these two necessary elements. Other factors of lesser consequence, such as the catalytic activity of species specific organizers and the differences in microenvironment within the cell, may contribute to other species variation.

The Role of the Cartwheel

The mechanism that forms the basis for organizer organelle activity is a mystery, the only clue to which is the apparent continuity between the corona of the deuterosome, or the corona of the centriole, and the cartwheel. The organizer organelle may form the cartwheel from the corneal elements of the deuterosome or the corona of the diplosomal centriole. If the cartwheel acts as a structural intermediate between organizer activity and procentriole morphology, then it may control the initiation and positioning of each triplet set.

The cartwheel is as ubiquitous a structure in procentriole development as the organizer or-

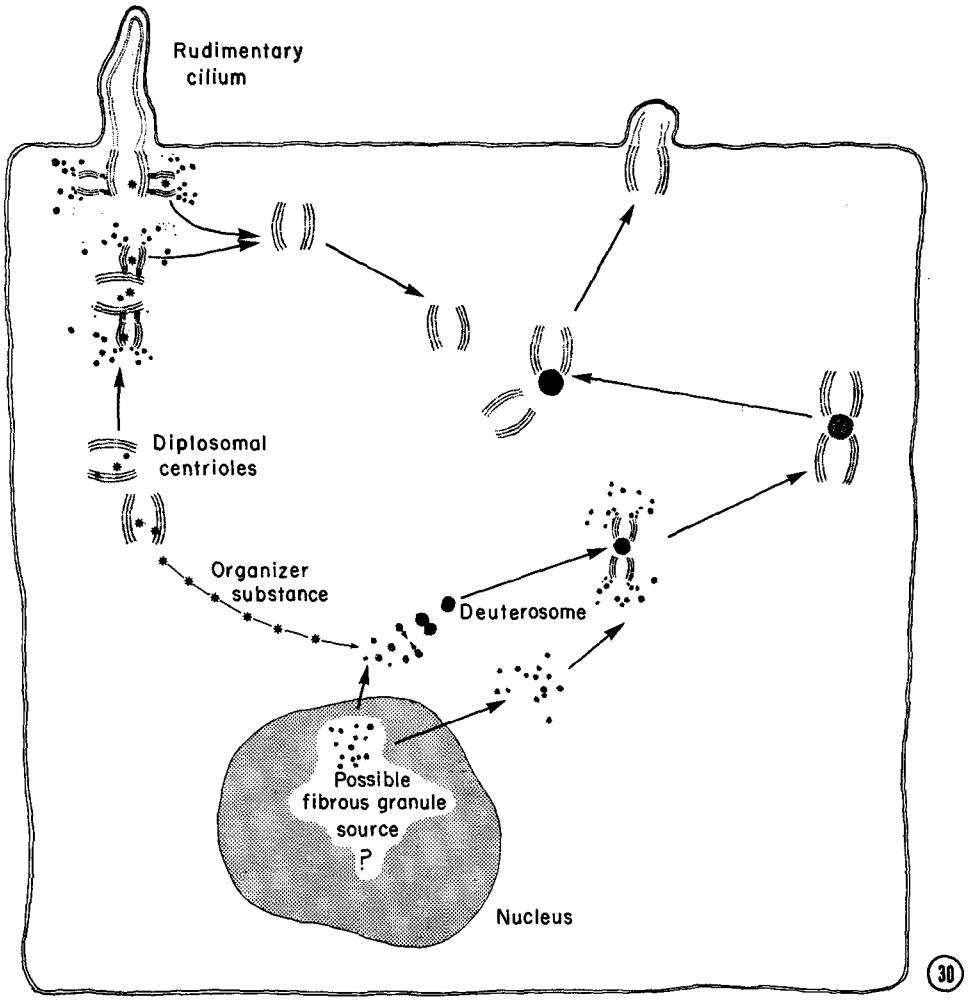


FIGURE 30 A hypothetical scheme for acentriolar and centriolar basal body formation. The diagram suggests that the fibrous granules originate in the nucleus and the organizer substance (stars) originates in the diplosomal centrioles. Some of the granules are arranged into the deuterosome by the organizer substance while the rest of the fibrous granules form the procentrioles. The procentrioles form either at the walls of the diplosomal centrioles or at the cortex of the deuterosome.

ganelle; there is only one reported case where it is absent during procentriole development (30). In many cells (23, 29, 35, 40), the cartwheel disappears shortly after the basal body or centriole is formed; during its brief existence in the newly formed organelle, it is located in the most basal region (the former procentriole lumen). These observations indicate that this structure functions only during the procentriole formation phase. Those organisms in which the cartwheel is an integral part of the mature basal body or centriole (1, 15, 20, 22, 40) may be examples of a system that is unable to eliminate an unnecessary accessory.

In monkey oviduct, the cartwheel is first detected before the annulus of amorphous material is formed. Image-enhanced photographs of a fully formed annulus show that the basic architecture of the cartwheel is established, although weakly, before any tubules are present in the wall material. Likewise, a cartwheel is present at similar stages of procentriole formation in *Labyrinthula* (29) and in chicken trachea (23). By contrast, Dippell states that in *Paramecium* the cartwheel appears between

the time of formation of the A and the B tubules (15).

In monkey oviduct the first tubule is juxtaposed to a spoke of the cartwheel and new A tubules are synthesized sequentially every 40° around the annulus in a clockwise direction. Except for the presence of the cartwheel, this is the same as tubule formation in *Paramecium* (15). Since the ninefold symmetry is established by the cartwheel before the tubules appear, it seems reasonable that this initial symmetry should dictate the placement of the A tubules. After the tubules are initiated, they become attached to the luminal attachment site. The spokes of the cartwheel may provide the support necessary to hold the tubules in place for the subsequent formation of the B and C tubules. The cartwheel structure becomes more robust during procentriole development, as more tubules are added to each triplet and as each triplet lengthens, which also suggests that the cartwheel functions to provide structural support.

Procentriole Development

Triplet tubule formation consists of an initiation and a growth phase. The former processes occur sequentially clockwise (apex to base view) around the annulus; the same is true for A tubule initiation in *Paramecium* (15) and chicken trachea (23). However, the tubule growth phase occurs nonsequentially. This may account for Dippell's observation that B and C tubule formation occurs nonsequentially, i.e., her observations may be based on observations of the growth phase rather than of the initiation phase.

Several structural modifications are made either while the basal body is forming or after the tubular system is complete. In the mature basal body, the angle of each triplet to the lumen gradually changes from ~40° at the base to ~10° at the apex (2, 3). Serial sectioning shows a slight decrease in angle from base to apex of the procentriole. However, measurements of the basal triplet angle in procentrioles and basal bodies during various stages of maturation indicate that the proper shift is not established until after the cartwheel breaks down (Table I). Since the base-to-apex angle change results from a centripetal rotation of the triplet on the axis of the A tubule (2, 3), each triplet may develop a tendency to twist centripetally as it lengthens, and first the cartwheel and then the triplet linkers may maintain the proper angle at the base. The helical pitch followed by each triplet from base to apex is introduced some time during the development phase (2, 3), but we do not know how this occurs. Finally, there must be sites on the wall of the basal body to correctly position the formation of the basal foot, the alar sheets, and the rootlet.

There are a considerable number of gaps in our understanding of centriole formation, especially about the nature and function of the organizer substance. What sets centriole formation apart from the replication of other organelles is the apparent multipotentiality of the precursor material. All of the tubules, connections, and accessory structures may be formed from a basic protein type whose organization into distinguishable structures is due to the influence of one or more organizer molecules. Moreover, the rapid synthesis of basal bodies in *Paramecium* (1–2 hr) compared with that in oviduct (24 hr) suggests that various enzymes control the assembly rate of this organelle. The synthesis of the basal body appears to result from a precise temporal and spatial interaction between various precursor materials and an organizer substance rather than from a spontaneous organization of molecules that depend only on their concentration and tertiary or quaternary structure.

The authors would like to thank Carole J. Wortley for her excellent technical assistance. We also are grateful to Joel Ito and Donna Ryan for the drawings.

This is publication No. 493 from the Oregon Regional Primate Research Center.

This investigation was supported in part by United States Public Health Service Training Grant GM-00445-09 to the Department of Anatomy, University of Oregon Medical School; USPHS Grant HD-02853 to Dr. Brenner; and USPHS Grant FR-00163 to the Oregon Regional Primate Research Center.

Received for publication 8 September 1970, and in revised form 30 November 1970.

BIBLIOGRAPHY

1. ALLEN, R. D. 1969. The morphogenesis of basal bodies and accessory structures of the cortex of the ciliated protozoan *Tetrahymena pyriformis*. *J. Cell Biol.* **40**:716.
2. ANDERSON, R. G. W. 1970. The formation and structure of the basal body (centriole) from the rhesus monkey oviduct. Ph.D. Thesis, University of Oregon Medical School, Portland, Oregon.

3. ANDERSON, R. G. W. 1970. The structure of the basal body from the rhesus monkey oviduct. *Anat. Rec.* **166**:272. (Abstr.)
4. ANDERSON, R. G. W., and R. M. BRENNER. 1971. Accurate placement of ultrathin sections on grids. Control by sol-gel phases of a gelatin flotation fluid. *Stain Technol.* **46**:1.
5. ANDRÉ, J., and W. BERNARD. 1964. The centriole and the centriole region. *Exerpta Med. Int. Congr. Ser.* **77**:9. (Abstr.)
6. BERNHARD, W., and E. DE HARVEN. 1960. L'ultrastructure du centriole et d'autres élément de l'appareil achromatique. *Proc. Int. Conf. Electron Microsc.* **2**:217.
7. BESSIS, M., and J. BRETON-GORIUS. 1958. Sur une structure inframicroscopique pericentriolaire. Etude au microscope électronique sur des leucocytes de mammiferes. *C. R. Acad. Sci. Ser. D.* **246**:1289.
8. BESSIS, M., J. BRETON-GORIUS, and J. P. THIÉRY. 1958. Centriol, corps de golgi et astre des leucocytes. Etude au microscope électronique. *Rev. Hematol.* **13**:363.
9. BIAVA, C. G., and S. MATSUURA. 1967. Morphogenesis of cilia from polyribosomes in differentiating tracheal epithelium of rats. *J. Cell Biol.* **35**(2, Pt. 2):13A. (Abstr.)
10. BRADBURY, P., and D. R. PITELKA. 1965. Observations on kinetosome formation in an apostome ciliate. *J. Microsc.* **4**:805.
11. BRENNER, R. M. 1969. The biology of oviduct cilia. *In* The Mammalian Oviduct. E. S. E. Hafez and R. J. Blandau, editors. University of Chicago Press, Chicago, Ill. 203.
12. BRENNER, R. M. 1969. Renewal of oviduct cilia during the menstrual cycle of the rhesus monkey. *Fert. Steril.* **20**:599.
13. BRENNER, R. M. 1970. Hormonal control of cilia renewal in the primate oviduct: ultrastructural studies. *Progr. Gynecol.* **5**:77.
14. DINGLE, A. D., and C. FULTON. 1966. Development of the flagellar apparatus of *Naegleria*. *J. Cell Biol.* **31**:43.
15. DIPPELL, R. V. 1968. The development of basal bodies in *Paramecium*. *Proc. Nat. Acad. Sci. U.S.A.* **61**:461.
16. DIRKSEN, E. R., and T. T. CROCKER. 1966. Centriole replication in differentiating ciliated cells of mammalian respiratory epithelium. An electron microscope study. *J. Microsc.* **5**:629.
17. FRISCH, D. 1967. Fine structure of the early differentiation of ciliary basal bodies. *Anat. Rec.* **157**:245. (Abstr.)
18. GALL, J. G. 1961. Centriole replication. A study of spermatogenesis in the snail *Viviparus*. *J. Biophys. Biochem. Cytol.* **10**:163.
19. GRASSÉ, P. P. 1961. La reproduction par induction du blépharoplaste et du flagella de *Trypanosoma equiperdum* (Flagellé protomonadine). *C. R. Acad. Sci. Ser. B.* **252**:3917.
20. GIBBONS, I. R., and A. V. GRIMSTONE. 1960. On flagellar structure in certain flagellates. *J. Biophys. Biochem. Cytol.* **7**:697.
21. HOAGE, T. R., and R. G. KESSEL. 1968. An electron microscope study of the process of differentiation during spermatogenesis in the drone honey bee (*Apis mellifera* L.) with special reference to centriole replication and elimination. *J. Ultrastruct. Res.* **24**:6.
22. JOHNSON, U. G., and K. R. PORTER. 1968. Fine structure of cell division in *Chlamydomonas reinhardii*. Basal bodies and microtubules. *J. Cell Biol.* **38**:403.
23. KALNINS, V. I., and K. R. PORTER. 1969. Centriole replication during ciliogenesis in the chick tracheal epithelium. *Z. Mikrosk. Anat. Forsch.* **100**:1.
24. MARKHAM, R., S. FREY, and G. J. HILLS. 1963. Methods for the enhancement of image detail and accentuation of structure in electron microscopy. *Virology*. **20**:88.
25. MARTÍNEZ-MARTÍNEZ, P., and W. T. DAEMS. 1968. Les phases précoces de la formation des cils et le probleme de l'origine du corpuscule basal. *Z. Mikrosk.-Anat. Forsch.* **87**:46.
26. MIZUKAMI, I., and J. GALL. 1966. Centriole replication. II. Sperm formation in the fern, *Marsilea* and the cycad, *Zamia*. *J. Cell Biol.* **29**: 97.
27. MURRAY, R. G., A. S. MURRAY, and A. Pizzo. 1965. The fine structure of mitosis in rat thymic lymphocytes. *J. Cell Biol.* **26**:601.
28. OUTKA, D. E., and B. C. KLUSS. 1967. The amebato-flagellate transformation in *Tetramitus rostratus*. II. Microtubular morphogenesis. *J. Cell Biol.* **35**:323.
29. PERKINS, F. O. 1970. Formation of centrioles and centriole-like structures during meiosis and mitosis in *Labrynthula*. An electron microscope study. *J. Cell Sci.* **6**:629.
30. PHILLIPS, D. M. 1967. Giant centriole formation in *Sciara*. *J. Cell Biol.* **33**:73.
31. RANDALL, J., T. CAVALIER-SMITH, A. MCVITTE, J. R. WARR, and J. M. HOPKINS. 1967. Developmental and control processes in the basal bodies and flagella of *Chlamydomonas reinhardii*. *In* Control Mechanisms in Developmental Processes. M. Locke, editor. Academic Press, Inc, New York. 43.
32. RENAUD, F. L., and H. SWIFT. 1964. The development of basal bodies and flagella in *Allomyces arbusculus*. *J. Cell Biol.* **23**:339.
33. ROBBINS, E., G. JENTZSCH, and A. MICALI. 1968.

The centriole cycle in synchronized HeLa cells. *J. Cell Biol.* **36**:329.

34. SCHUSTER, F. L. 1963. An electron microscopy study of the amoeba-flagellate, *Naegleria gruberi*. I. The amoeboid and flagellate stages. *J. Protozool.* **10**:297.
35. SOROKIN, S. P. 1968. Reconstruction of centriole formation and ciliogenesis in mammalian lungs. *J. Cell Sci.* **3**:207.
36. STEINMAN, R. 1968. An electron microscopic study of ciliogenesis in developing epidermis and trachea in *Xenopus laevis*. *Amer. J. Anat.* **122**:19.
37. STOCKINGER, L., and E. CIRELI. 1965. Eine bisher unberkannte Art der Zentriolenvermehrung. *Z. Mikrosh.-Anat. Forsch.* **68**:733.
38. STUBBLEFIELD, E. 1968. Centriole replication in a mammalian cell. *In* The Proliferation and Spread of Neoplastic Cells. The Williams and Wilkins Company, Baltimore, Md. 175.
39. STUBBLEFIELD, E., and B. R. BRINKLEY. 1967. Architecture and function of the mammalian centriole. *In* Formation and Fate of Cell Organelles. K. B. Warren, editor. Academic Press, Inc., New York. 175.
40. TURNER, F. R. 1968. An ultrastructural study of plant spermatogenesis. *J. Cell Biol.* **37**:370.