THE THREE-DIMENSIONAL STRUCTURE OF THE BASAL BODY FROM THE RHESUS MONKEY OVIDUCT

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ABSTRACT

The structure of the oviduct basal body has been reconstructed from serial, oblique, and tangential sections This composite information has been used to construct a three-dimensional scale model of the organelle The walls are composed of nine equally spaced sets of three tubules, which run from base to apex pitched to the left at a 10°–15° angle to the longitudinal axis. The transverse axis of each triplet set at its basal end intersects a tangent to the lumenal circumference of the basal body at a 40° angle (triplet angle). As the triplet set transverses from base to apex, it twists toward the lumen on the longitudinal axis of the inner A tubule; therefore, the triplet angle is 10° at the basal body-cilium junction. Strands of fibrous material extend from the basal end of each triplet to form a striated rootlet. A pyramidal basal foot projects at right angles from the midregion of the basal body. In the apex, a 175 mμ long trapezoidal sheet is attached to each triplet set. The smaller of the two parallel sides is attached to all three tubules while the longitudinal edge (one of the equidistant antiparallel sides) is attached to the C tubule. The sheet faces counterclockwise (apex to base view) and gradually unfolds from base to apex; the outside corner merges with the cell membrane.

INTRODUCTION

Basal bodies belong to a general class of organelles that includes centrioles (19), kinetosomes (12), and basal granules (24). Except for the giant centriole in *Sciara* (32), all of these organelles have similar cylindrical structures with walls composed of nine sets of three tubules or fibers (12, 19, 22) The cylindrical bodies of these organelles (150–250 mμ diameter, 300–8000 mμ length [14, 16]) often have accessory structures attached at specific sites on their walls, e g., basal bodies usually have a basal foot attached in the midregion (22). The function of these organelles is not clear; however, the centrioles are associated with spindle formations during cell division (19), while the basal bodies, kinetosomes, etc , are associated with cilia formation (12) and cilia movement (22, 30, 35). The centriole can function as a basal body (5, 37); some investigators have proposed that the mitotic spindle functions in part as a centriole distributor, the primary function of the centriole being to serve as the basal body of a cilium (17, 33).

There has not been any comprehensive study of the structure of a mammalian basal body. Knowledge of the structure of this organelle is needed to complement the ongoing study of basal body genesis in the rhesus monkey oviduct (5) being carried out in this laboratory. Although a model for the fibroblast centriole exists (38), a three-dimensional model of the basal body has not been presented. Consequently, it was considered worthwhile to carry out a detailed analysis of oviductal basal body structure, and to build a scale model of this organelle.

The analysis was carried out by examining

numerous sections which represented different regions of the organelle sectioned at various angles In several cases, serial sections of the organelle were used to obtain accurate measurements. The composite information was used to construct a scale model. The whole model, or a scale section of a plastic-embedded model, was compared with representative micrographs of the basal body to corroborate the electron micrograph analysis. In addition to confirming the three-dimensional interpretations derived from two-dimensional photographs, the models contributed to a better understanding of how the organelle is constructed, as well as how the morphology of this structure is maintained.

A preliminary report of this study has been published (2) The results present a total three-dimensional view of a mammalian basal body, indicate new structural features that may be relevant for other basal bodies and centrioles, and provide information that is needed for a phylogenetic analysis of basal body structure.

MATERIALS AND METHODS

Rhesus monkeys, *Macaca mulatta* (Woodard Research Corp., Herndon, Va.), weighing 4–8 kg were ovariectomized 6 wk before use to insure complete atrophy of the oviduct epithelium. 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 on the fifth and tenth days of estrogen stimulation (9, 10). Tissue was fixed for 30 min at room temperature in a cacodylate-buffered 0.75% glutaraldehyde – 3% formaldehyde mixture. Subsequently, the tissue was 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 DuPont diamond knife (I. E. DuPont de Nemours & Co, Wilmington, Del.). Serial sections were prepared by the methods of Anderson and Brenner (4). Electron micrographs were taken with a Philips 200 microscope.

Multiple measurements on representative thin sections of basal bodies were made. Information accrued from these measurements was used to construct scale models (330 A = 1 inch) of the basal body from polyethylene tubes and sheets (Fig. 11). The tubes were inserted into holes drilled in a wooden platform at a 14° angle to the longitudinal axis of the structure. Elmer's glue was used to attach the triplet tubules and the various polyethylene sheets. Several of the models were embedded in a plastic resin (R. B. Howell Co, Portland, Or.), and scale transverse and oblique sections (1000 A = 3 inches) were cut with a band saw. Sections from plastic-embedded scale models and complete models were compared with actual sections Where two interpretations were possible, models constructed according to both concepts were compared

RESULTS

General Morphology

The general appearance of the ovidcut basal body is quite similar to other centrioles (19) and basal bodies (1, 12, 22). In a longitudinal view (Fig. 2) the proximal end of the cylindrical body is dense and robust while the apical end, in continuity with the cilium, is less dense. The walls are composed of continuous sets of tubules embedded in an amorphous matrix; the circumferential distribution of the tubule system is seen in transverse sections (Fig. 1) It is apparent that the organization of the tubules changes from base to apex. Nine sets of three tubules (triplet set) are equally spaced around the cylinder wall. The tubules within each set are attached in a row, although a line connecting the centers of tubules (the axial plane) is slightly curved The C and B tubules (outer and middle) within each set (20) are crescent shaped, sharing a portion of their wall with the B and circular A tubule (inner), respectively, each tubule is approximately 200 A in diameter. The transverse or axial plane of each triplet set intersects a tangent to the lumenal circumference at the A tubule to form an acute angle (triplet angle) that faces clockwise when the section is viewed from the apex. In the base region, 200 mμ long, 80 A thick, sheets of material interconnect the triplet sets, extending from the B or the C tubule of each triplet set to the A tubule of the triplet set clockwise to it (apex to base view) (Fig. 5). These sheets are covered by intertriplet amorphous material in the mature organelle (Figs. 1 *h*, 1 *i*). There is a similar intertriplet connecting sheet in the midregion (approximately 150 mμ long) which encircles the lumen of the organelle, interconnecting all of the A tubules (Figs. 1 *f*, 1 *e*).

Attached to the cylindrical body of the organelle at the base, the midregion and the apex are the rootlet (Figs. 1 *k*, 1 *m*, 2), the basal foot (Figs 1 *g*, 3), and the alar sheet accessory structures (Figs. 1 *b*–1 *d*, 4). The former two appendages resemble

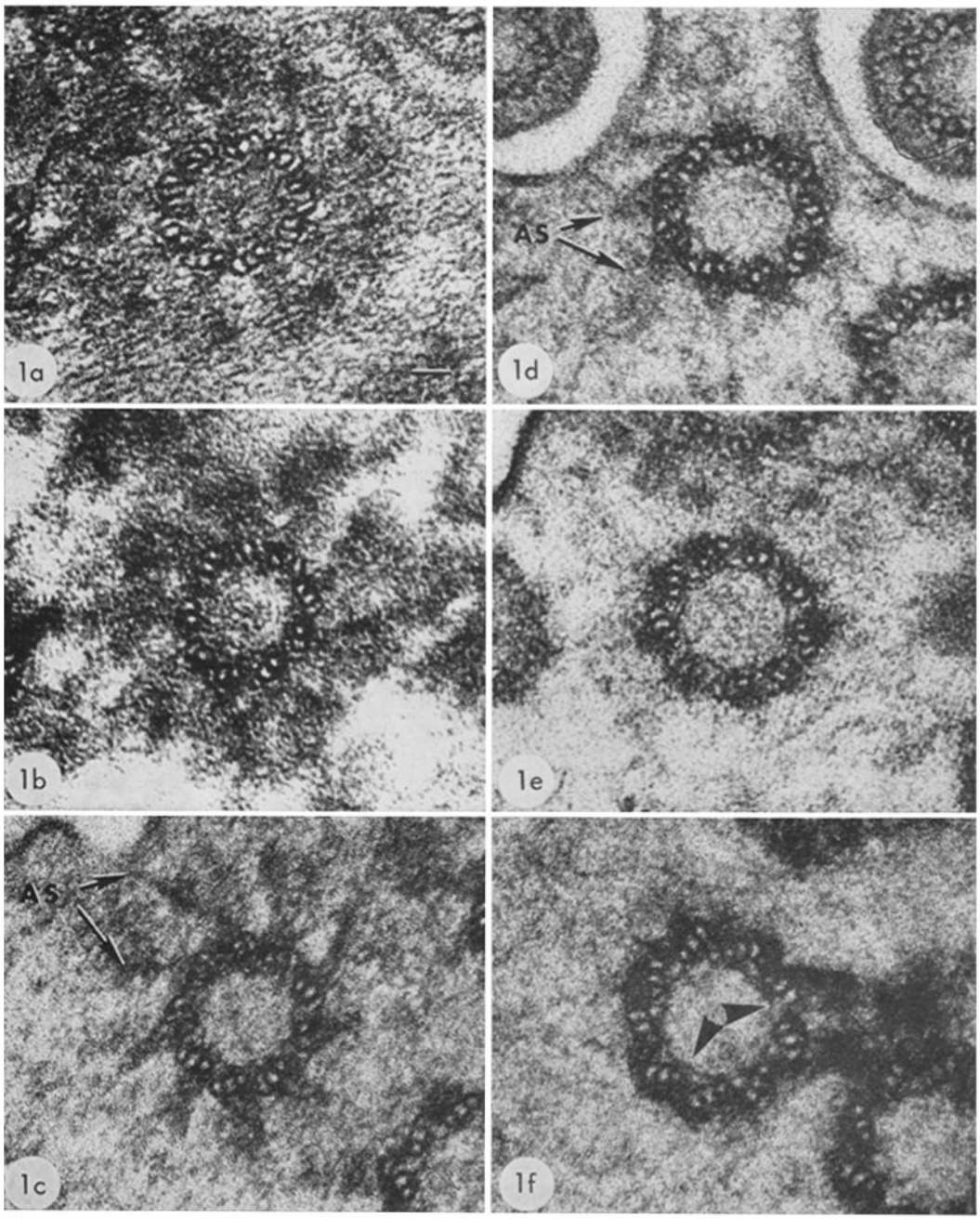
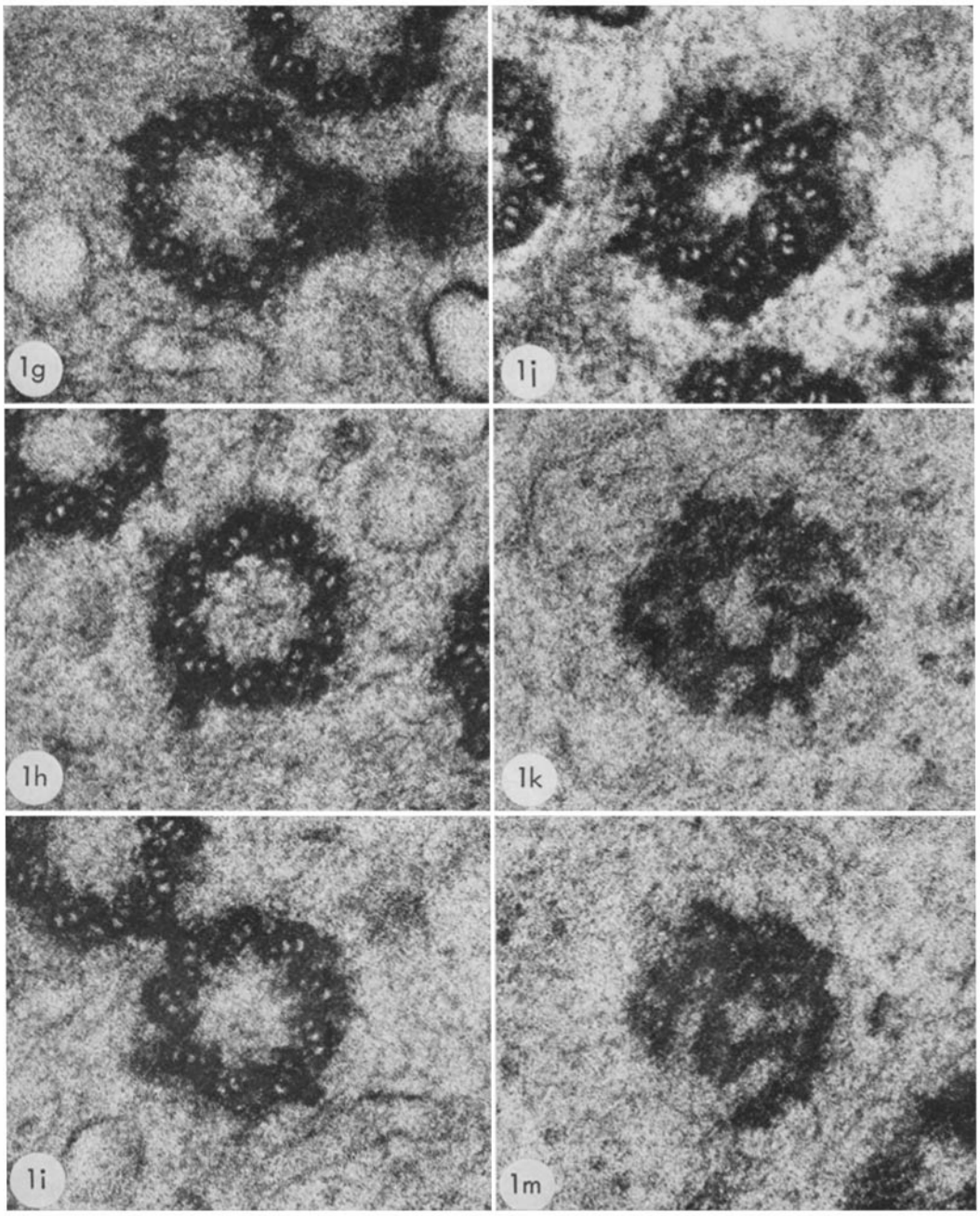


FIGURE 1 Transverse sections through the length of the basal body (apex to base orientation) The regional distribution of such structural features as triplet arrangement, intertubule amorphous material, and accessory structures permits a positive identification of the level of any transverse section: apex, *a–d*; midregion, *e–g*, base, *h–j*; and rootlet, *k* and *m*. The gradual decrease in the triplet angle can be followed from *j* through *a*. Intertubule amorphous material is more concentrated in the basal half of the organelle (*f–j*). This material continues into the rootlet, *k* and *m*. Lower and midportion of the basal foot are seen in *g* and *f*. The lumenal band of material interconnecting the A tubules (arrow heads) begins in the midregion (*f*) and continues into the lower apical region (*d*). The alar sheets (*AS*) are sec-



tioned at successively higher levels from *d* to *a*. Sectioned at the level of their origin from the triplet wall, the alar sheets appear triangular (*d*) at higher levels (c, b). After they have unfolded, the three appears as a fiber. The dense plaques (*a*) radially distributed ~130 mμ from each triplet are the regions where the sheets merge with the cell membrane (see Fig. 16). The heterogeneous appearance of the sheets in Fig. 1 *c* (some look like fibers, [arrows] while others look triangular) may indicate that the sheets are sectioned at different levels and, therefore, do not all originate at the same level on the basal body trunk. Scale 500 A. × 115,000.

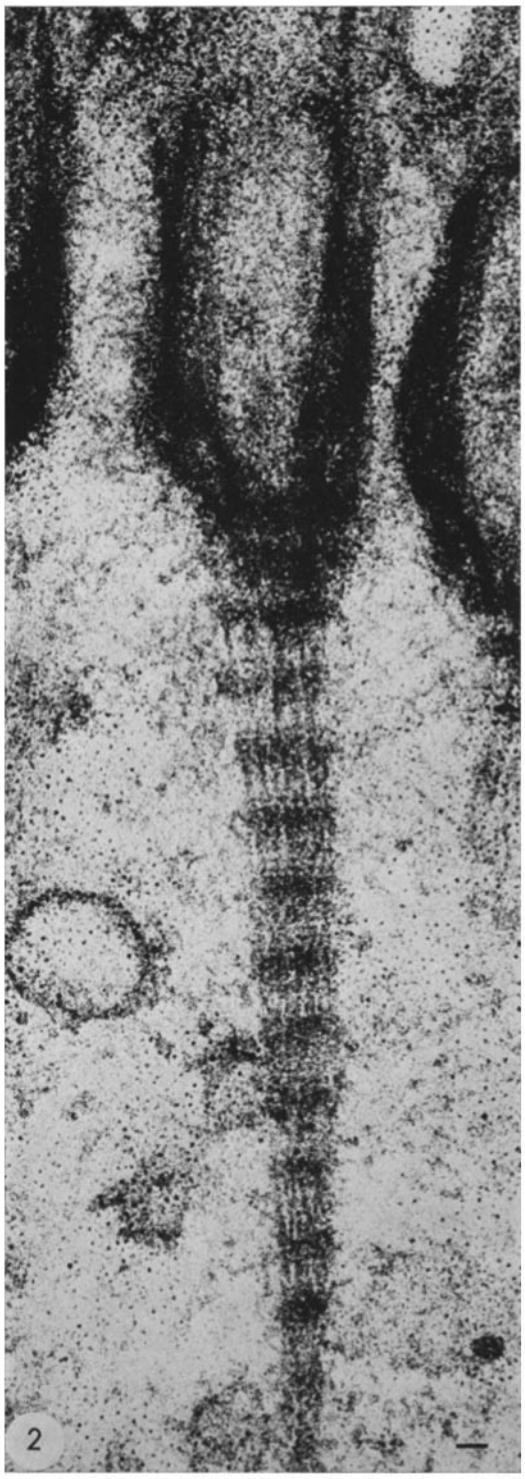


FIGURE 2 The fibrous construction of the rootlet is seen in the longitudinal view. The periodic dense regions probably correspond to plates of material which transect the rootlet. Scale 500 A. × 95,000.

structures described by numerous other investigators (13, 15, 22, 30, 34, 35); however, the alar sheets have never been described before, although in some views they resemble transitional fibers (20, 34).

Organization of the Tubule System

An examination of Fig. 1 reveals that the triplet angle decreases from base to apex of the organelle. Measurements of the triplet angle in the base, midregion, and apex compared with the outside diameter (C tubule to C tubule) and lumenal diameter (A tubule to A tubule) in these regions are presented in Table I. Since the continuous angle reduction of 30° is accompanied by a decrease in the outside diameter of 85 mμ, the angle change must be due to a centripetal rotation of each triplet on the longitudinal axis of the A tubule. In addition, the decrease in lumenal diameter indicates that the whole triplet is so positioned in the wall that it slants toward the center of the lumen as it traverses from base to apex.

Several investigators have noted that a basal body or centriole in cross-section rarely has all nine triplets sharply in focus (6, 7, 14, 19, 22, 32). This is the usual pattern in transverse views of oviduct basal bodies (Fig. 6). This has been interpreted to mean that the triplet sets do not run parallel to the longitudinal axis of the basal body but follow a helical path instead (6, 7, 14, 19, 32). It has also been suggested that this configuration occurs because the tubules do not run parallel to each other through the entire length of the organelle (22)

If each triplet set followed a helical path along the longitudinal axis, then in a transverse view the tubules on one side of the basal body would leave the plane of section in one direction and those on the other side would exit in the opposite direction. If such a section were tilted in relation to the electron beam, the tubules pitched in the direction of the tilt would become less sharp because the incident electron beam would no longer pass through their lumens. After the tilt, the view would be through the inner walls of the tubules. However, on the other side of the basal body in this tilted view, the lumens of the tubules would become more aligned with the path of the electron beam and the outlines would appear sharper.

Fig. 7 *b* is a transverse section of a centriole

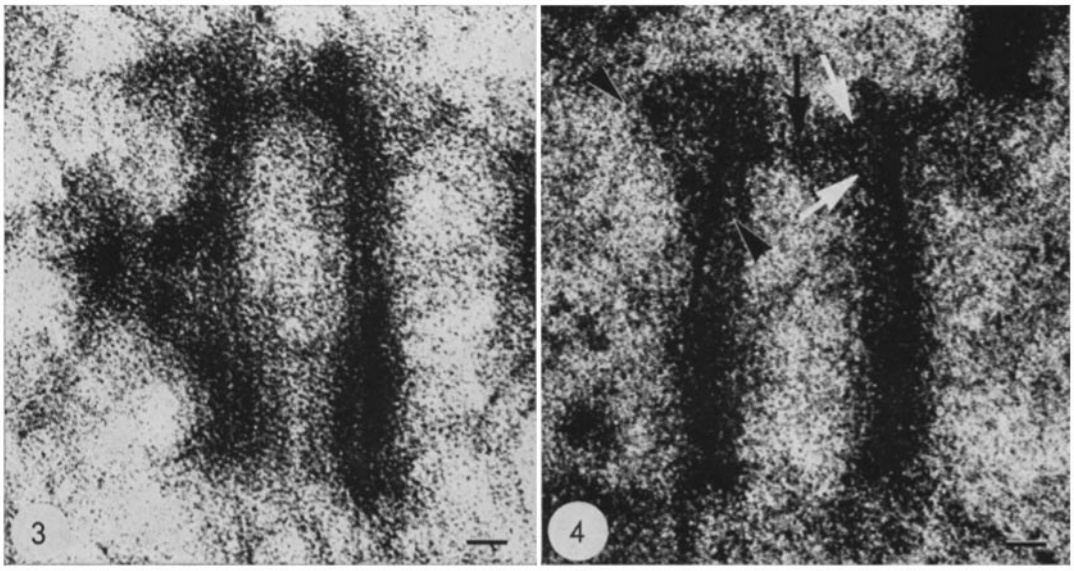


FIGURE 3 Longitudinal section of a newly formed basal body showing the basal foot. The wall of the foot is arranged into light and dark regions. Scale 500 A × 110,000.

FIGURE 4 The full sheet morphology is seen in the longitudinal view of the alar sheet. The angle of the section is such that the unfolding of the sheet at its base (darker region below a line connecting the two arrow heads) can be clearly seen. Also, the fibrous substructure of the apical ring structure is seen in the apex of the lumen. The horizontal fiber (black arrow) is the fiber which encircles the lumen in transverse view (Fig. 15 *c*), while the fibers running at an angle (white arrows) from the horizontal fiber to the triplet units are the secondary fibers. Scale 500 A × 120,000.

TABLE I

Measurements of the Triplet Angle in the Base, Midregion, and Apex Compared with the Lumenal Diameter and the Outside Diameter

	Triplet angle	Lumenal diameter	Outside diameter
		mμ	mμ
Base	40°	150	250
Midregion	30°	140	200
Apex	15°	130	165

engaged in basal body production (5). The section is through the midregion of the centriole, and almost all of the triplets are clearly seen When the stage of the microscope was tilted backwards 6° (Fig. 7 *a*), the triplets on the left side of the centriole no longer appeared sharp whereas those on the opposite side became sharper. Tilting the stage in the reverse direction 6° made the formerly sharp tubules appear fuzzy and the opposite tubules sharper (Fig 7 *c*). The tubules on each side of the tilting plane respond to the tilt as if they were pitched in opposite directions. This experiment establishes that each triplet is pitched, and the response of the tubules to the direction of tilt indicates that the triplets are pitched to the left (clockwise in the apex to base view).

Scale sections of the model can be manipulated in the same way as the centriole in Fig. 7. Fig. 8 shows a transverse section of a plastic-embedded model tilted backward (Fig. 8 *a*), and forward (Fig 8 *c*), and untilted (Fig. 8 *b*). Notice that in the untilted section the tubules are uniformly illuminated whereas the tubules on the left in the backward-tilted section or the tubules on the right in the forward-tilted section no longer transmit as much light. As in the case of the real centriole, the change in transmittance of light is due to the displacement of the tubules away from the axis of the light source The model was constructed so that the tubules were pitched from the longitudinal axis in a clockwise direction (apex to base view). Since it responded to tilting in the same way as the centriole (Fig. 7), the tubule

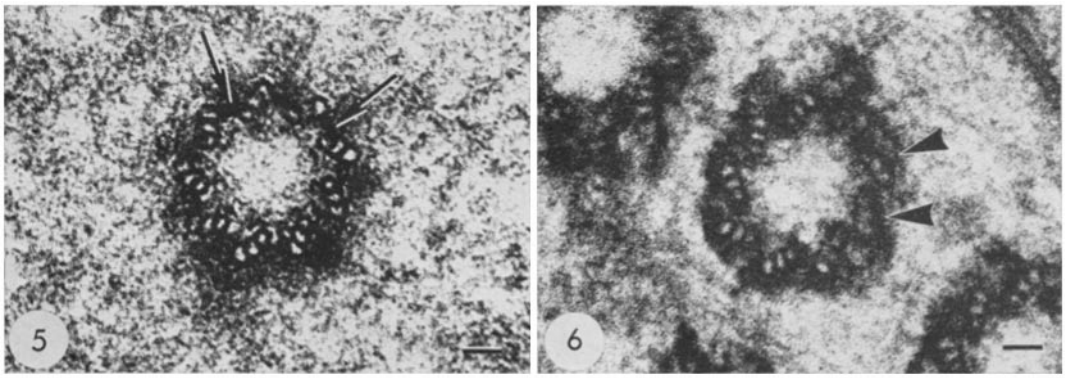


FIGURE 5 Transverse section through the base region of a newly formed basal body. The lack of intertubular amorphous material at this stage allows one to see the A-C tubule connectors (arrows). Scale 500 A. × 115,000.

FIGURE 6 Typical transverse view of a basal body. The unclear triplets (arrow heads) are tubules that have been sectioned obliquely so that the electron beam does not pass through their lumen Since oblique and transversely sectioned tubules occur within the same section of a basal body, the longitudinal axis of at least some of the triplets must not run parallel to the long axis of the basal body. Scale 500 A. × 115,000

system of the real structure must be similarly arranged.

Other views of the basal body establish the degree of pitch Fig. 9 *a* is a fortunate longitudinal section which grazes through either the front or back tubules but contains mostly side tubules These act as a reference for the longitudinal axis of the basal body; it is obvious that the tubules in the back wall are disposed at a 10°–15° angle to this axis (arrows). Fig. 9 *b* is a model constructed to represent a longitudinal section through a whole model, each set of tubules was pitched 14°. The set in the back wall appears to be arranged like those seen in a grazing section through an actual basal body (Fig. 9 *a*), sections of models constructed with unpitched tubules do not show slanted tubules in this part of the wall

The longitudinal axis of an obliquely sectioned basal body (Fig 10 *a*) is represented by a line that bisects the ellipse created by such a section. Enough of the triplet sets can be seen in the front and back wall of this section to establish that they are oriented at a 10°–15° angle to the axis. Oblique sections of a model constructed with pitched tubules (14° from longitudinal) compare favorably with the actual oblique section (Fig. 10 *b*). The degree of accuracy of the pitch measurements from Fig. 10 *a* is indicated by the fact that measurements of tubule pitch in the photograph of an oblique section through the model give values of 10°–15° even though the model was constructed with tubules pitched 14°.

Although the evidence for a uniform helical arrangement of the tubules is substantial, transverse photographs showing nonuniform triplet clarity (Fig. 6) could be obtained if only a few of the wall tubules were pitched, i e , if not all of the tubules ran parallel to each other throughout the length of the basal body (22). However, models constructed with mixed pitched and unpitched tubules (Figs. 12 *b*, 12 *c*) show that the circumferential spacing between triplets is not uniform throughout the length of the structure Since transverse sections of basal bodies always show a uniform distance between triplet sets, the tubules must run parallel to each other from base to apex Transverse views like Fig 6 occur when the plane of section is not exactly perpendicular to the longitudinal axis of the structure

Oblique sections provide the most reliable view for detecting and measuring the helical arrangement of the triplet sets. However, in a few cases, oblique sections of basal bodies do not show slanted tubules. Either some basal bodies are constructed with unpitched triplet sets or the pitch varies during the functional life of the organelle.

Some longitudinal sections show the walls tapering from base to apex, giving the structure the appearance of a truncated cone (Fig. 13 *a*). In

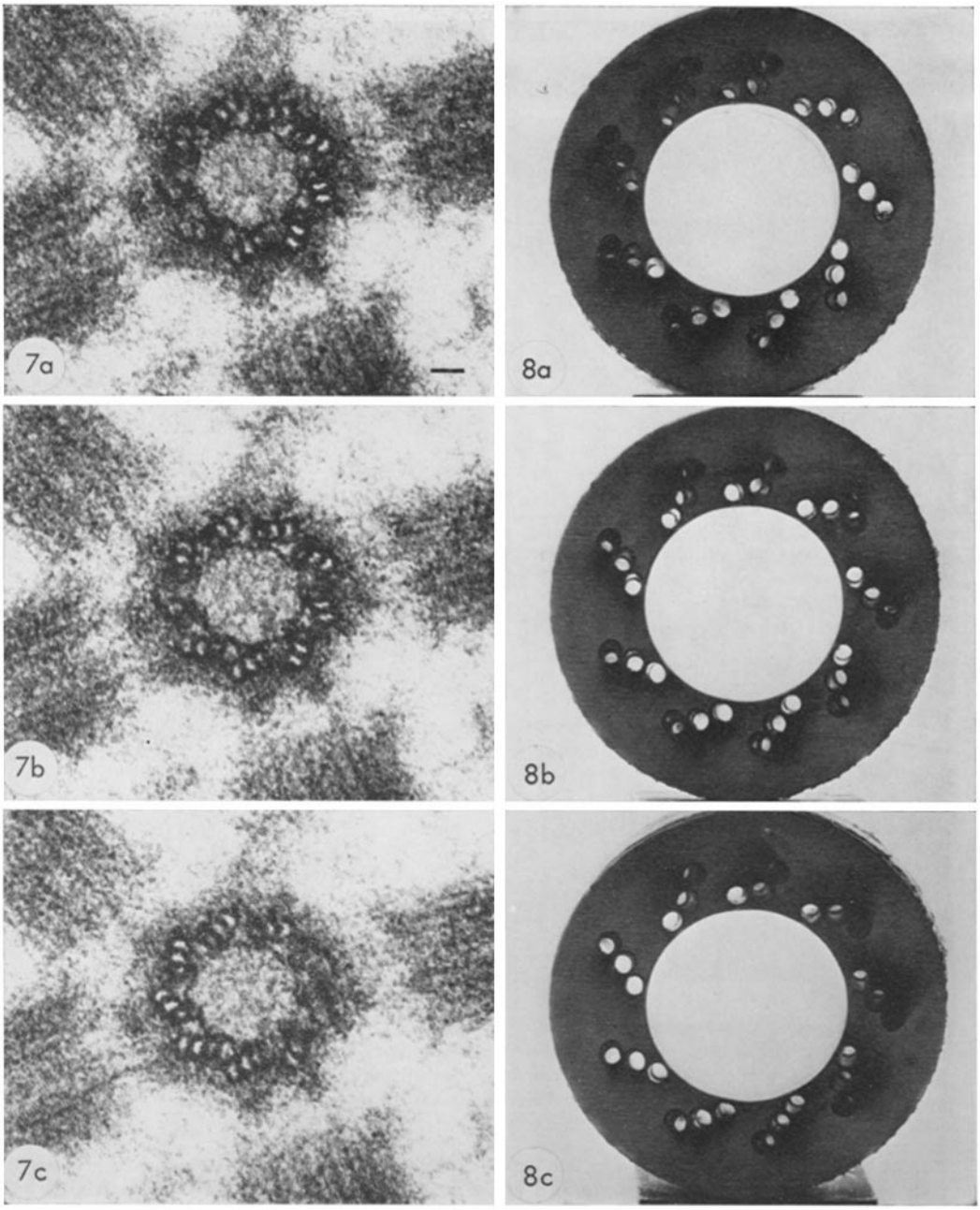


FIGURE 7 The middle photograph is of a diplosomal centriole (structure same as a basal body) sectioned so that the transverse axis is perpendicular to the axis of the electron beam. Almost all of the tubules are clearly seen. Tilting the stage of the microscope backwards 6° (*a*) causes the tubules on the left to appear fuzzy, whereas tilting the stage forward 6° (*c*) causes the tubules on the right to appear fuzzy. The tubules on the opposite side in each case are sharper than in the level section (*b*). Scale 500 A. × 100,000.

FIGURE 8 Three views of a scale transverse section through a scale model which demonstrates that the model responds to a backward tilt (*a*) and a forward tilt (*c*) in a similar way to the section through the centriole (Fig. 7). Since the model was constructed with each triplet pitched clockwise (apex to base view), the triplets of the basal body must be pitched in the same direction. The similar response of the model and the real structure to the tilting also confirms that every triplet set in the basal body is pitched.

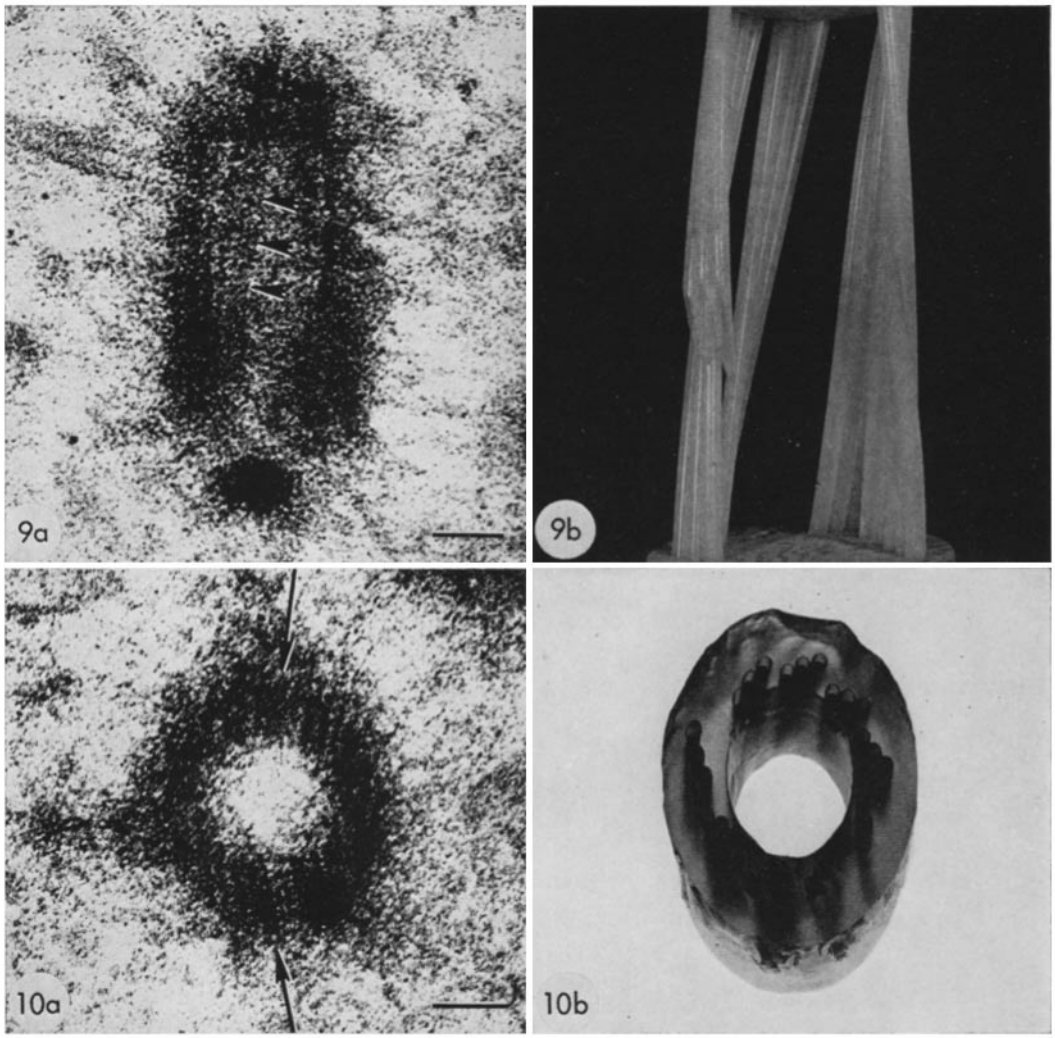


FIGURE 9 A model (*b*) constructed to represent a longitudinal section that includes side and back walls is compared with a similar longitudinal section through a basal body (*a*). The slanted arrangement of the tubule in the back wall of the model (14°) compares favorably with the tubule slant seen in the micrograph of the basal body (arrows) This slanted arrangement is not evident in models that are constructed with unpitched triplets. Scale 1000 A. × 95,000.

FIGURE 10 Comparison of an oblique section through a model (*b*) with a similar section through a basal body (*a*). The model was constructed with each triplet set pitched 14° from the longitudinal axis of the structure. The tubules in the model appear to be as slanted as those in the basal body, an indication that the pitch of each basal body triplet set is 14°. In addition, lines drawn parallel to the long axis of the front and back triplet sets (arrows) of the basal body (*a*) intersect to form a 20°–30° angle, therefore, each triplet is pitched 10°–15° from the long axis of the organelle. Scale 1500 A. × 105,000.

other sections, the diameter of the organelle is greater in the middle than at the two ends, a barrel-shaped configuration (Fig. 14 *a*). Figs. 13 *b* and 14 *b* are comparable pictures of the whole model showing that both barrel-shaped and truncated profiles can be obtained, depending on how the model is oriented for photography. In these photographs, if some of the front and back tubules of the model were removed, the replica would appear identical with the longitudinal

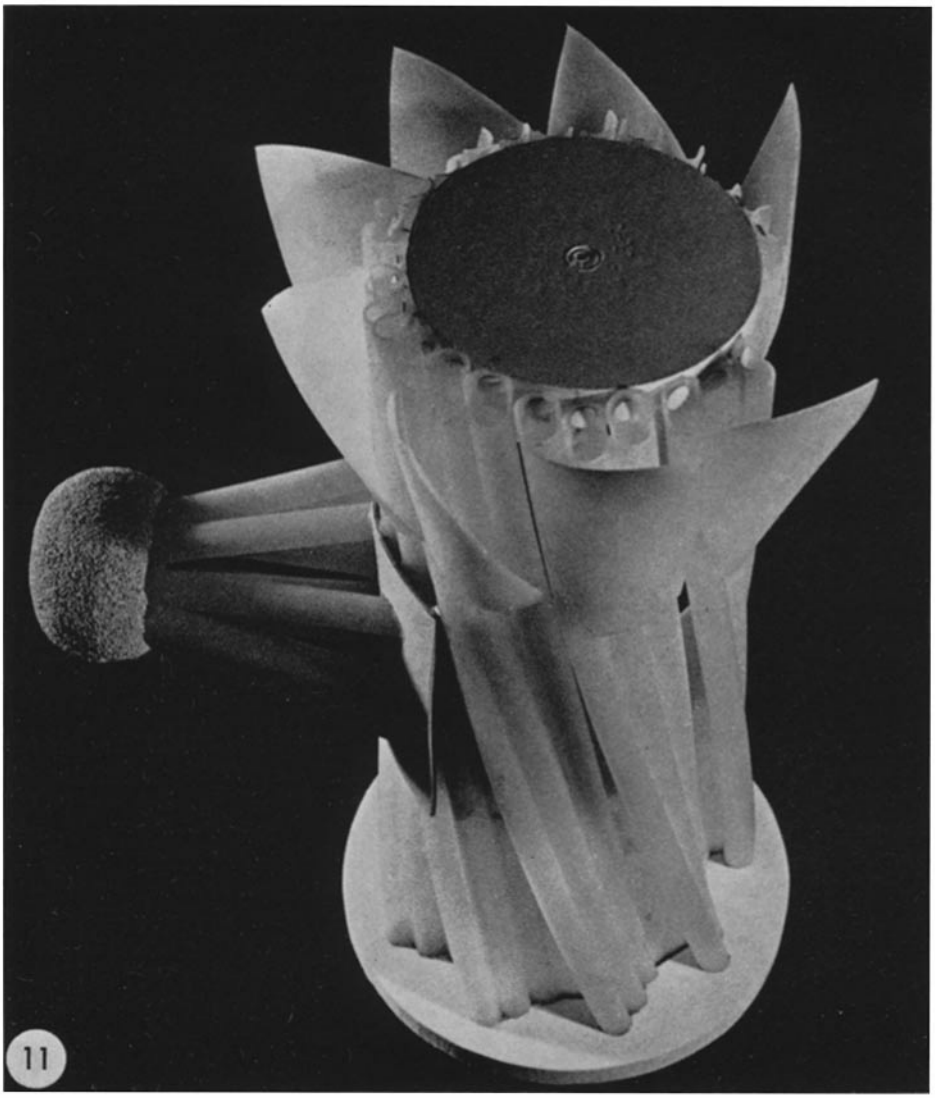


FIGURE 11 Scale model of the oviduct basal body (1 inch = 330 A).

sections of the real structure. Therefore, the two different configurations represented by Figs 13 *a* and 14 *a* can be produced from structurally identical basal bodies, depending on the position of the longitudinal section along the transverse axis of the organelle.

One would expect from the data (Table I) that all longitudinal sections would show the truncated cone configuration. However, a study of the model showed that the barrel-shaped geometry is due to the combination of a pitched arrangement of the tubules and a continuous base-to-apex centripetal rotation of each triplet set on the longitudinal axis of the A tubule. As further evidence, a model constructed with tubules that run parallel to the long axis of the structure (unpitched tubule arrangement) never shows a barrel-shaped profile, regardless of how it is oriented Fig 12 *a* is a photograph of a model constructed with pitched (left side) and unpitched tubules. Notice that the wall appears barrel-shaped on the left side but truncated on the right side.

Accessory Structures

The rootlet is a tapering bundle of longitudinally arranged fibers embedded in a light amorphous

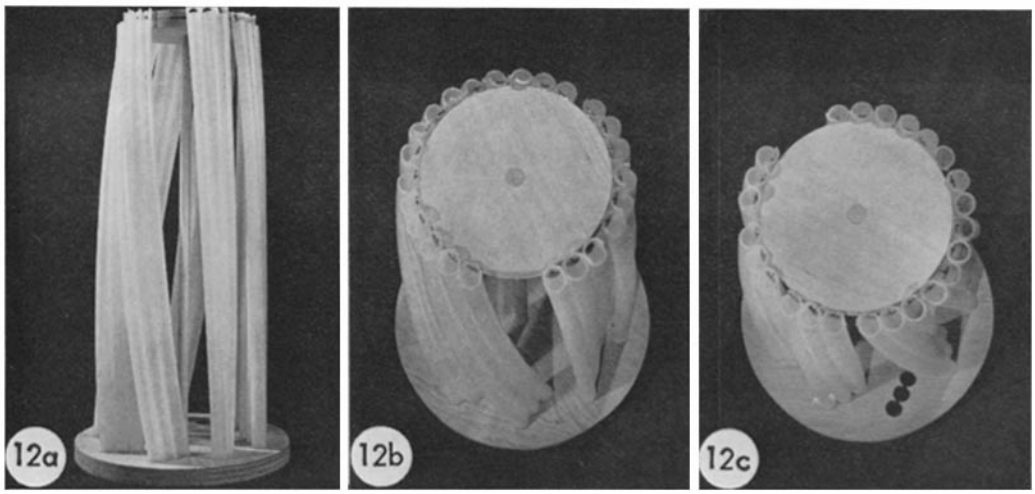


FIGURE 12 Three views of a model constructed with five triplets pitched 14° and three unpitched. Fig *a* shows that the barrel-shaped appearance of some longitudinally sectioned basal bodies (Fig. 14 *a*) is in part due to the pitched configuration (left side) of the triplet set. Going clockwise in Figs. *b* and *c* at the transition between pitched and unpitched triplets (*c*), there is not enough room for one of the triplets at the apex of the structure. Likewise, at transition between unpitched and pitched (*b*), a space appears at the apex which is not present at the base. Since the spacing between triplets is uniform in a basal body, the triplets in the actual organelle must be all pitched or unpitched at any one time.

material (Figs. 1, 2) The attached end is continuous with the cylindrical wall of the trunk and the whole structure gradually decreases in diameter as it extends into the cytoplasm. The fibers are not continuous throughout the length of the rootlet At periodic intervals (approximately 700 A), 550 A thick bands are perpendicularly oriented to the longitudinal axis of the rootlet. These bands are probably dense plates which transect the rootlet at these points.

The basal foot is a cone measuring 150 mμ wide by 130 mμ high (Figs. 1 *f*, 1 *g*, 3). The base attaches to two or three of the triplet sets within the wall. Rods of fibrous material approximately 100 A in diameter extend at approximately a 55° angle from the walls of the basal body (Fig. 3). All of these fibers are surrounded by an amorphous matrix which periodically varies in density from apex to the base of the foot. The rods of material merge like the struts of a tepee into a spherical mass about 125 mμ from the wall. From this spherical structure, microtubules often splay out into the cytoplasm.

In the upper apical region, the lumen contains an accumulation of amorphous material approximately 500 A thick (Figs. 1 *a*, 1 *b*, 3, 4). Embedded in this material is a thin fiber that encircles the lumen approximately 150 A from the A tubules (Fig. 15 *c*). Nine secondary fibers extend radially from the circular fiber, one to each A tubule. These secondary fibers extend at an angle from the circular fiber (Fig 4). This apical ring structure is shown diagrammatically in Fig. 16

The alar sheet accessory structures are nine pieces of material, each of which is attached to a triplet set in the apical region The appearance of these appendages changes with the level of the transverse section. In a section that includes the upper midregion and lower apical region (Fig 1 *d*), a broad, triangular-shaped piece of material projects radially 70 mμ counterclockwise (apex to base view) from each triplet set. The clockwise edge of the triangle, which is very sharply defined, intersects the transverse axis of the triplet set at the C tubule to form a 50° angle. Sections that include mid- and lower portions of the apex (Fig 1 *c*) have thinner triangles extending from the triplet set 90–100 mμ, and the clockwise edge now intersects the triplet axis at 60°–65°. The material does not appear triangular in sections through the upper apex (Fig. 1 *b*), instead fibers seem to extend 120 mμ from each C tubule. Finally, at the level of transition from basal body to cilium (Fig. 1 *a*),

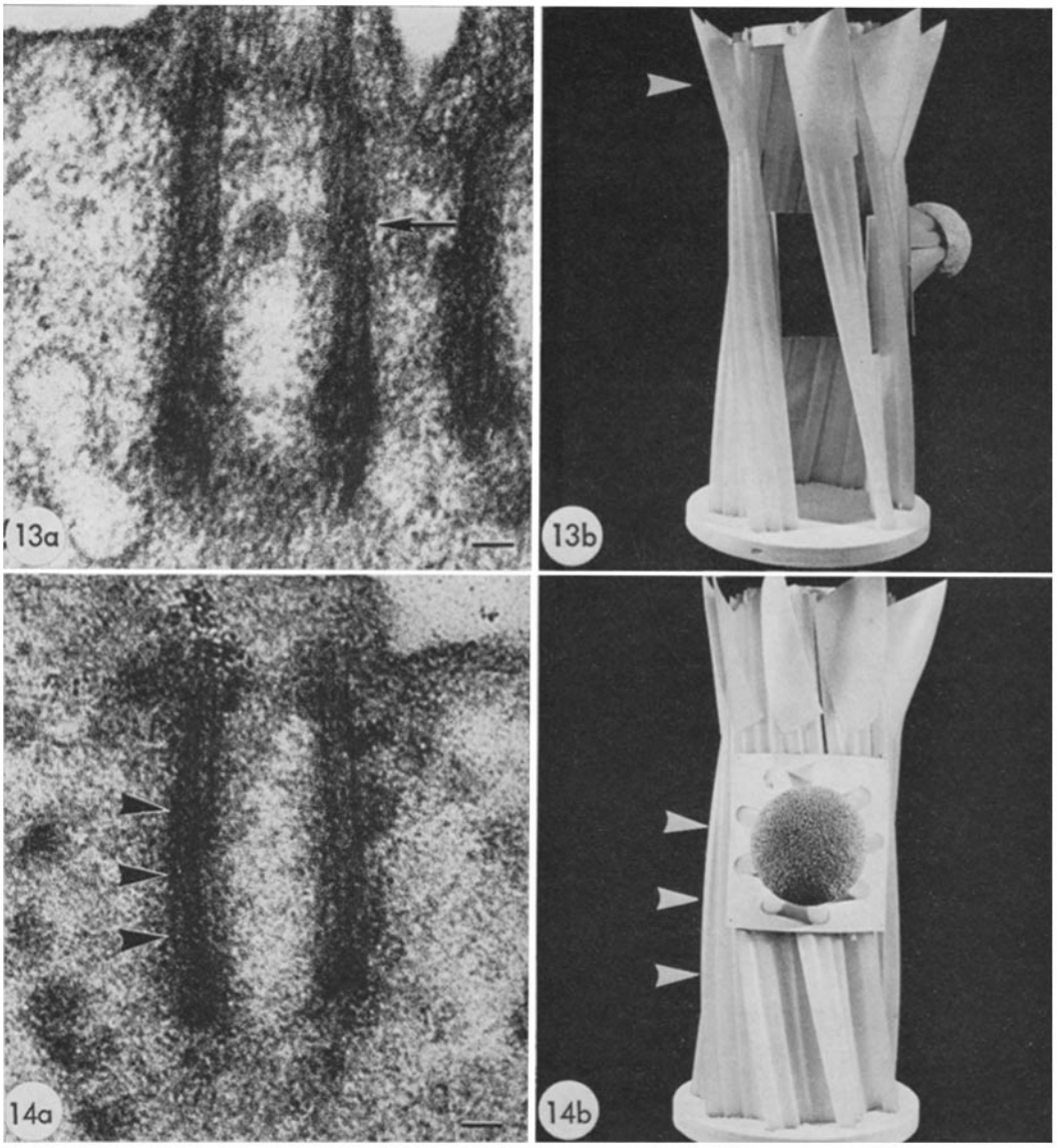


FIGURE 13 The model (*b*) can be oriented so that the walls appear to taper from base to apex. This truncated cone appearance is sometimes seen in longitudinal sections of a basal body (*a*) Also, in Fig. *a*, the arrow points to region where the triplet set leaves the plane of section, indicating that the triplets do not run parallel to the long axis of the organelle. The longitudinal view of the model alar sheets in Fig. *b* (arrow head) is quite similar to the longitudinal view of the actual structure (Fig 4). Scale 500 A. × 100,000.

FIGURE 14 The model (*b*) can also be oriented so that the walls appear barrel-shaped, i e , the outside diameter appears greater in the middle than at the ends of the structure (arrows). Longitudinal sections of the basal body (*a*) often show this type of wall arrangement (arrows). Scale 500 A. × 115,000

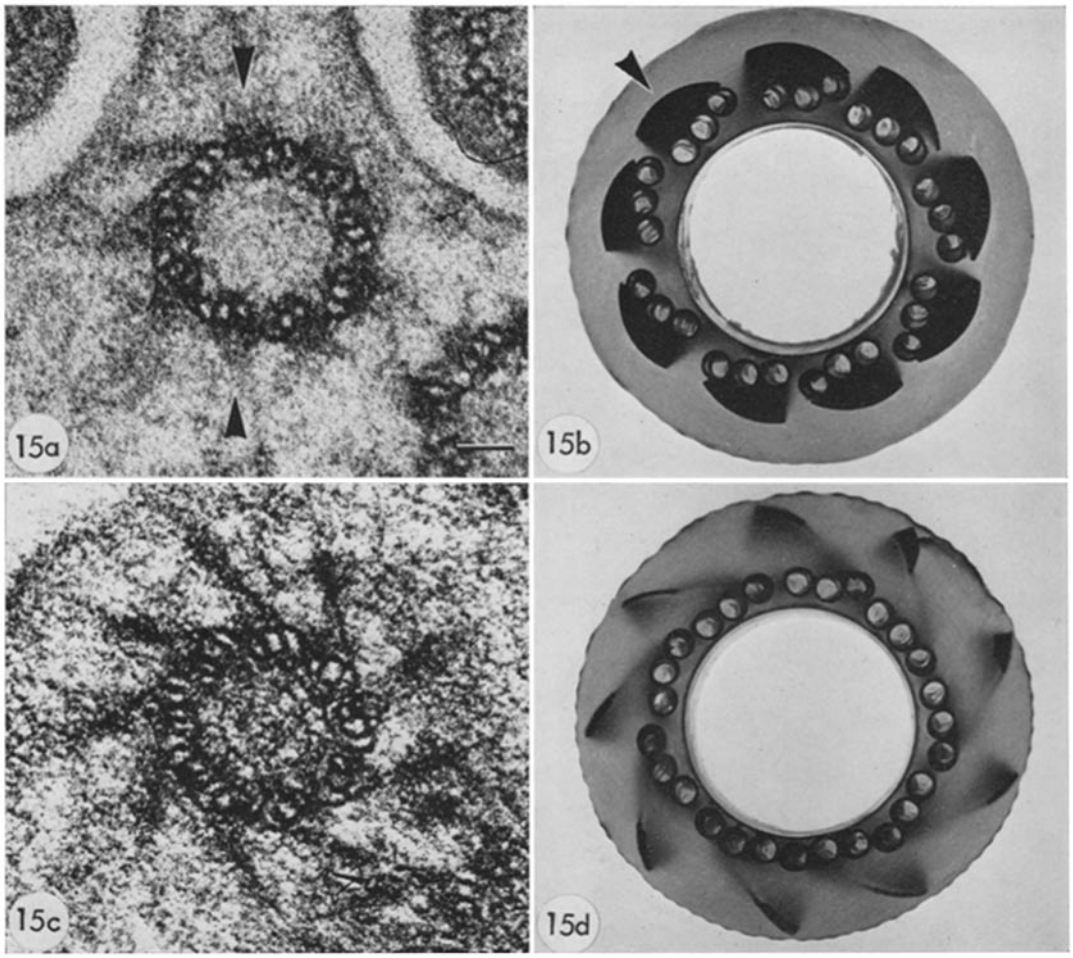


FIGURE 15 Two sets of photographs comparing transverse sections of the actual alar sheet (*a*, *c*) with two serial sections of model alar sheets constructed like those seen in Fig. 11. Set *a-b* compares transverse views of the base region of the sheet (arrows), while set *c-d* compares the apical region. Some artifacts introduced during the construction of the model alter the appearance of the model sheet; for example, the model sheets appear curved in Fig. 15 *b*. However, the comparison does show that the model sheets appear in transverse view like the actual sheets, thus confirming the structural interpretations presented in the text. Also, a transverse view of the apical ring structure is present in Fig. *c*. A fiber encircles the basal body lumen 150 A from the A tubules. On the left side, a secondary fiber can be seen extending to the A tubule. Scale 500 A. × 135,000.

plaques of material 130 mμ away from each triplet set merge with the ciliary membrane.

The third dimension of this accessory structure is seen in the longitudinal view (Fig. 4). The structure appears to be a 170 mμ long sheet of material shaped like an equilateral triangle, the base is at the apex of the basal body, and one side is attached to the wall of a triplet set. Below a line extending between two arrow heads in Fig. 4, the sheet appears denser than above, as if it were double thickness in this region. Their wing-like appearance has suggested the name alar sheets.

From these various views, it is deduced that the alar sheet is a trapezoid sheet attached to the three tubules of the triplet set by its basal edge (the smaller of the two parallel sides) and to the C tubule by one of its entire longitudinal edges (the equal antiparallel sides). The sheet faces to the right and gradually unfolds from base to apex, creating a variable angle with the transverse axis

of the triplet set. The outside corner merges with the ciliary membrane. Thus, the triangular appearance in transverse sections through the lower part of the sheet (Fig. 1 *d*) is due to the unfolding of the sheet, likewise, in longitudinal views the unfolding portion shows as a region (below plane of two arrow heads, Fig. 4) that is denser than the rest of the sheet

Manipulation of the scale model substantiates the interpretation of alar sheet morphology. Each replica was a sheet of polyethylene shaped like a long trapezoid, i.e., the sheet was wider at one end. It was attached to the triplet set by two edges—the clockwise lateral edge to the C tubule and the basally located edge, or the narrow side, to the whole triplet set. Since the other two edges were free, the whole sheet unfolded from base to apex (Fig. 11) The unfolding was emphasized by the increase in width of the sheet at its apical end. In a longitudinal view (Fig. 13 *b*), the overlap created by this unfolding of the alar sheet looks very similar to the overlapping of the real sheet seen in longitudinal sections of the basal body (Fig. 4). When a replica of the apical region is embedded in plastic and serial transverse sections are made (Figs 15 *b*, 15 *d*), the appearance of the sheets in both sections is similar to the actual sheets sectioned at the same level (Figs. 15 *a*, 15 *c*). Although there are some discrepancies between the appearance of model and real sections of the alar sheets, it is quite clear that this accessory structure is a sheet rather than a fiber

In longitudinal sections, the alar sheets often look like fibers running obliquely from the basal body wall to the cell membrane, typical of the descriptions given by other investigators (13, 15, 20, 22, 34) The sheets appear this way because they are thin (50 A) and tend to blend with the surrounding cytoplasm; thus, only the transition between sheet and cytoplasm—the outer edge—is distinguishable. Probably the plane of section also contributes to this optical illusion. The alar sheets of isolated basal bodies (3), where the cytoplasm has been removed, always appear as sheets, rather than fibers.

DISCUSSION

In general, the structure of the triplet unit and its position in the wall is the same for all basal bodies and centrioles that have been studied (19). The triplet angle has been noted in transverse sections of all such organelles, and the direction of this angle in relation to the longitudinal axis is always the same (21, 29). In addition, the base to apex reduction in the angular disposition of the triplet set has been established for several different centriolar structures (11, 34, 38).

The base to apex decrease in the triplet angle must produce corresponding dimensional changes in the body of all organelles that have this type of tubule organization. Depending on how the triplet angle changes, there are three possible types of dimensional change: (*a*) an angle change caused by a centrifugal twisting of the transverse axis on the longitudinal axis of the C tubule will cause an increase in the lumenal diameter; (*b*) a transverse twisting on the longitudinal axis of the B tubule will cause a decrease in the outside diameter and a corresponding increase in the lumenal diameter, and (*c*) a centripetal twisting on the longitudinal axis of the A tubule will cause a decrease in the outside diameter. Regardless of the type of angle change that actually occurs, the theoretical dimensional changes can be calculated. Fig. 17 shows a trigonometric analysis of the relationship between angle change and basal body diameter change. The calculations assume a 30° angle change and a triplet set transverse axis length of 600 A According to these calculations, under ideal measuring conditions one should detect a base to apex change in either the lumenal diameter, outside diameter, or both, of about 560 A.

Several criteria have been used to determine the nature of the triplet angle change in this study. The truncated appearance of the basal body in a longitudinal section (Fig 13 *a*) could only occur if the outside diameter were greater at one end than the other. If the cilium-basal body junction is in the same longitudinal plane as the basal body (Fig. 13 *a*), the diameter of the proximal part of the cilium is equal to the distal diameter of the basal body but less than the proximal diameter of the basal body. The proximal diameter of the basal body must decrease if the organelle is to be continuous with the cilium. The diameter decrease as measured in serial transverse sections is about 850 A (Table I) Part of this change (~200 A) is due to a decrease in the lumenal diameter. The 30° change in the triplet angle results in an approximate 650 A change in the outside diameter. This concurs with the theoretical change of about 560 A.

Although there is evidence for centripetal rotation of the triplet set in other basal bodies (11,

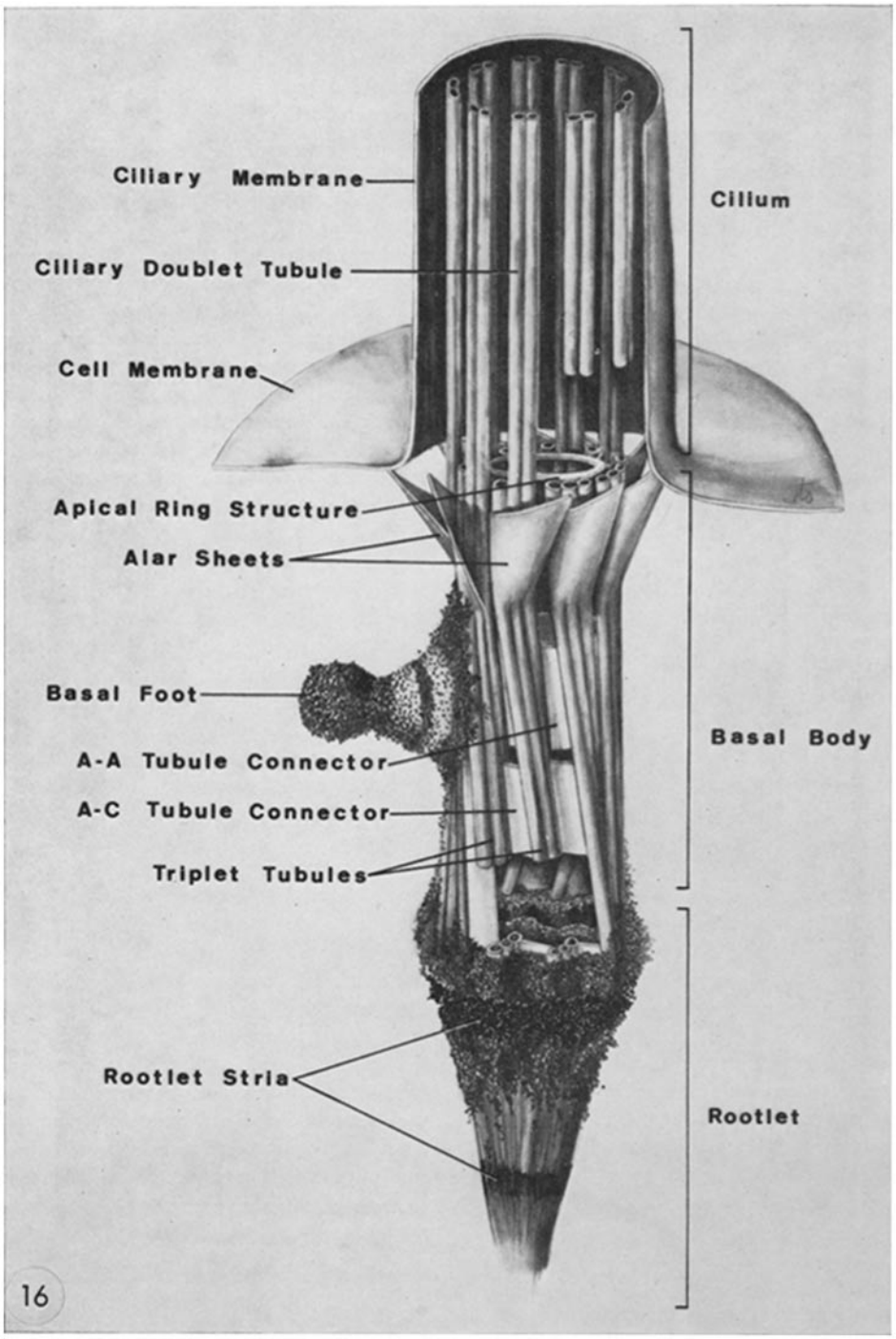


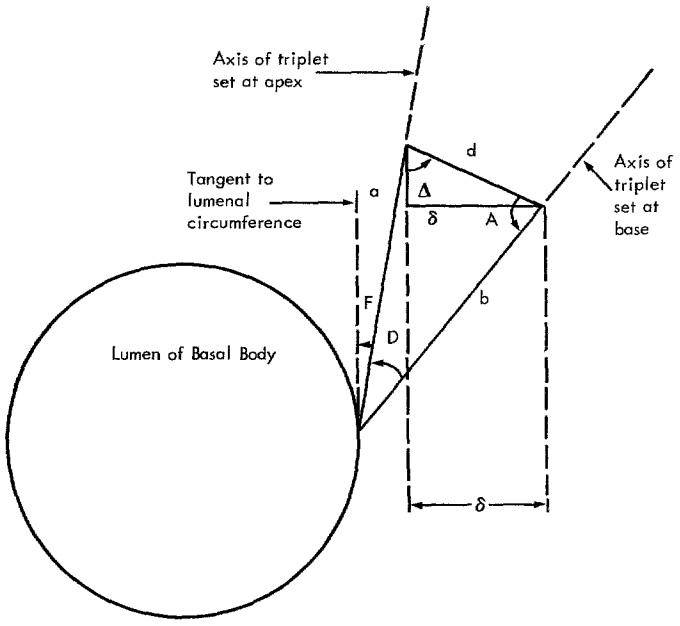
FIGURE 16 Three-dimensional diagram of the basal body showing the relationship of the organelle to the basal portion of the cilium and the cell membrane. The structural details of the cilium are not included in this drawing.

22, 25), different conclusions were reached in two other studies on the structure of centriolar organelles (20, 38). In a classic study on basal body structure in certain flagellates, Gibbons and Grimstone (20) compared the outside diameter of the basal body with that of the cilia and found that they were equal They concluded that the triplet angle change (~40°) was the result of a centrifugal twisting of the triplet set on the longitudinal axis of the C tubule. However, since they did not compare the proximal diameter with the distal diameter of the basal body, it was not clear in what region of the organelle the measurements were made for the comparison with the cilium diameter. Obviously, if the basal body diameter was measured near the distal end, it would be nearly equal to the cilium diameter, even though the C tubule does not continue into the cilium. On the other hand, quite possibly the angle change could be different in flagellate basal bodies since the structure of this organelle differs in other respects from that of oviduct basal bodies. Similar conclusions were presented by Stubblefield and Brinkley (38) for the angle changes in fibroblast centrioles. These investigators measured a proximal to distal angle change of 47° accompanied by an increase in the lumenal diameter of 350 A; the outside diameter was uniform from end to end. Although the tubule arrangment in the fibroblast centriole could differ from that in oviduct basal bodies, two observations should be considered. As far as can be determined, the construction of the cylindrical trunk of the oviduct centriole is like that of the trunk of the basal body, and it seems unlikely that such a major difference in tubule arrangement would exist between two mammalian centrioles. More importantly, if the triplet angle change is 47° in the fibroblast centriole, then according to the calculations in Fig. 17 the lumenal diameter increase should be considerably greater than 350 A.

The results of this study firmly establish that the longitudinal axis of each triplet set follows a helical course in the wall of the oviduct basal body According to measurements on oblique sections, the triplets are pitched 10°–15° from the longitudinal axis of the organelle, and tilting experiments show a uniform pitch for each triplet in the same direction as the triplet angle. Clearly, in a transverse section which is exactly perpendicular to the longitudinal axis of the basal body, all of the triplets will be sectioned obliquely (a 10°–15° angle of obliqueness). Therefore, slight deviations from perpendicularity will increase the angle of obliqueness through some triplets and diminish the angle for triplets on the opposite side. The result is that rarely do all the triplets appear clearly in transverse sections of the basal body.

Gibbons was the first to note that not all of the triplets are clearly seen in transverse sections of molluscan basal bodies (22). In his opinion, this was because they do not run parallel to each other in the wall However, the models constructed in the present study show that if this were true the intertriplet spacings would be greater in the regions where triplets change from a parallel to an antiparallel arrangment; this is not seen in transverse sections of molluscan basal bodies. More than likely when triplet tubules are nonuniformly clear in a transverse section, the basal body or centriole has helically arranged tubules.

André (6) and André and Bernhard (7) came to similar conclusions about the disposition of the triplet sets in centrioles. Fawcett (14) and Phillips (32) extended these observations to other centrioles and to insect basal bodies Although numerous other studies on basal bodies and centrioles have not described helically organized triplets, micrographs of transverse sections presented by these investigators indicate that triplets may be arranged this way. Nonuniformly clear triplet tubules are seen in micrographs of centriolar structures from such unrelated organisms as the unicellular flagellates, *Euplotes eurystomus* (23), a marine protist, *Labyrinthula* (31), the jellyfish *Phialidium gregarium* (39), the fungus *Anthoceros laevis* (27), and the rat trachea (37). Oblique and longitudinal photographs of fibroblast centrioles show similarly arranged tubules (38). In contrast, transverse micrographs of kinetosomes from *Tetrahymena* (1), *Paramecium* (12), *Tokophrya infusionum* (26), and *Trichonympha* (20) do not intimate helically arranged triplets In these latter studies transverse sections that indicated a pitched tubule organization could have been improperly classified as poor sections and therefore ignored. Wolfe has studied negatively stained whole kinetosomes from *Tetrahymena* (42), and some of his photographs show triplet units overlapping as if they were helically disposed in the wall of the organelle (Fig 8, page 695 and Fig. 13, page 697 of reference 42). On the other hand, photographs from a more recent study on whole *Tetrahymena* kinetosomes (28) do not show this overlapping phenomenon, so that the appearance in Wolfe's micrographs may be a preparation artifact This aspect



Known Values	Calculations for value d	Calculations for value δ
a = 600 A	$\frac{d}{\text{Sin } D} = \frac{a}{\text{Sin } A}$	$\text{Sin } \Delta = \frac{\delta}{d}$
b = 600 A	$\frac{d}{\text{Sin } 30°} = \frac{600 \text{ A}}{\text{Sin } 75°}$	$\text{Sin } 65° = \frac{\delta}{310 \text{ A}}$
D = 30°	$\frac{d}{0.5000} = \frac{600 \text{ A}}{0.966}$	$0.906 = \frac{\delta}{310 \text{ A}}$
Δ = 65°	d = 310 A	δ = 280 A
A = 75°		
F = 10°		

FIGURE 17 Theoretical formulation of the expected basal body diameter change due to the reduction in the triplet angle. In the diagram, line *a* represents the transverse axis of the triplet set at the apex (angle = 10°) and line *b* the axis of the same set at the base (angle = 40°). Therefore, the triplet angle change, 30°, is the angle between line *a* and *b* (*D*). Since triangle *abc* is an isosceles triangle, $A = 75°$. Because $F = 10°$, $\Delta = 65°$. The value of δ equals one-half of the total outside diameter change (measuring from C tubule to C tubule); therefore, the total theoretical diameter change $2\delta = 560$ A. Similar calculations could be made if the A or the B tubule changed position rather than the C tubule.

of kinetosome structure should be clarified because of the evolutionary and functional implications of centriolar structure.

In addition to the nine triplet units, the basal body wall contains two sets of intertriplet connector sheets and an amorphous material surrounding all of the triplet sets. These elements seem to be ubiquitous components of centrioles and basal bodies (19). During the construction of the model, it was found that the intertubule linking material could easily function as a stabilizing structure to maintain the position of the triplet units in the wall. The A-to-C linkers could maintain the position of the 40° triplet angle (12, 20) while the A-to-A linkers in the midregion could act as a collar to maintain tubules in their circular distribution. Wolfe (42) has shown that the A-C linkage is fairly strong. The apical ring structure also may

be important for maintaining tubule arrangement. Since this structure seems to be bound to each triplet set, it is analogous to a wooden plate used in the model to hold the tubules in position at the apex (Fig. 11). The amorphous material may be related to centriole replication or rootlet attachment; both functions are associated with the basal portion of the basal body where this material is the most prominent.

Except for the apical ring structure, the lumen of the average oviduct basal body does not contain any structures. However, different structures have been seen in this region at different times in the life history of this organelle During oviduct basal body formation (5), newly formed organelles have a cartwheel structure in the proximal half of the lumen. Shortly after the organelle is completely formed, this cartwheel breaks down to form a vesicle that eventually disappears. Finally, a certain percentage of the older basal bodies accumulate an electron-opaque material in the lumen.

Despite a recent statement to the contrary (19), the cartwheel structure is not present in all mature centriolar structures This lumenal structure is not found in metazoan basal bodies that have been described (13, 15, 22, 34, 37) and Turner (41) reports that in *Nitella* it disappears when the centriole transforms into a basal body. Some investigators have published micrographs of metazoan centrioles containing cartwheels (19), and Stubblefield and Brinkley (38) have concluded that this structure is an integral part of the fibroblast centriole. However, these studies have not satisfactorily established that the micrographs showing the cartwheel were of mature centrioles. In oviduct cells (5), newly formed basal bodies or centrioles retain the cartwheel established during procentriole formation but the cartwheel soon breaks down into a vesicle. Transverse sections of organelles at this stage can be easily misidentified as mature centrioles One must be certain of both the developmental stage of the organelle and the level of the transverse section before drawing conclusions about such a transient structure as the cartwheel.

The basal foot, the rootlet, and the alar sheets are attached to the wall tubules at specific sites, and these appendages accentuate the longitudinal and lateral polarity of the organelle. The basal foot and rootlet have a characteristic structure that seems to be common to most basal bodies found in metazoan cells (8, 13, 15, 18, 34, 36, 37). The alar sheets, however, have not been described before. They occupy the same position as the transitional fibers first described by Gibbons and Grimstone (20) and subsequently noted in a variety of basal bodies and centrioles (11, 13, 15).

Two observations indicate that transitional fibers in other metazoan basal bodies are actually alar sheets. The transitional fibers of frog olfactory (34), rat choroid plexus (13), and lamprey olfactory (40) basal bodies have the same triangular appearance in cross-section as the alar sheets; a sectioned fiber would not have this appearance. Second, geometric considerations suggest that if it were truly a fiber radiating outward from a point on the C tubule (~30° to the longitudinal axis [13]) and traveling upwards to join the cell membrane (a vertical distance of ~150 A [13]), then some transverse sections (~800 A thick) should show a discontinuity between the fiber and the C tubule Yet all published micrographs of transverse sections show continuity between the "fiber" and the C tubule. In fact, Reese (34) presents two adjacent serial photographs through transitional fibers of the frog olfactory basal body; both photographs clearly show the fibers joining the C tubule. This would not occur unless these transitional fibers were actually sheets. A more complete study should be made of this accessory structure in other basal bodies to verify this analysis

Much more information is required for an adequate analysis of basal body function. For example, what is the significance of the orientation of basal foot to the direction of beat (22)? Are the rootlets involved in coordinating interciliary activity (35)? If basal bodies are important for proper ciliary motion, how do many sperm flagella function without any basal bodies (32)? On the other hand, why do some nonmotile, sensory (8, 15, 40) cilia have complete basal bodies with accessory structures? The answers to these and many related questions will come with the development of more direct techniques for studying basal body activity.

The author wishes to thank Dr. Robert M. Brenner for his invaluable advice and assistance, Carole J. Wortley and Jacqueline Gellatly for their technical assistance, and Joel Ito for the drawings.

This is publication No. 585 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, United States Public Health Service Grant HD-02853 to Dr. Robert M. Brenner, and United States Public Health Service Grant FR-00163 to the Oregon Regional Primate Research Center.

Received for publication 19 October 1971, and in revised form 12 April 1972.

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