

ULTRASTRUCTURAL ORGANIZATION OF CILIA AND BASAL BODIES OF THE EPITHELIUM OF THE CHOROID PLEXUS IN THE CHICK EMBRYO

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ABSTRACT

Ultrastructural studies were performed on normal and abnormal cilia and basal bodies associated with the choroidal epithelium of the chick embryo. Tissues were prepared in each of several fixatives including: 1% osmium tetroxide, in both phosphate and veronal acetate buffers; 2% glutaraldehyde, followed by postfixation in osmium tetroxide; 1% potassium permanganate in veronal acetate buffer. Normal cilia display the typical pattern of 9 peripheral doublets and 2 central fibers, as well as a system of 9 secondary fibers. The latter show distinct interconnections between peripheral and central fibers. Supernumerary fibers were found to occur in certain abnormal cilia. The basal body is complex, bearing 9 transitional fibers at the distal end and numerous cross-striated rootlets at the proximal end. The distal end of the basal body is delimited by a basal plate of moderate density. The tubular cylinder consists of 9 triple fibers. The C subfibers end at the basal plate, whereas subfibers A and B continue into the shaft of the cilium. The 9 transitional fibers radiate out from the distal end of the basal body, ending in bulblike terminal enlargements which are closely associated with the cell membrane in the area of the basal cup. One or 2 prominent basal feet project laterally from the basal body. These structures characteristically show several dense cross-bands and, on occasion, are found associated with microtubules.

INTRODUCTION

The choroid plexus system in the chick embryo consists of four distinct concentrations of plexus tissue, one associated with each major brain ventricle. Histologically, the plexus consists of an inner choroidal epithelium, which remains contiguous with the ependyma, and an outer component of highly vascularized connective tissue, which is derived from the leptomeningeal membrane (4). In most areas, the choroidal epithelium, consisting of one principal cell type, varies from simple cuboidal to columnar, and is

associated with a well defined basement membrane.

In birds and mammals, the ventricular surface of the choroidal epithelium is complex, generally consisting of numerous microvilli and moderate concentrations of cilia. The cilia usually occur singly or in small groups, though the number and distribution may vary in different species (5, 7, 21, 33, 35). In the monkey and rabbit, Wislocki and Ladman (35) reported 11 to 16 cilia grouped centrally in the ventricular surface of each epithe-

lial cell. In the rat and opossum they found the epithelial cells to bear cilia grouped in single or multiple annuli, with 4 to 8 cilia per annulus. They reported cilia to be less abundant in the dog. In the chick embryo, vigorous ciliary activity has been demonstrated in cultured explants of the choroidal epithelium (6, 18, 31). Though exact determinations have not been made regarding the number and distribution of cilia in the choroidal epithelium of the chick embryo, it appears that most epithelial cells bear at least a few cilia. Counts as high as 15 to 18 cilia per cell have been made in unreported observations by the present authors.

The function most commonly ascribed to the choroid plexus involves a role in the production of at least a substantial fraction of the cerebrospinal fluid. Although the specific mechanism of fluid production remains somewhat enigmatic, the choroidal epithelial cells possess many features in common with cell types known to engage actively in fluid transport and/or secretory activity. Tennyson and Pappas (33) have reported that the microvillous cell surface, large concentrations of mitochondria, and the suggestion of pinocytotic activity may be important factors in ascribing a secretory function to the choroidal epithelial cells in the rabbit. Pease (24) has suggested that cells involved in fluid transport are characterized by extensive invaginations or interdigitations of the plasma membranes, such as those displayed by cells in the proximal convoluted tubules of the kidney, the choroid plexus epithelium, the ciliary epithelium of the eye, and the striated duct of the salivary gland. In later embryonic stages of the chick embryo, the choroidal epithelial cells display a profusely microvillous apical surface, distinct basal interdigitations between contiguous cells, numerous mitochondria, pinocytotic vesicles, multivesiculate bodies, and dense bodies. An elaborate ribosome-laden endoplasmic reticulum is localized largely in the basal cytoplasm, and in the apical cytoplasm there is an extensive Golgi complex with numerous associated microvesicles (5, 7).

In the present study, principal emphasis will be given to a consideration of the ultrastructure of basal bodies associated with the cilia of the choroidal epithelium in the chick. In addition, certain detail will be presented on the structural organization of the axial filament complex of normal and abnormal cilia.

MATERIALS AND METHODS

Choroid plexus tissues were collected from chick embryos (White Leghorn strain) of 6 to 20 days of development. Incubation and age determination were achieved by methods previously noted by Birge (3).

In preserving the choroidal epithelium, ice-cold fixative was perfused through the ventricular system, after the method described by Tennyson and Pappas (33, 34). Immediately upon perfusion, tissue samples were excised with the aid of a dissecting microscope and immersed in fresh ice-cold fixative. Tissue samples taken from the telencephalic plexus at 2-day intervals were preserved in each of the following fixatives: 1% osmium tetroxide in phosphate buffer, adjusted to pH 7.35 and 332 milliosmols/kg, for 1 to 2 hr, after the method of Millonig (22); 1% osmium tetroxide in 0.1 M phosphate buffer, adjusted to pH 7.6 and 443 milliosmols/kg, for 1 to 2 hr, after the method of Karlsson and Schultz (14); 1% osmium tetroxide in veronal acetate buffer, adjusted to pH 7.2-7.4 with a tonicity of 0.34, for 1 to 2 hr, after the method of Zetterqvist (36); 2% glutaraldehyde (biological grade) in 0.05 M cacodylate buffer, adjusted to pH 7.2 and 355 milliosmols/kg, for 10 to 20 min, after the method of Gordon et al. (13); 1% potassium permanganate in veronal acetate buffer, adjusted to pH 7.4-7.6 and 372.2 milliosmols/kg, for 1 hr, after the method of Luft (16).

All tissues preserved in glutaraldehyde were post-fixed in osmium tetroxide by the method of Millonig or Karlsson and Schultz. Postfixation was carried out either immediately after initial fixation or following a 24- to 48-hour wash in 4°C buffer (0.08 M cacodylate, 0.18 M sucrose, pH 7.2, 414.2 milliosmols/kg). Osmolarities were determined on all fixatives and buffer washes by a freezing-point depression osmometer ("Advanced Osmometer," Advanced Instruments, Inc., Newton Highlands, Massachusetts). The unit of measurement of salt concentration is milliosmols/kg H₂O.

Tissues were dehydrated through graded concentrations of cold ethanol, passed through cold propylene oxide, and embedded in Epon 812, after the method of Luft (17).

Sections were cut with glass and diamond knives on a Servall Porter-Blum microtome (model MT-2) and mounted on substrate-free copper grids (300 mesh). The tissue sections were stained in uranyl acetate and lead hydroxide (15), and examined under an RCA EMU 3F electron microscope, equipped with a 35- μ objective aperture and operated at 50 kv. Photographs were taken at a direct magnification of 1,500 to 33,000 diameters, using both medium and contrast grades of Eastman Kodak Lantern Slide Plates.

OBSERVATIONS AND DISCUSSION

The ventricular surface of the presumptive choroidal epithelium exhibits a relatively simplified ultrastructure in the telencephalic plexus of the 6-day chick embryo, including a sparse array of incipient microvilli and occasional cilia. However, by 8 to 10 days of development, an irregular border of microvilli and cilia is much in evidence. In later embryonic stages, the development of irregular microvilli is profuse and cilia are numerous (5).

Concerning the various fixatives used in this study, the best over-all preservation was obtained with glutaraldehyde, followed by postfixation in osmium tetroxide.

Axial Filament Complex

The shaft of the cilium contains the classical pattern of 9 peripheral double fibers arranged cylindrically around 2 central fibers (Fig. 1), as noted by numerous other investigators as existing in cilia and flagella of various plant and animal species (1, 8, 9, 11, 12, 19, 20, 27, and others). The central fibers are single and are surrounded by a central sheath which appears similar to that described by Gibbons and Grimstone (12) and Gibbons (11).

As in flagellates (12), molluscan gill cilia (11) and sea urchin spermatozoa (1), the peripheral doublets are oriented in a manner which consistently places 1 subfiber slightly closer to the center of the axial filament complex. This subfiber consistently bears 1 or 2 armlike projections which point in a clockwise direction when the cilium is viewed from base to tip. Using the terminology of Gibbons and Grimstone, this unit of each doublet will be designated as subfiber A and the other member of the doublet as subfiber B. Typically 2 "clockwise" arms are associated with subfiber A (1, 8, 11, 12). In the cilia of the chick plexus, the outer arm consistently is found to be prominent but the inner arm often is less conspicuous and frequently indistinguishable on 1 or more fibers. A somewhat similar condition may exist in the olfactory cilia of the frog (*see* Fig. 17 in reference 26). Afzelius (1) shows 2 arms on subfiber B of doublet 6 which project counterclockwise and interconnect with the arms of doublet 5. In Fig. 1 of the present study, a similar interconnection appears to exist but involves only the inner arms.

Frequently, linear densities are found to extend

from 1 or more of the doublets out to the ciliary membrane. In Fig. 1 such extensions appear opposite several doublets, especially 3 and 7. At present, it is difficult to determine to what extent these lateral extensions may correspond to the radial arms described in sensory cell kinocilia of teleosts by Flock and Duvall (10). Such extensions may provide further stabilization for the axial filament complex.

Though subfibers A and B normally appear of similar diameter, A frequently is found to be slightly larger. While this is in general agreement with the findings of Afzelius (1), Gibbons and Grimstone (12) found the reverse relationship in certain flagellates.

In cross-sections of choroid plexus cilia, 9 dotlike or spokelike condensations are found situated between peripheral doublets and the central fibers. As seen in Fig. 1, these condensations, when dotlike, normally lie closer to the inner margins of the A subfibers. When they appear as spokes (*see* doublet 9, Fig. 1), they normally interconnect A subfibers with the closest central fiber. Though the dot profile is more common in cross-section, 1 to several spokelike linear densities are noted in most cross-sections. On occasion, complete sets of spokes are observed in chick plexus cilia. Similar structures have been noted briefly by Flock and Duvall (10) and Reese (26). These dotlike and spokelike profiles which appear in cross-sections undoubtedly correspond to the "secondary fibers" described in the flagella of flagellates by Gibbons and Grimstone (12) and the "spokes" described in the flagella of sea urchin spermatozoa by Afzelius (1).

Gibbons and Grimstone describe a set of 9 secondary fibers situated between the outer doublets and the central fibers. Speaking of the secondary fibers, they state (12, p. 703), "... there seem to be fine lines extending from them to the outer and central fibers (e.g., Fig. 3), and it is entirely possible that these represent radial connections between the different sets of fibers." More recently in a study of molluscan gill cilia, Gibbons has given further evidence supporting the existence of a system of secondary fibers (11). Here again the secondary fibers were found interconnected with central and peripheral fibers by fine spokelike radial links. These radial links undoubtedly correspond to the spokes of Afzelius and the linear spokelike densities described in the present study.

Structures shown in Figs. 1 through 5 were fixed in glutaraldehyde and postfixed in osmium tetroxide.

FIGURE 1 Cross-section through cilium showing the classical pattern of 9 double fibers surrounding 2 single central fibers which are enclosed by a central sheath. The arrow denotes doublet 1; the remaining doublets should be numbered counterclockwise, in the direction of the arms, since this section is viewed from tip to base. The outer arms of the A subfibers are distinct, but inner arms are not evident on all doublets. The A subfibers lie slightly closer to the central fibers than do the B subfibers. Doublets 3 and 7 appear connected to the membrane of the cilium by thin strands. Doublet 5 appears to be met by an inner arm projecting back from doublet 6. Situated between the central fibers and the A subfibers of the surrounding doublets are condensations which appear either as dots (secondary fibers) or less frequently as strands or spokes. Subfiber A of doublet 9 appears connected to the adjacent central fiber by such a strand. $\times 72,000$.

FIGURE 2 This cross-section represents an anomalous condition in which only 8 doublets are present. Apparently, doublet number 1 is lacking and is replaced, at least at this level, by 3 single fibers. The single fibers do not appear to possess distinct arms. $\times 81,000$.

FIGURE 3 The arrow denotes a cross-section through the tip of a cilium. The fibers all appear to be single and to lack arms. There is an outer circle of 11 single fibers and an inner group of 4 single fibers. The cross-section on the left illustrates an anomalous condition in which 6 single supernumerary fibers are present external to the conventional 9 doublets. $\times 81,000$.

FIGURE 4 Cross-section through another cilium showing 6 supernumerary single fibers. $\times 81,000$.

FIGURE 5 Longitudinal section through the rootlet of a basal body, showing prominent cross-striations. The center-to-center distance between the major periods is approximately 575 to 625 A. $\times 127,000$.

Structures shown in Figs. 6 to 9 were fixed in osmium tetroxide, after method of Millonig (22).

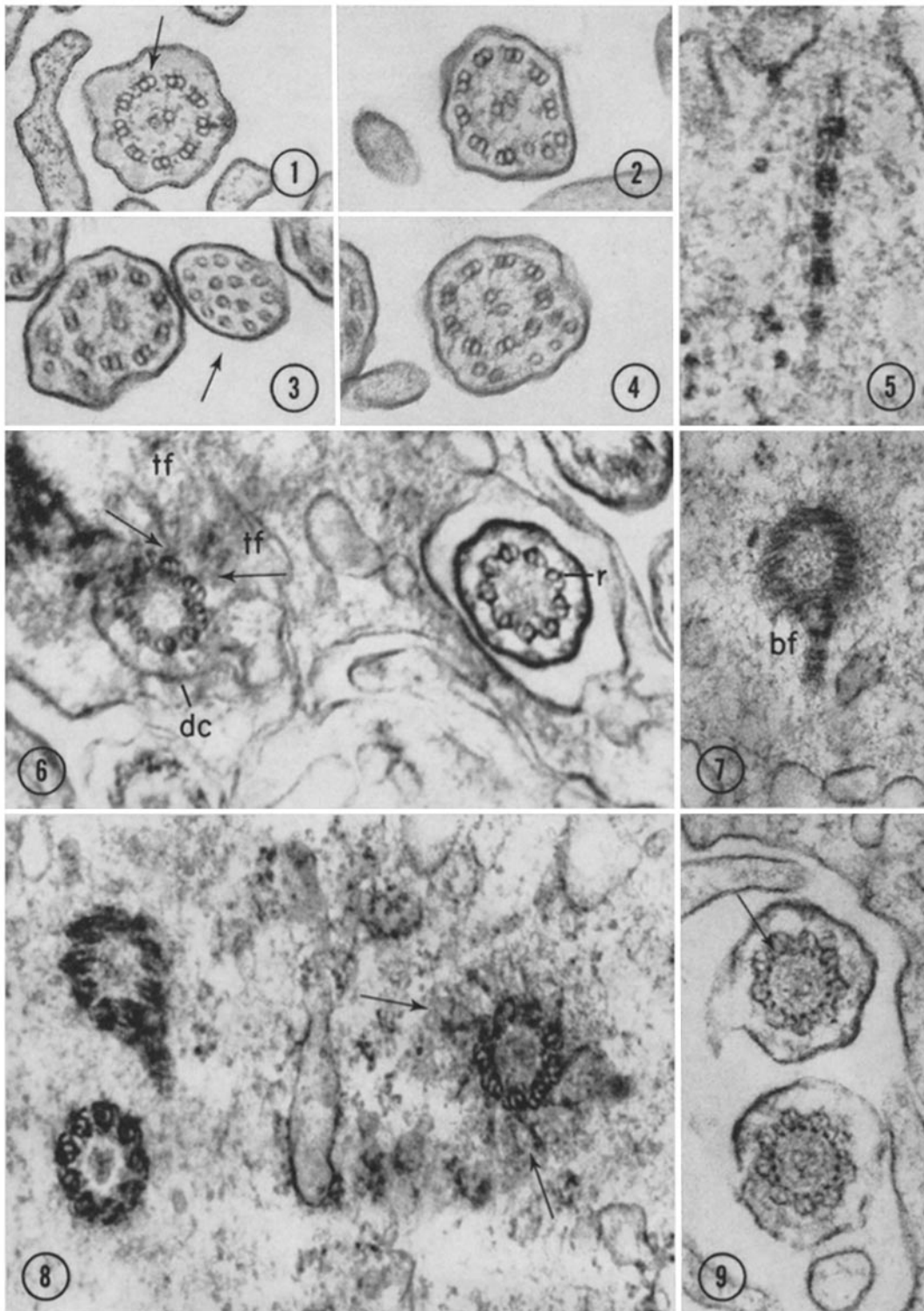
FIGURE 6 Cross-section to the left represents an oblique cut through the transitional zone, between basal body and cilium. On one side of this section, 3 triple fibers are evident, each associated with a transitional fiber complex (*tf*). Two transitional fibers appear to be associated with each of 2 triplets (see arrows). Opposite the triple fibers are several discernible doublets, each connected to a dense condensation (*dc*), adjacent to the ciliary membrane. The cross-section to the right is just above the basal plate and shows 9 doublets, each with a dense arm or ridge (*r*) along the outer side. The ridges appear to connect tenuously to corresponding condensations (peripheral ridges) located at the inner surface of the ciliary membrane. No central fibers are discernible. $\times 85,000$.

FIGURE 7 Section through a basal body at the level of the cross-striated basal foot (*bf*). $\times 68,000$.

FIGURE 8 The section to the right is through the upper part of a basal body and shows 9 triple fibers, each associated with a transitional fiber complex. Each transitional fiber complex usually consists of 1 dense fiber, as well as 1, or possibly 2 less dense fibers (see arrows). The basal plate is evident at this level, extending across the lumen of the basal body. The sections to the left are through basal bodies at levels below or proximal to the transitional zone. The 9 triple fibers are evident in each. A basal foot extends from one basal body. $\times 85,000$.

FIGURE 9 Cross-section through 2 cilia at, or just above the upper surface of the basal plate. Central fibers are not evident. However, 18 small dense fibers (or granules) appear in a circle, adjacent to the inner margins of the 9 peripheral doublets. Tenuous strands appear to interconnect at least some of the small fibers (arrow). $\times 88,000$.

Except for Fig. 13, structures shown in Figs 10 to 15 were fixed in glutaraldehyde, followed by postfixation in osmium tetroxide.



Judging from the studies of Gibbons and Grimstone (12) and Gibbons (11), it seems likely that fine secondary fibers are situated between the central and peripheral fibers, and that radial links interconnect the secondary fibers with the adjacent central and peripheral fibers. The results of the present study tend to support this position, even though the existence of secondary fibers is still open to some question. Nevertheless, it becomes increasingly evident that radial interconnections of some sort are established between peripheral and central fibers, as originally presumed by Afzelius.

Transition from Cilium to Basal Body

Several specialized structural features are evident near the proximal end of the free shaft of the cilium, where it joins the apical cell surface. A study of cross-sections through this level reveals 9 peripheral dense condensations adjacent to the

inner margin of the ciliary membrane, situated opposite the 9 double fibers. These condensations are connected by fine filaments to corresponding small dense granules or ridges which appear along the outer margins of the doublets (Figs. 6, 9). Such connections between the peripheral doublets and the ciliary membrane have been shown in cross-section in various other studies (10, 11, 12, 26). However, a study of longitudinal sections gives a more complete understanding of the complexity of this area (Figs. 10 to 13, 16). The outer or peripheral condensations appear as ridges which extend along the inner surface of the ciliary membrane, paralleling and opposing the doublets. Proximally, these *peripheral ridges* are continuous with the bulblike terminal enlargements of the 9 transitional fiber complexes which arise from the peripheral triplet fibers of the basal body. Distally, these ridges taper and end approximately 0.28 to 3.5 μ above the basal plate (Figs.

FIGURE 10 Longitudinal section through cilium. At the base of the cilium, the cell membrane is reflected to form a distinct basal cup (*bc*). The basal plate occurs at or just below the floor of the cup. $\times 68,000$.

FIGURE 11 Longitudinal section through the basal body and transitional zone of a cilium. Two basal feet (*bf*), which display prominent cross-bands, are evident. Transitional fibers (*tf*) extend from the distal end of the basal body to the cell membrane, where the latter reflects away from the base of the cilium to form the basal cup. The terminal ends of the transitional fibers are enlarged or bulblike where they join the membrane. Each terminal enlargement is continuous with a dense ridgelike condensation (peripheral ridge) which extends around under the inner surface of the ciliary membrane (see arrow). The peripheral ridges are seen in cross-section in Fig. 6, in which they appear as dense condensations immediately adjacent to the ciliary membrane. Though a basal plate is evident at the distal end of the basal body, a distinct closing plate is not seen at the proximal end. $\times 68,000$.

FIGURE 12 Section through basal body revealing transitional fibers, as described above. Linear densities, which possibly may represent the small dense fibers shown in Fig. 9, are sometimes evident along the inner margins of the peripheral doublets (see arrow). $\times 68,000$.

FIGURE 13 Longitudinal section through cilia and basal bodies showing transitional fibers. Cross-striated rootlets project from the proximal ends of the basal bodies. Preservation was with osmium tetroxide, after Millonig (22). $\times 48,000$.

FIGURE 14 Tangential section through the edge of a basal body showing a basal foot (*bf*) which connects with a microtubule (arrow). A cross-striated rootlet extends well into the apical cytoplasm. $\times 85,000$.

FIGURE 15 Longitudinal section through 2 nonciliated centrioles, situated adjacent to a developing junctional complex (*jc*). Fiber arrangement in the centriolar cylinder is similar to that of the ciliary basal bodies shown in Figs. 11 and 12. Pericentriolar bodies (*pb*) project laterally from the centrioles. Arrows denote other projections which may possibly correspond to the transitional fibers of ciliary basal bodies. $\times 68,000$.

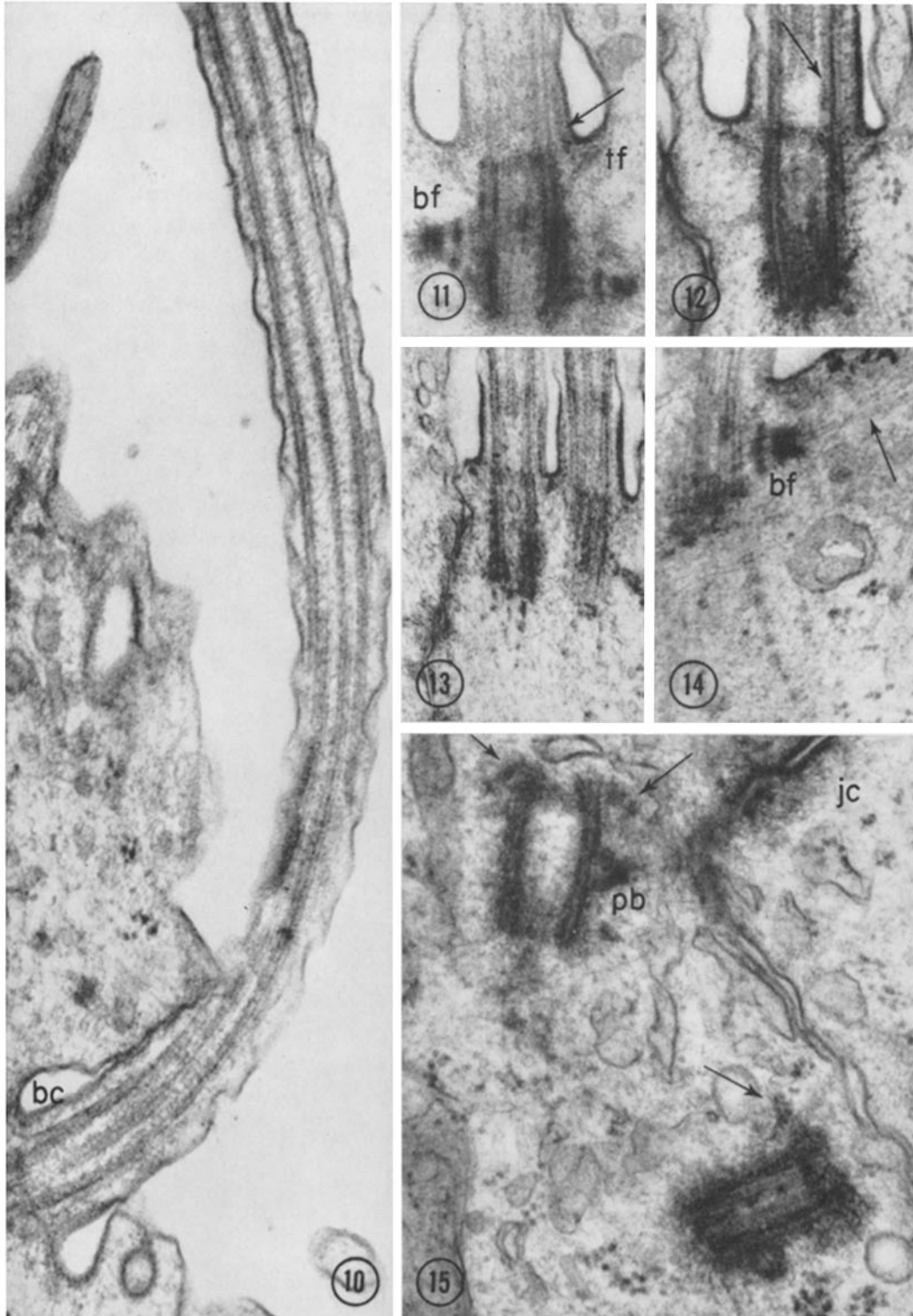




FIGURE 16 Tangential section through 3 basal bodies, showing cross-striated rootlets. Four rootlets originate from the proximal end of each of 2 basal bodies. Arrows indicate transitional fibers. $\times 119,000$.

11 to 13, 16). The radial connections between these peripheral ridges and the corresponding double fibers and their proximal connections with the transitional fibers seemingly would substantially reinforce the basal support of the cilium.

In sections through the transitional region in which the connections between the peripheral doublets and the ciliary membrane are evident, usually there appear interconnections joining the 9 doublets (Fig. 6). Similar interconnections have been described in molluscan gill cilia by Gibbons (11).

In cross-sections taken at or just above the upper surface of the single basal plate, 18 dense granules or rods are arranged in a circular profile, just inside the circle of peripheral doublets (Fig. 9). Fine strands appear to interconnect at least some of these units. On occasion, linear rodlike densities, which may represent these structures (Fig. 12), are seen in longitudinal sections. They may possibly extend into the basal body, since dense, dotlike profiles are sometimes discernible adjacent to the inner margins of the triplet fibers (Fig. 8). Though an interpretation of the exact structural organization of these units is not possible with present data, they possibly may represent proximal components of the secondary fiber complex. It is interesting to note that Gibbons has also described a similar circular array of dense dots (probably eighteen in number) just above the basal plate area in the transitional region of the gill cilia of a lamellibranch mollusc. He states (11, p. 190), "... it is not yet clear whether this feature is related to the secondary fibers."

Though the central fibers end just above the basal plate, the peripheral doublets can be traced into the basal body (Figs. 12, 13), as noted in various other studies (8, 10-12, 27).

Basal Body

The distal end of the basal body is delimited by a well formed basal plate of moderate density (Figs. 8, 10 to 13), similar to that described by Gibbons and Grimstone. This differs from the condition described in molluscan gill cilia by Gibbons, in which two basal plates are situated in the transitional region, about 0.2 μ above the distal end of the basal body (11).

The tubular cylinder of the basal body in chick plexus cilia consists of the usual array of nine triple fibers (Figs. 6, 8). The innermost and middle subfibers of the triplets continue into the ciliary

shaft, representing respectively A and B subfibers of the 9 peripheral double fibers. The outermost or C subfibers, generally characteristic of the basal bodies, terminate at or just below the level of the basal plate, as noted in other systems (8, 10-12, 26, 32). As seen in cross-section, the triple fibers are rotated in such a manner as to place the A subfibers substantially closer to the central axis of the basal body, typical of descriptions given by Fawcett, Gibbons and Grimstone, Gibbons, and others.

The proximal end of the basal body appears open and the inner matrix of the central lumen consists of a material of light-to-moderate density, frequently containing granules or densities of circular profile. These may correspond to the "irregular cylinder" described in the distal portion of the basal body of flagellates (12). The inner "cartwheel" structure found within the proximal segment of basal bodies in flagellates is absent.

In the epithelial cells of the chick choroid plexus, the ciliary basal body displays a complex array of processes, consisting of three distinct types in all. At the distal end of the basal body, transitional fibers extend from the triplets to the cell membrane at the base of the ciliary shaft. In addition, one or two prominent basal feet, sometimes associated with cytoplasmic microtubules, project laterally from the intermediate region of the basal body, and multiple cross-striated rootlets project down into the cytoplasm from the proximal end of the basal body.

Transitional Fibers

Nine transitional fiber complexes arise from the distal end of the basal body. They radiate out, resembling a cartwheel (Figs. 6, 8), and end in cup-shaped or bulblike terminal enlargements which fuse to, or come into close proximity with the cell membrane where it is reflected at the base of the ciliary shaft. As noted above, these terminal enlargements are continuous with nine longitudinal peripheral ridges which extend distally along the inner surface of the ciliary membrane (Figs. 10 to 13, 16), occupying positions opposite to the peripheral fibers with which they are interconnected. Most commonly, the transitional fibers seem to originate from the outer subfibers (B or C) of the triplets, as shown by Gibbons and Grimstone, and Gibbons. One prominent fiber appears to be associated with each triplet though, in addi-

tion, one or sometimes two less distinct extensions also may occur (Figs. 6, 8).

Studying flagellates, Gibbons and Grimstone were the first to note transitional fibers. They described 9 such fibers, one fiber arising from each triplet at, or near the distal end of the C sub-fiber and extending outwards toward the cell membrane, where the latter becomes the flagellar membrane. They could not determine for certain whether the fibers ended on the cell membrane. In his study of molluscan gill cilia, Gibbons found a similar array of transitional fibers. In this case they extend to the cell membrane, and each fiber seemed to consist of two fine filaments. In a recent study of olfactory cilia in the frog, Reese (26) has described an arrangement of transitional fibers similar to that proposed by Gibbons, except that he found three fibrous components apparently arising from each triplet. Flock and Duvall (10), studying kinocilia of the sensory cells of the teleost lateral line system, have described spokelike structures which extend from the triplet tubules of each basal body toward the cell membrane. In all likelihood, these *spokes* correspond to transitional fibers. Flock and Duvall, as well as Reese, present figures very similar to Fig. 8 of the present study.

It becomes increasingly evident that transitional fibers constitute a basic feature of the ultrastructural organization of cilia and flagella. They apparently serve to connect or anchor the distal end of the basal body to the cell membrane. These fibers appear distinct from the basal feet described below.

Basal Feet

One or 2 prominent basal feet project laterally from the intermediate region of each basal body of chick plexus cilia. As seen in longitudinal section, they are typically wedge-shaped and bear several (usually 3) dense cross-bands (Figs. 11, 14). Basal feet are shown in cross-section in Figs. 7 and 8, in which the cross-bands are evident. Similar basal feet have been described by various investigators, including Gibbons, Flock and Duvall, Reese, and Satir (30). Szollosi has recently described satellites associated with the basal bodies of flagella of spermatids and of testicular surface epithelial cells of the testes of jellyfish. In several longitudinal sections of flagellar basal bodies (Figs. 2, 9, and 10 of reference 32) he identifies cross-striated satellites which may well correspond to the ciliary basal feet described above. However, in certain other

figures of flagellar basal bodies in the study by Szollosi, the structures identified as satellites may possibly correspond to the transitional fibers of ciliary basal bodies (see Figs. 3, 4, and 6 of reference 32). In these figures, representing cross-sections, there are 9 structures identified as satellites associated with each basal body. These structures radiate outwards from the distal portion of the basal body and appear to anchor to the cell membrane adjacent to the base of the flagellar shaft (compare with Figs. 6, 8, 11, and 12 of the present study).

In sections taken parallel to the ventricular surface of the chick choroidal epithelium, rows of 4 to 6 basal bodies have been observed within a given cell. Usually 2 basal feet are evident per basal body, and the feet tend to show uniform orientation. This alignment of basal feet may bear a relationship to the plane of ciliary beat. As Gibbons has shown in molluscan gill cilia, the basal feet lie in the plane of the effective stroke.

Occasionally, microtubules, such as those described by Behnke (2), are found to attach to the basal feet in the chick plexus (Fig. 14). Behnke has reported microtubules in association with both filament-bearing basal bodies and mitotic centrioles. Reese also has described microtubules in association with ciliary basal feet, and Szollosi has shown spindle fibers attached to satellites of flagellar basal bodies. This relationship with microtubules suggests that the basal feet of filament-bearing basal bodies are homologous with the similarly shaped lateral projections (pericentriolar bodies) of nonciliated centrioles (Fig. 15). However, the full significance of this relationship remains somewhat obscure at present. As pointed out by Szollosi, it is not known how long such fibers or microtubules remain attached to satellites (or basal feet) after cell division, or whether all such structures are associated with spindle fibers at one time or another.

Rootlets

Apparently, the cross-striated rootlets constitute one of the more variable structural features of filament-bearing basal bodies. Though they were not reported in flagellates (12), cross-striated rootlets with major periods of 550 to 700 Å have been described in various invertebrates (8, 9, 11). Rootlets also have been reported in epithelial cells of the testes of the jellyfish (32). These exhibit a

complex pattern of bands, with a major period of 850 to 900 A.

According to Fawcett (8), rootlets are less evident in vertebrate forms, showing greater prominence in epithelia which bear particularly long cilia. Prominent rootlets are associated with the basal bodies of the choroidal epithelium of the chick embryo (Figs. 5, 14, 16). Preliminary measurements indicate a major period of 575 to 700 A. The rootlets appear to originate from the proximal ends of the triplet fibers. As many as 4 rootlets may appear in a single longitudinal section (Fig. 16). This seemingly would indicate a substantially larger number for each basal body, perhaps 1 for each of the 9 triplets.

Wislocki and Ladman have described prominent ciliary rootlets in the choroidal epithelium of the monkey and rabbit (35). These rootlets, reported usually to be single or sometimes double as viewed in sections, were distinctly cross-striated, with a major period of approximately 60 μ . Compared with the chick embryo, there are fewer rootlets per basal body in the choroidal epithelium of these mammalian forms, though in the latter the rootlets are distinctly larger and appear to extend deeper into the cytoplasm (see Fig. 6 of reference 35).

As suggested by Fawcett, the rootlets possibly may serve to stabilize the basal body, particularly when they are associated with long cilia. It is of interest to note that Sakaguchi (28) has recently described "pericentriolar filamentous bodies" associated with centrioles of nonciliated cells. These structures consist of parallel bundles of filaments, showing cross-striations with a period of 700 A. They apparently correspond to the rootlets of filament-bearing basal bodies.

Nonciliated Centrioles

In this study, nonciliated centrioles have been encountered in only one cell (Fig. 15). Their structural similarity to the basal bodies (Figs. 11, 12) is evident.

The lateral, wedge-shaped projections very likely represent pericentriolar bodies. Similar structures have been reported recently by other investigators (see Figs. 4, 10 of reference 23; Fig. 14 of reference 32). Often such bodies are found attached to or associated with spindle fibers. They possibly may correspond to the basal feet of the filament-bearing basal bodies, such as those shown

in Fig. 11. As noted earlier, the basal feet of filament-bearing basal bodies are sometimes observed in close association with microtubules.

In Fig. 15, other processes, occupying more terminal positions, appear to extend from the centrioles (see arrows). One may question whether these processes may correspond to the transitional fibers of the filament-bearing basal bodies (compare with Figs. 11 and 12). In this regard, Reese has reported (26, p. 219), "Transitional fibers were also found on centrioles whether or not they were in close proximity to the cell membrane. . . ." Also Murray et al. have shown certain cross-sections of nonciliated centrioles (Figs. 2, 6 of reference 23) which bear up to 9 radially orientated processes which are very similar in appearance to the transitional fibers shown in Figs. 6 and 8 of the present study.

Supernumerary Fibers

In 4 instances, cilia were encountered which possessed supernumerary peripheral fibers. In 3 of these cases, the 9 peripheral doublets and the 2 central fibers appeared essentially normal, displaying typical spatial arrangements. In each of 2 of these 3 cases, 6 single supernumerary fibers are identifiable, loosely grouped external to the peripheral doublets in a broad, outward fold of the ciliary membrane (Figs. 3 and 4). The 3rd case is essentially similar, except that the number of supernumerary fibers cannot be determined exactly, but there appear to be either 5 or 6. In the 4th case, only 8 peripheral doublets are present. Apparently, number 1 is absent and is replaced by 3 single fibers (Fig. 2). Though the evolutionary stability of the 9 + 2 pattern has been well established (8, 9), it is now evident that certain anomalies do occur. Satir (29) has reported 1 case of supernumerary fibers in a cilium from the gill tissue of the mussel, *Elliptio complanatus*. In this case, 12 peripheral fibers were reported instead of the usual 9. Pitelka (25) has reported aberrant cilia in *Paramecium* in which 1 or 2 peripheral fibers were lacking.

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