THE FINE STRUCTURE OF EPENDYMA

IN THE BRAIN OF THE RAT

M. W. BRIGHTMAN, Ph.D., and S. L. PALAY, M.D.

From the Laboratory of Neuroanatomical Sciences, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Maryland. Dr. Palay's present address is Department of Anatomy, Harvard Medical School, Boston

ABSTRACT

The ciliated ependyma of the rat brain consists of a sheet of epithelial cells, the luminal surface of which is reflected over ciliary shafts and numerous evaginations of irregular dimensions. The relatively straight lateral portions of the plasmalemma of contiguous cells are fused at discrete sites to form five-layered junctions or *zonulae occludentes* which obliterate the intercellular space. These fusions occur usually at some distance below the free surface either independently or in continuity with a second intercellular junction, the *zonula adhaerens*. The luminal junction is usually formed by a *zonula adhaerens* or, occasionally, by a *zonula occludens*. The finely granular and filamentous cytoplasm contains supranuclear dense bodies, some of which are probably lysosomes and dense whorls of perinuclear filaments which send fascicles toward the lateral plasmalemma. The apical regions of the cytoplasm contain the basal body complexes of neighboring cilia. These complexes include a striated basal foot and short, non-striated rootlets emanating from the wall of each basal body. The rootlets end in a zone of granules about the proximal region of the basal body, adjacent to which may lie a striated mass of variable shape. All components of the basal body complex of adjacent cilia are independent of each other.

INTRODUCTION

The parenchyma of the central nervous system confronts pools of extracellular fluid along three broad interfaces: the capillary wall, the pia-glial (external limiting) membrane, and the ependyma. The first two interfaces, known as the blood-brain barrier and the cerebrospinal fluid-brain barrier, are the subject of an enormous literature which has been critically reviewed recently by Dobbing (10). The ependymal interface has received somewhat less attention, probably because of its relatively inaccessible position deep in the brain and spinal cord. Nevertheless, it has been demonstrated that various substances can cross this barrier into the brain tissue. For example, Roth et al. (56) have shown that acetazoleamide introduced into the blood stream is secreted by the choroid plexus into the ventricular fluid whence it passes into the gray matter that forms the walls of the ventricles and the aqueduct. In more direct experiments, Draškoci et al. (11) perfused histamine through the ventricles and found that it penetrates into the walls of the third ventricle to a depth of at least 2.5 mm within 1 hour. Feldberg and Fleischhauer (18) studied the uptake of the sodium salt of bromophenol blue and showed that the dye, like acetazoleamide and histamine, penetrates more readily into gray than into white matter and that there are regional differences in depth of penetration; for example, the pineal gland stains very deeply whereas the neurohypophysis does not

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stain at all. They added the important observation that uptake of the dye does not occur when perfusion of the ventricles is carried out in a freshly killed animal.

As Feldberg and Fleischhauer pointed out, one of the factors controlling the transfer of substances from the ventricular fluid to the brain parenchyma is the structure of the ependyma itself, which, as a continuous epithelium lining the ventricles of the brain and the central canal of the spinal cord, is the first barrier to be met. Detailed knowledge of the structure of this epithelium is, therefore, necessary to an understanding of the mechanism which regulates exchange of substances between ventricular fluid and the parenchyma of the central nervous system.

In 1900 Studnička (60) gave a comprehensive summary of the morphology and interrelationships of ependymal cells in a variety of species. So far as optical microscopy is concerned, this work remains the classic account of the subject. With the electron microscope, Tennyson and Pappas (62) have examined embryonic and early post-natal stages in the development of the ependyma. Fleischhauer (19) has recorded some electron microscopic observations on the structure of ependyma in the turtle. The following account is a description of the fine structure of ependymal cells in the brain of the adult rat, with particular emphasis on specializations of the cell surface, e.g., the free border with its cilia, and the lateral cell surfaces with their interrelationships. Some of our findings have been presented in earlier preliminary reports (4, 39).

MATERIALS AND METHODS

The brains of nine rats were fixed by perfusion through the aorta with a solution of 1 per cent OsO_4, buffered with acetate-veronal (pH 7.1–7.4) and containing 0.54 gm per cent of calcium chloride (41). The perfusion lasted for about 30 minutes. Except for the initial 5 minutes of perfusion with warm fixative, the remainder of the perfusate was maintained at about 5°C. *In situ* fixation was allowed to proceed for an additional 30 to 60 minutes. Dissection and dicing of the brain into 1- to 2-mm square blocks was then performed, care being taken to keep the tissue moist with cold fixative throughout this variable time. The total time of fixation was from $1\frac{1}{2}$ to 3 hours. The brains of two additional rats were fixed by perfusion with a 3 per cent solution of potassium permanganate (34). The tissue blocks were then rinsed briefly in acetate-veronal buffer, dehydrated in ascending concentrations of methanol, and em-

bedded in Epon 812 (35). Samples were taken mainly from the lateral walls of the third ventricle, the aqueduct of Sylvius (iter), and the fourth ventricle.

Sections approximately 2 to 3 μ thick were mounted in glycerol and photographed with phase contrast optics. Thin sections were cut on a Porter-Blum microtome, mounted on carbon-coated grids, and stained with a saturated, aqueous solution of uranyl acetate for $1\frac{1}{2}$ to 2 hours. Sections of osmium tetroxide–fixed tissue were also stained with a 1 per cent solution of potassium permanganate (32). The sections were examined in the RCA EMU 3D or 3E electron microscope, at initial magnifications of about 5,000 to 13,000 times.

OBSERVATIONS

Optical Microscopy

The ependyma is composed of cuboidal or columnar cells having an irregular striated border at their free or ventricular surface. The apex of each cell is furnished with a tuft of about 40 (35 to 60) parallel cilia aligned in uneven rows (Figs. 1 to 3). The cilia terminate in rows of basal bodies situated directly beneath the striated border. These were noticed by Purkinje (46), who wrote the earliest paper on the ependyma and who described them as a layer of granules to which the cilia are fastened and from which they could be stripped off without disturbing the continuity of the epithelium. Terminal bars can be seen at the intercellular junctions near the apices of the cells.

Beneath the layer of basal bodies, the apical cytoplasm is occupied by other granules of various dimensions. The smaller and more numerous appear to be mitochondria. The larger and less numerous ones probably correspond to the lysosomes described by Becker *et al.* (2) in the supranuclear cytoplasm. The round or oval nucleus is finely and homogeneously granular and is bounded by a thin, but distinct, smooth membrane.

In some cells (Figs. 1 and 2), the cytoplasm appears to be condensed into dark perinuclear laminae. These correspond to systems of filaments that can be seen in the electron micrographs.

The basal cytoplasm is usually finely granular, but frequently it appears to be clear, as may be seen in Figs. 1 and 2. In electron micrographs these clear zones turn out to be localized expansions of the perinuclear cistern and are clearly artifactitious. In the present study we were unable to obtain any specimens that did not have a few of these poorly fixed cells in each section.
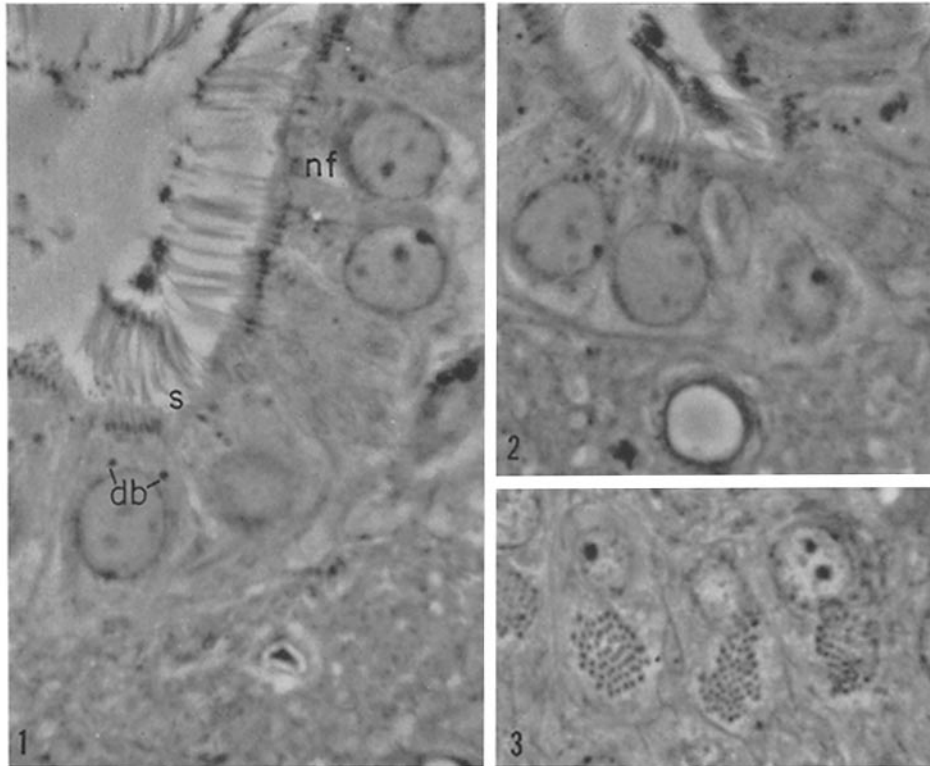
FIGURES 1 TO 3   The ependyma of the iter. Photomicrographs were taken with phase contrast optics at a magnification of 1,660.

FIGURE 1   Both cilia and basal bodies have been sectioned transversely in the lower left cell. Continuity between cilia and basal bodies cut longitudinally is illustrated in the neighboring cells. A portion of the striated surface (S) is evident in the adjacent cell. Note the large, round, osmiophilic dense bodies (db) in the supranuclear regions. In the upper cell, the perinuclear material (nf) consists of masses of filaments which follow the contour of the nuclear membrane. The apparently empty basal region of this cell is indicative of poor preservation.

FIGURE 2   Five ciliary shafts continuous with their basal bodies are well shown on the cell to the left of center. A subependymal capillary appears near the lower margin of the figure, illustrating the proximity of vessels to the bases of ependymal cells.

FIGURE 3   The basal bodies of several contiguous ependymal cells are cut transversely and form aggregates distal to the nuclei.

The ependyma rests directly upon the underlying neuropil of the brain without an intervening basement membrane or obvious intercellular space.

Electron Microscopy

CELL SURFACE: The striated apical cell border of the phase contrast image is resolved by the electron microscope into a highly folded surface.

The cell membrane is reflected over broad, short ridges and numerous longer, tortuous, finger-like projections which are too irregular in their dimensions to be termed microvilli (Figs. 4, 5, 14, and 21). These projections partially or completely encircle cilia (see below), which extend relatively far into the ventricle. Frequently, processes of contiguous cells overlap parts of the apical surface that would otherwise have bordered the ventricle.

Most of the projections contain small vesicles and fine filaments, but some of them are so slender that they consist of little more than folds of the cell membrane. The general effect of all these irregularities is to produce a shallow labyrinth at the free surface, through which the tufted cilia project.

Not all of the cell processes seen at the free surface belong to ependymal cells. Occasionally, isolated profiles are encountered, the content of which is quite unlike any part of the ependymal cells (Fig. 14). They contain small tubular elements about 200 A in diameter, vesicles enclosing single dense droplets about 400 A in diameter, and quite distinctive small mitochondria. These mitochondria, in contrast to those of ependymal cells, are characterized by broad, dense cristae that are regularly spaced and parallel with one another. In addition, these mitochondria do not contain the large dense granules typical of mitochondria in ependymal cells and neuroglial cells. Since this type of mitochondrion, the narrow tubules, and the droplet-containing vesicles (69) all resemble elements found in neurons, these cell processes probably belong to slender expansions arising from nerve cells lying in the subependymal tissue. This identification is made more likely by the clusters of fine unmyelinated nerve fibers frequently encountered in the interstitial spaces between the bases or sides of adjacent ependymal cells.

In the ependyma the lateral cell surfaces are comparatively simple, without the elaborate folds and interdigitations commonly seen in the surfaces of other columnar epithelial cells, such as those lining the intestine. Near the apices of contiguous cells, the apposed surface membranes contribute to the formation of complex intercellular junctions which in optical microscopy have been given the name terminal bars. Farquhar and Palade (12, 14) have recently analyzed these junctions very carefully in a wide variety of tissues and have proposed a new series of names to designate three different

morphological types of junctions seen electron microscopically in the position of the terminal bar of optical microscopy. Because their terminology seems to us to be clearer than earlier formulations, we shall follow it in this description of the ependyma.

The most obvious type of junction is the *zonula adhaerens* (Figs. 4, 12, 14, 15, and 18), clustered in twos and threes near the apices of contiguous cells. These junctions are so extensive as to form a girdle about the cell. They frequently encircle completely the invaginated processes of neighboring ependymal cells (Fig. 14). The segments of plasmalemma comprising the junction are denser than the plasmalemma elsewhere. It is likely that this dense portion represents the inner, cytoplasmic leaflet (45 A thick) of the plasmalemma. A dense cytoplasmic plaque, about 300 A thick, lies parallel to the cell membrane. It can be directly applied against the plasmalemma or separated from it by an interval of 30 A. Cytoplasmic filaments insert into the plaque at an angle. The total width of the *zonula*, measured between the cytoplasmic interfaces of apposing cell membranes, is about 340 A (305 to 400 A). The width of the intercellular space, which is about 230 A (170 to 270 A), is the same as that beyond the junction. The interspace of the junction is occupied by a fluffy, filamentous material which, infrequently, is condensed into an indistinct, thin lamella lying parallel to the plasmalemmas in the middle of the space.

In addition to having attachments provided by the *zonulae adhaerentes*, adjacent ependymal cells are bound together by a second variety of intercellular junction, in which the confronted lateral surface membranes are more closely apposed. Such junctions occasionally occur directly at the ventricular surface (Fig. 14), but more frequently between two apical *zonulae adhaerentes* (Figs. 4, 12, 15, and 17), or at some distance from the free sur-

Figure 4   Three adjacent ependymal cells of the third ventricle are illustrated. The free surface is thrown into numerous irregular folds which partially or completely envelop the ciliary shafts (c). The apical cytoplasm contains basal bodies (bb) and their rootlets (r), mitochondria, scattered elements of the granular endoplasmic reticulum, small vesicles, Golgi complex, and dense bodies (db). The smooth, oval nuclei are surrounded by whorls of filaments sectioned longitudinally and transversely. Two apically situated *zonulae adhaerentes* (za) are joined by a *zonula occludens* (zo). One of the cell processes (n) lying within the ventricle may be neuronal in origin. A centriole (ce) appears adjacent to the nucleus of the lower cell. × 17,000.
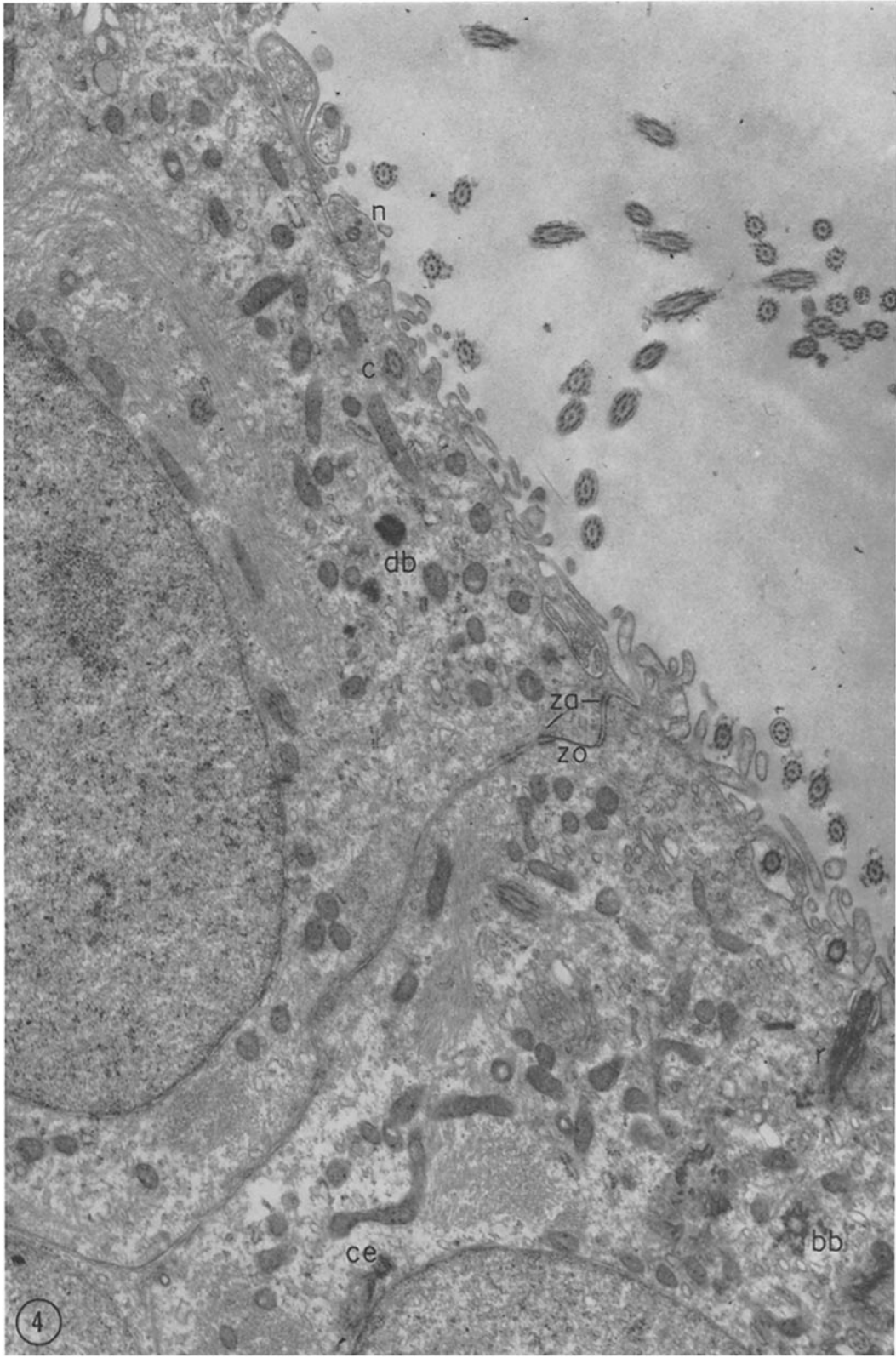
face and independently of the *zonula adhaerens* (Figs. 14 and 16). Occasionally, this second type of junction participates in the formation of peculiar dove-tail joints near the basal region of two contiguous cells, as illustrated in Fig. 16. In some instances, it completely surrounds an invagination of one ependymal cell into another and appears in transverse section as a perfect circle.

The structure of this type of intercellular connection is shown at high magnification in Figs. 17 and 18. The two apposing membranes are

but 35 A wide in tissue fixed in permanganate) formed by the contact or "fusion" of the outer leaflet of each cell membrane. Thus, the light intervals between the two dense cytoplasmic leaflets are not intercellular space (which is obliterated by the fusion of the outer leaflets) but rather consist of the light intermediate laminae of either cell membrane. These junctions, therefore, are composed of five layers, namely, two confronted, very dense cytoplasmic leaflets between which lie two intermediate, light leaflets
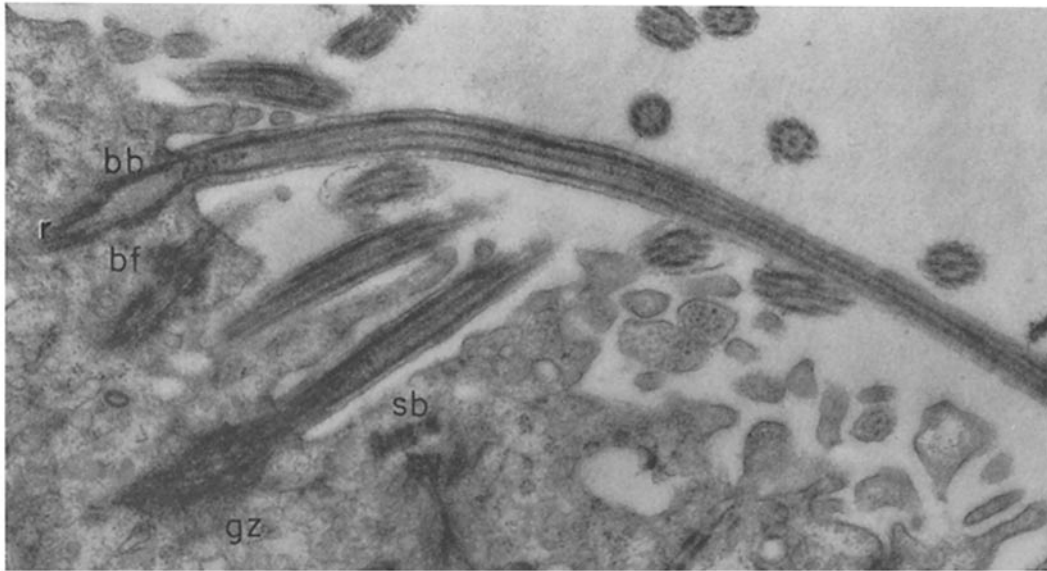

FIGURE 5 The cell surface is reflected as the ciliary membrane over the ciliary shaft. The peripheral fibers of the shaft are continuous with those of the basal body (*bb*). Short, non-striated rootlets (*r*) emanate from the wall of the basal body, and merge with an amorphous mass of dense, fine granules (*gz*). The rootlets and wall of the lower left basal body have been sectioned tangentially so that its interior is invisible. A basal foot (*bf*) is in contact with one basal body. To the right lie fragments of rootlets surrounded by their granular zone and a striated body (*sb*). $\times$ 24,000.

represented by sharp lines, highly regular, and perfectly parallel with each other. These dense lines represent only one layer of the usual trilaminate unit membrane (52, 53). The inner or cytoplasmic leaflet of this second type of junction is 45 A wide and is even denser than that of the *zonula adhaerens*. In tissue which has been either fixed or stained with permanganate, these leaflets are about 90 A apart, but in tissue fixed in osmium tetroxide and stained with uranyl acetate, the interval is only 60 to 70 A. This interval is bisected by a median lamella (20 A wide in tissue fixed in osmium tetroxide and stained with uranyl acetate,

separated by a dense median layer. Neither filaments nor an increase in density occur within the cytoplasm bordering the junctions. The total width of the junction between the cytoplasmic interfaces of both inner leaflets is 200 A (180 to 235 A).

Intercellular junctions of this general configuration were first noticed in the cardiac muscle of the guinea pig (58). Karrer (30), who found them in normal human cervical epithelium, gave them the name "quintuple-layered cell interconnections" and predicted that by careful study they would be discovered in other tissues. They have
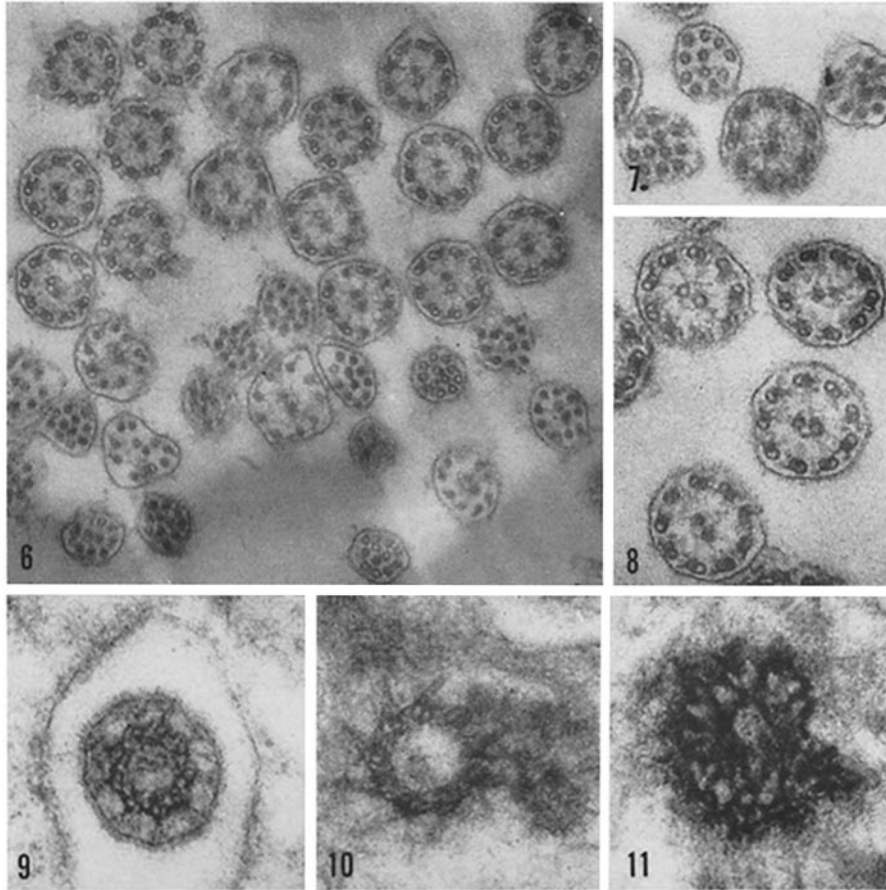
Figures 6 and 7 Cross-sections of ciliary shafts at different levels along their lengths. Near the distal tip, the diameter of the shaft diminishes and the nine peripheral doublets become nine singlets. One of the nine peripheral single subfibers is usually closer to the center of the shaft. The two central, axial subfibers persist at this level. There are no connecting filaments between the axial or peripheral subfibers near the tip of the cilium. This level corresponds to plane A of Fig. 21. Fig. 6, $\times$ 50,000. Fig. 7, $\times$ 67,000.

Figure 8 The shafts of several cilia are seen in cross-section. Of each doublet, subfiber A usually has a dense core and a pair of short arms that project toward subfiber B of the succeeding doublet. Short "filaments," which may actually be plates (see text, p. 428 and Fig. 21), connect the peripheral subfibers with the axial pair. The latter are joined to each other by an arcuate filament. $\times$ 67,000.

Figure 9 Transverse section through the ciliary shaft near its junction with the basal body. A granule lies between the subfibers of each pair on the surface facing the ciliary membrane. A short filament radiates from the granule and bifurcates to make contact with the ciliary membrane. The central fibers are displaced. The section corresponds to plane D of Fig. 21. $\times$ 74,000.

Figure 10 Transverse section, at plane E of Fig. 21, through the proximal region of the basal body. Rootlets emanate from the peripheral subfibers into the cytoplasm in pin-wheel fashion. The amorphous mass is made up of rootlets and basal foot process. $\times$ 74,000.

Figure 11 Transverse section through the anastomosing rootlets of the basal body at level F of Fig. 21. $\times$ 58,000.

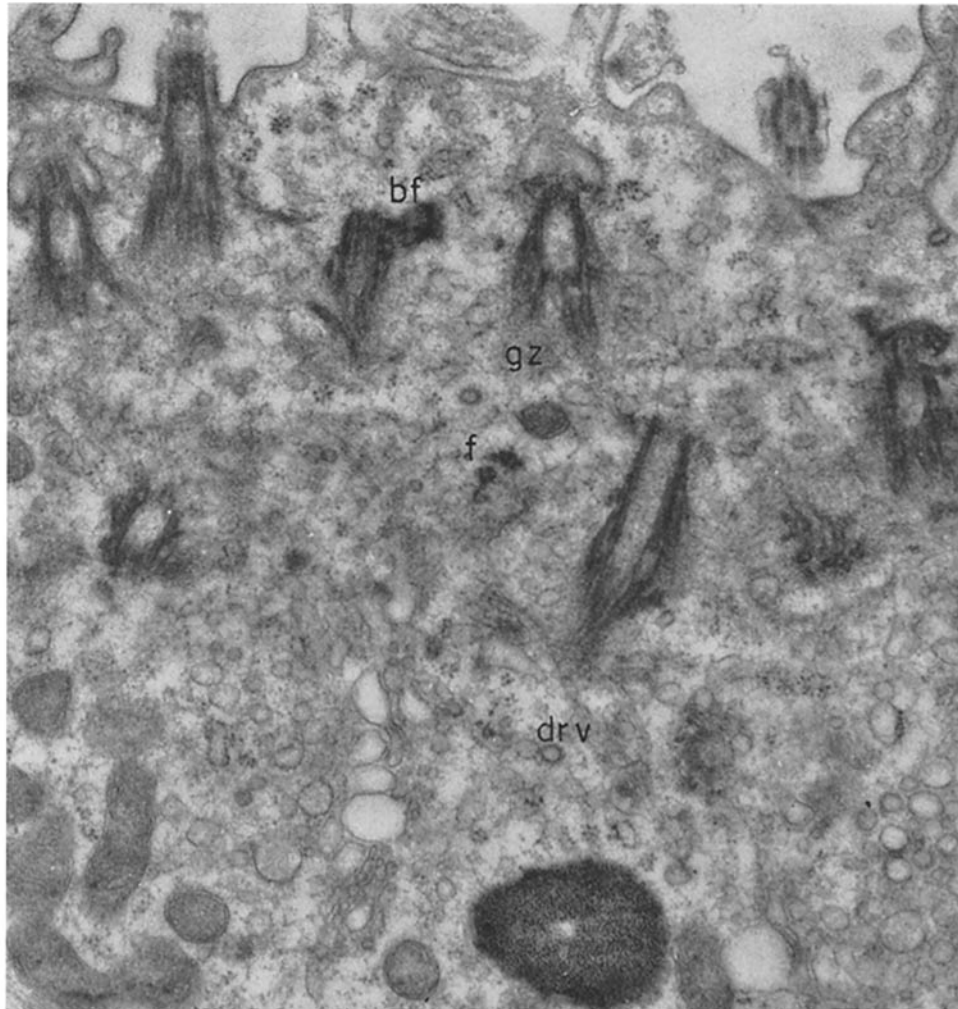M. W. Brightman and S. L. Palay *Fine Structure of Ependyma*   421
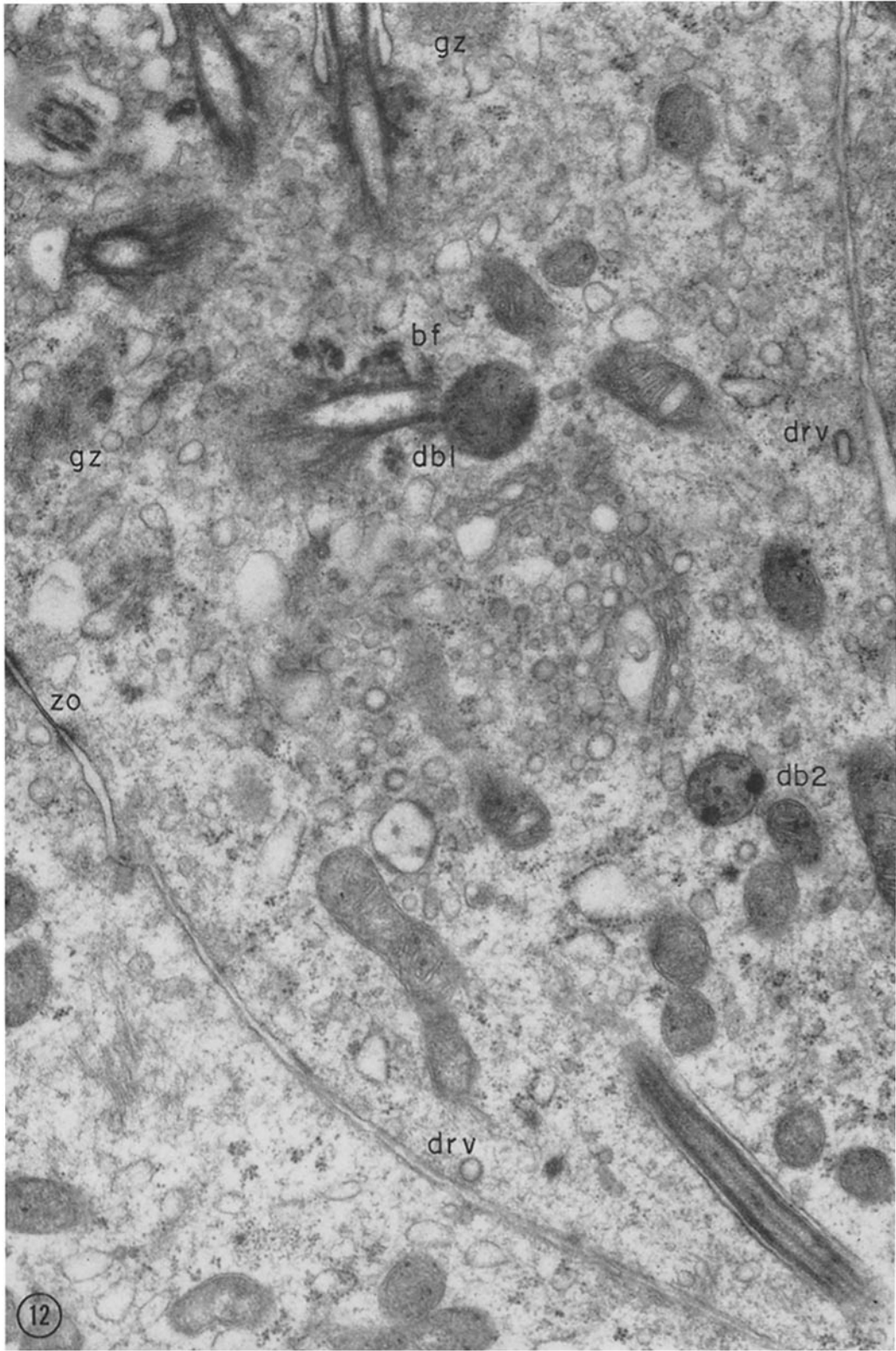
FIGURE 13   Apical cytoplasm of an ependymal cell, showing basal bodies in longitudinal section. The basal bodies have parallel walls and one of them is in contact with a basal foot (*bf*). The individual rootlet filaments have light interiors and are embedded within the granular zone (*gz*). Fragments (*f*) of basal bodies and their adnexa are scattered throughout the cytoplasm. A densely rimmed vesicle (*drv*) and a coarsely granular dense body at the bottom lie above the nucleus and adjacent to a Golgi complex. × 30,000.

FIGURE 12   Apical cytoplasm of an ependymal cell. Two dense bodies (*db 1* and *2*) are situated on opposite sides of the Golgi complex. In the apical region one of the four longitudinally cut basal bodies is directed parallel to the free surface. Rootlets, a granular zone (*gz*), and a unilateral, conical, striated basal foot (*bf*) are associated with this basal body. The shaft and membrane of a fifth cilium lie deep within the cell. Two other ciliary complexes are represented merely by their granular zones (*gz*). A zonula occludens (*zo*) joins two zonulae adhaerentes. Two densely rimmed vesicles (*drv*) lie close to the lateral contact surfaces and a third is situated in the Golgi zone. × 33,000.
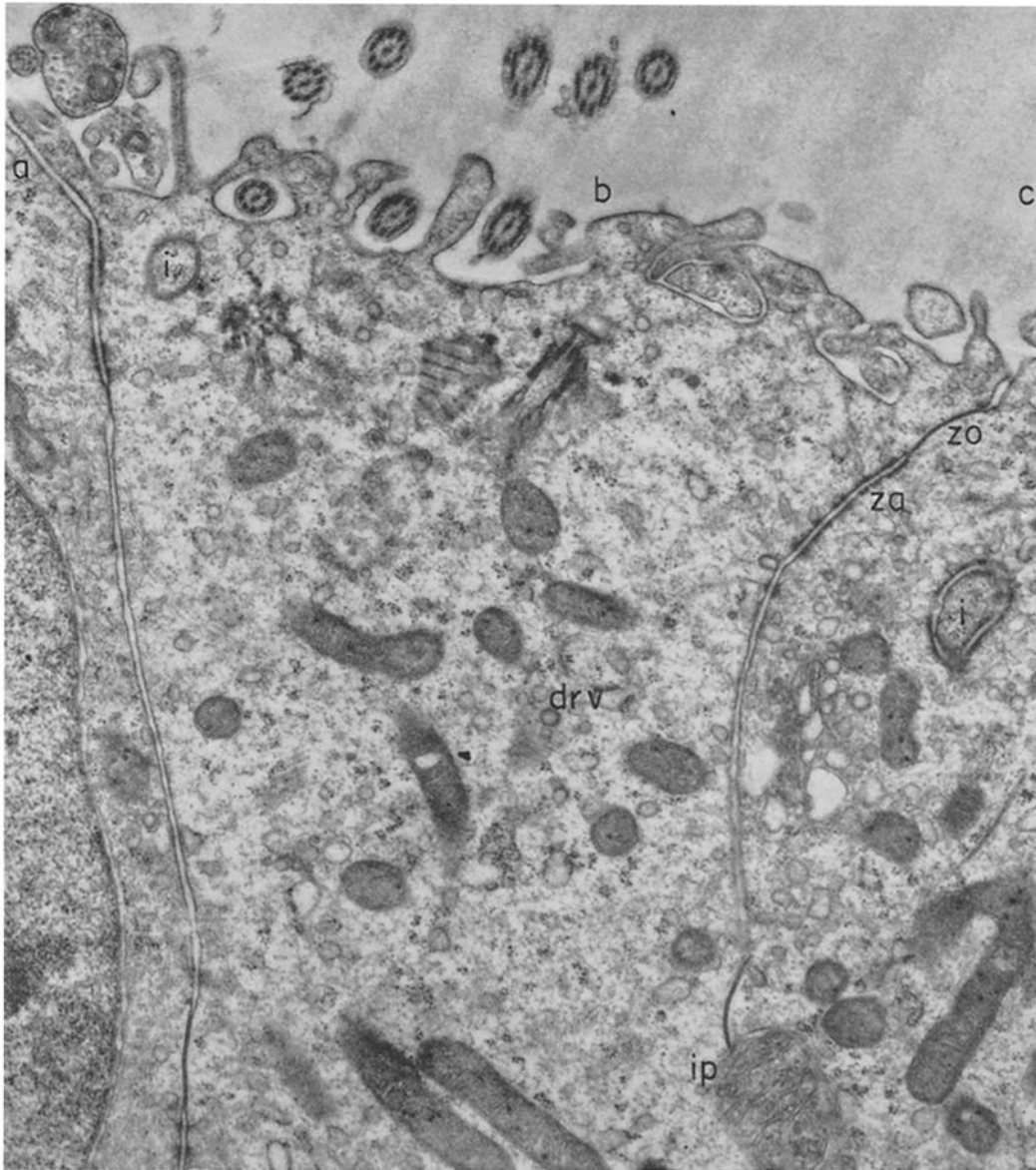
FIGURE 14   The apical cytoplasm of three contiguous cells (*a*, *b*, and *c*) are crowded with vesicles interspersed among mitochondria and fine, cytoplasmic granules. A striated parabasal mass having a major and minor period lies to the left of a longitudinally sectioned basal body. The luminal surfaces of cells *b* and *c* are joined by the *zonula occludens* (*zo*) which is continuous with a *zonula adhaerens* (*za*). Three additional *zonulae occludentes* appear in this figure. Directly beyond one of them the intercellular space widens abruptly to accommodate an aggregate of interstitial processes (*ip*) probably neuronal in origin. The process at the upper left corner lies free within the ventricle and may also be neuronal (see text). Islands of ependymal cytoplasm occur in cells *b* and *c*; in cell *c* the island (*i*) is encompassed by a *zonula adhaerens*; in cell *b* the island (*i*) may be entirely enclosed by a *zonula occludens*. Also in cell *b*, one of two densely rimmed vesicles (*drv*) makes contact with the lateral cell wall. × 21,000.

since been found in the striated musculature of blood vessels by Karrer himself (31), toad bladder epithelium (6, 8, 44), loose myelin sheaths (54), renal tubules, pancreatic acini and ducts, hepatic parenchyma, intestinal epithelium (12, 14), and between glial cells in the central nervous system (23, 45). Farquhar and Palade (12) originally called them "tight intercellular junctions." In his first report, Karrer recognized that they were produced by fusion of the confronted plasma membranes belonging to two different cells, and that they were similar in their structure to the "external compound membrane" (51) of the mesaxon in peripheral nerve fibers. Because these junctions appear to be common wherever cells are closely apposed, a simple term, *haptomere* (portion of attachment), was suggested in an earlier report (5). In view, however, of the more systematic terminology proposed by Farquhar and Palade (14), the name *zonula* or *fascia occludens* has been adopted in the present account.

The basal surface of the ependymal cell will be described in more detail elsewhere in relation to the subjacent structures. The ependymal cell lies directly upon the underlying nervous tissue, and no basement membrane is interposed between them. Usually the first structure beneath the ependyma is an attenuated slip of astrocytic cytoplasm, separated from the epithelium by an intercellular cleft 200 to 250 A wide. Occasionally, however, small myelinated or unmyelinated nerve fibers, or even synapses, occur immediately beneath the ependyma (Fig. 20). There is no surface specialization between neuronal processes and the basal surface of ependymal cells.

CYTOPLASM: The matrix of the ependymal cell is permeated by a widely dispersed, finely granular, and filamentous component that gives the entire cytoplasm a diffuse, cottony appearance (Figs. 12 to 15). Although this material passes into the apical finger-like projections, it does not aggregate differentially in the apical cytoplasm to form a structure corresponding to the terminal web of other columnar epithelial cells, like that of the intestine (33, 40). The filaments attaching to the cytoplasmic surfaces of the *zonulae adhaerentes* apparently consist of this material.

The most striking cytoplasmic inclusion of ependymal cells is an ordered array of perinuclear filaments (Figs. 4, 19), which are 75 to 100 A in diameter, of indefinite length, and spaced about 100 A apart. These filaments run parallel to one another and are gathered into fascicles that are so densely packed that they exclude other organelles. The innermost filaments lie very close to the nuclear envelope and either follow its contours or sweep in toward it almost at right angles. The outermost filaments leave the fascicles in offshoots that can extend to the lateral cell membrane, but they do not contribute to the *zonulae adhaerentes* or to the filamentous appendages of the cilia. As in Fig. 19, the filaments sometimes form a sort of vortex in the perinuclear cytoplasm. In transverse section the fascicles appear as aggregates of dense granules, which are smaller and more distinct than ribosomes. We have not ascertained whether the filaments have a light core. In general, the rest of the cytoplasm is free of large fascicles, although it is often laced by short, randomly disposed bundles of filaments (Figs. 12 and 14).

The ependymal cell contains at least two kinds of dense bodies, located principally in the supranuclear cytoplasm (Fig. 4). All of them are spherical or ellipsoidal, 500 to 800 mμ in diameter, and are delimited by a single, smooth membrane. The first type is replete with extremely fine granules (Fig. 12), somewhat larger dense granules which may be ferritin (Fig. 13), or a mixture of both. The second type (Fig. 12) has a highly heterogeneous content consisting of various granules, vesicles, and lamellae. Many of them contain extremely dense and homogeneous droplets, 100 to 200 mμ in diameter, probably lipoidal in nature. Both of these types of dense body occur in the cells of other tissues, notably in the liver where they have been identified, by combined histochemistry and electron microscopy (29), with the lysosomes isolated by means of fractionation techniques.

Mitochondria are more numerous in the apical than in the basal regions of ependymal cells, an arrangement that is reflected in the distribution of succinic dehydrogenase activity as shown by histochemical preparations (7). The mitochondria are generally elongated and have the usual internal structure with transverse cristae and large, dense, randomly disposed granules. Although these mitochondria are somewhat smaller than those in astrocytes, they are otherwise similar in structure and can be distinguished from the mitochondria in neurons as mentioned below (p. 437 and Figs. 12, 14, 15, and 20).

The elements of the endoplasmic reticulum are

widely dispersed in the cytoplasm. Most of them take the form of smooth-surfaced vesicles and short canaliculi; interconnecting tubules are rarely encountered. Although ribosomes occur abundantly, chiefly in isolated clusters or rosettes, they are only infrequently associated with the membranes of the endoplasmic reticulum. Consequently, the granular reticulum is relatively sparse. The Golgi complex is confined to the supranuclear cytoplasm, often near one of the lateral cell membranes (Fig. 4). It consists of the usual dense cluster of vesicles together with a few flattened or dilatered cisterns, arranged in a stack. The whole apparatus is rather small and is not sharply demarcated from the more scattered vesicles in the surrounding cytoplasm (Figs. 12 to 14).

Among these vesicles is a small number with features distinctive enough to merit separate description (5). They are circular or oblate in profile, ~90 mμ in diameter, and are limited by a particularly dense membrane, ~90 A thick, which is fitted with a halo of fine granules or short radiating rods (Figs. 12 to 14, and 21). Occasionally, part of the membrane is continuous with the lateral cell membrane (Fig. 14). Although these densely rimmed vesicles also occur deep within the cell and in the region of the Golgi complex (Figs. 12 to 14), they are most obvious under the lateral plasmalemma (Figs. 12 and 14). Similar vesicles were recently noted by Wissig (68) in the cells of renal tubules and other organs. They appear to be common components of the cytoplasm in many cell types.

NUCLEUS: The nucleus of the ependymal cell is a simple, regularly oval structure occupying a relatively large part of the cell volume and often lying close to the lateral plasmalemma (Figs. 2, 4, and 14). It contains an eccentric nucleolus embedded in coarsely granular karyoplasm. The nuclear envelope consists of the ordinary fenestrated double membrane and displays no unusual features. Discrete patches of ribosomes are affixed to its outer, cytoplasmic, surface (Fig. 20).

CILIA: The cilia which crown the ependymal cells are about 15 to 20 μ long and 0.4 μ in diameter. Since the ciliary shaft is structurally similar to most of those found in protozoa and other metazoan forms (16, 17), only a brief description is given here, following the terminology proposed by Gibbons and Grimstone (22). The surface membrane, or sheath, of each cilium is a direct continuation of the plasmalemma of the cell from which it arises. Around the cilium, the plasmalemma dips abruptly to form a moat girdling the base of the shaft (Figs. 4, 5, 12 to 14). In transverse sections, the deepest portion of this periciliary moat appears as a crescentic or circular space about the shaft or basal body.

Each cilium contains 9 peripheral or outer fibers arranged in a regular circle about a central pair of fibers, in the usual pattern. In transverse section (Figs. 6, 8, and 21) each of the peripheral fibers is seen to be a doublet consisting of subfiber A, which bears two short arms and has a dense center, and subfiber B, which has a light center and no arms. The subfibers are about 190 A in diameter.

Unlike the outer subfibers the central fibers are not in contact with each other, but are about 110 A apart (as measured between their limiting membranes). The interior of either fiber can be light or dense (Figs. 6 and 8). Both central fibers are linked by an arcuate membrane extending from the outer surface of one fiber (i.e., the portion of the fiber facing the periphery) to the outer

FIGURE 15   This ependymal cell contains basal bodies sectioned at different levels and in different planes. Two adjacent basal bodies (bbd) have been sectioned transversely in their distal region, identified by nine peripheral sets of doublet subfibers. Rootlets arise from the subfibers in a pin-wheel arrangement. A third basal body (bbp) has been sectioned more proximally, as indicated by nine peripheral sets of triplet fibers set askew. The rootlets (r) of a fourth basal body have been sectioned obliquely and end within the surrounding granular zones (gz), a number of which appear in this figure. The structure to the upper left of the rootlets (r) may be the cross-section of a cilium at the level of transition between the shaft and the basal body, unique in having a central, dense core or plate. A cone-shaped, striated basal foot (bf) is in contact with the rootlets of the lowermost basal body and the one directly above it. Two zonulae adhaerentes with an interposed zonula occludens (zo) join this cell with its neighbor. × 37,000.
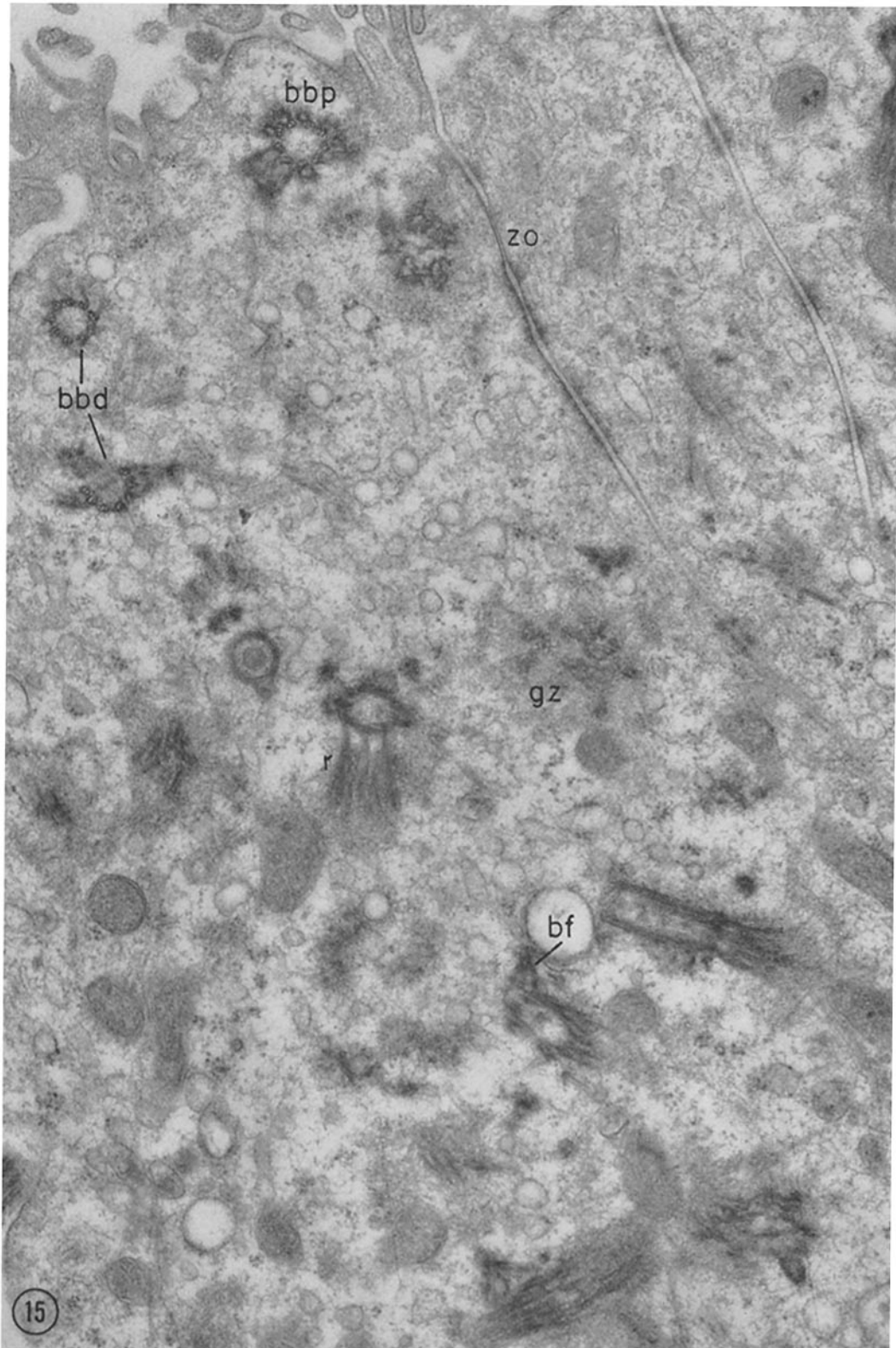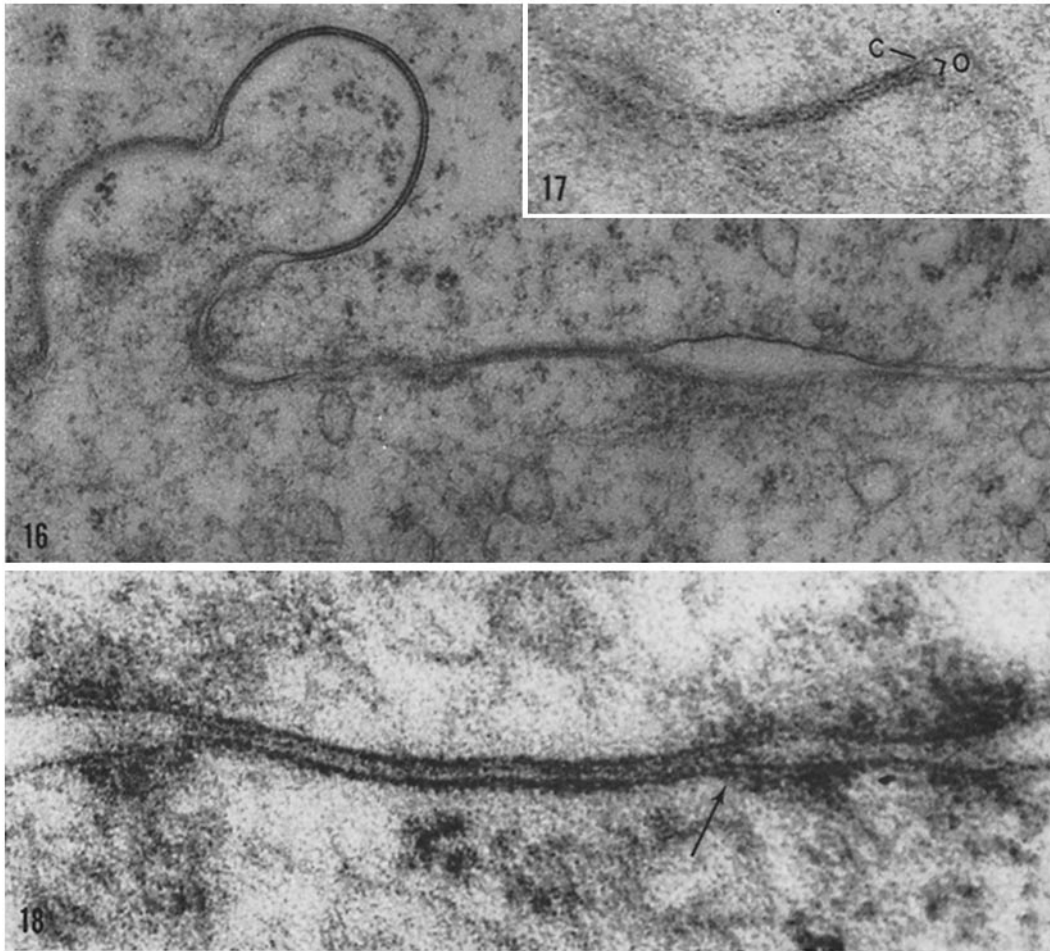
FIGURE 16 A *zonula occludens* forms a dove-tail type of junction between two ependymal cells. The interval between the two dense, completely parallel cell membranes may be compared with the usual intercellular space at the extreme right. × 49,000.

FIGURE 17 A *zonula occludens* joins two cells near their ventricular surfaces. The outer leaflets (*o*) converge to form a median lamella lying parallel to the dense cytoplasmic leaflets (*c*). Permanganate fixation. × 129,000.

FIGURE 18 A *zonula occludens* interposed between two *zonulae adhaerentes* displays the same construction as that of Fig. 18. The three lamellae of either plasmalemma are most evident at the arrow. Tissue fixed in osmium tetroxide and stained with uranyl acetate. × 144,000.

surface of the other (Figs. 6, 8, and 21). This membrane may actually represent only one-half of the central sheath described as enveloping the central fibers in other cilia (16). The other half either has been destroyed during preparation of the tissue or is absent from these cilia. Occasional vague densities on the side opposite the arcuate membrane suggest the former possibility. A short

"filament" extends radially between each peripheral doublet and the central pair. Because these "filaments" occur in all transverse sections between the regions of the tip and base of the shaft, it is likely that they represent longitudinal plates. Such plates, however, have not yet been distinguished from adjacent structures in longitudinal sections. Secondary fibers situated between the
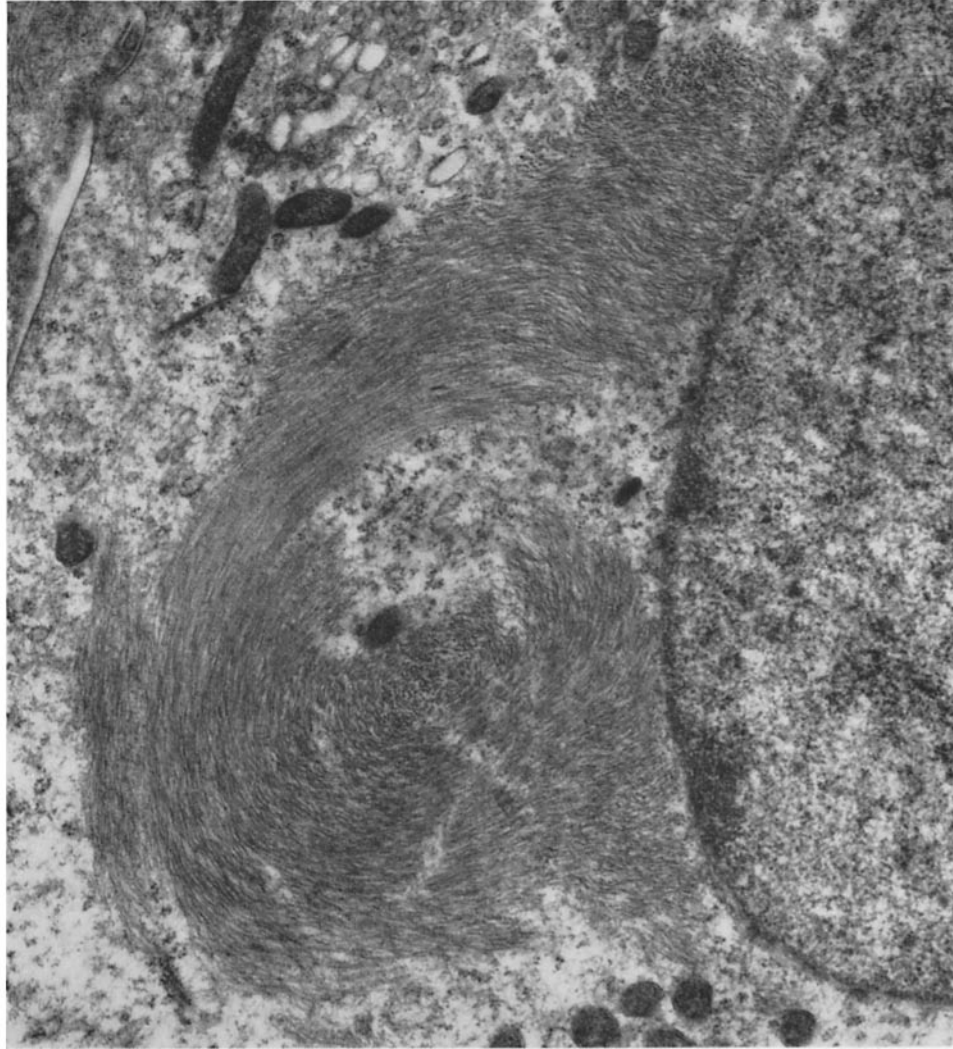
FIGURE 19   A perinuclear whorl of filaments fills the lateral part of this ependymal cell taken from the posterior region of the third ventricle. The filaments lie very close to the outer nuclear membrane. A cluster of mitochondria lies between the whorl and the basal cell membrane, which is not included in the picture. × 24,000.

peripheral and central fibers, as in certain flagellates (22), have not been found in these cilia.

Near the distal tip of the cilium, its diameter diminishes to almost one-half, and here a ring of 9 single fibers encircles the central pair. Since at any one transverse plane along the longitudinal axis of the shaft there are either 9 or 18 peripheral subfibers rather than any intermediate number, it may be concluded that all the outer doublets become singlets at about the same level. Probably subfiber A is the first to terminate, for the remain-

ing subfibers lack the paired arms of subfiber A. One of the remaining 9 singlets is usually crowded out of the peripheral ring to take up a position immediately adjacent to the central pair (Figs. 6 and 7). The diameter of the continuing subfibers remains unaltered. One or two short radial filaments sometimes persist between a singlet and the central fibers.

At the basal end of the cilium, the central fibers terminate near the floor of the periciliary moat (Figs. 5, 12, 13, and 21). This level marks

the transition from the shaft to the basal body of the cilium. No basal plate is present. The peripheral fibers continue their course into the cytoplasm, forming the slightly convex wall of the basal body (Figs. 12 and 13). In transverse sections through the level of transition, a small dense granule appears at the point of contact between the two subfibers of each doublet on the side facing the ciliary membrane. From each granule a short filament radiates peripherally and bifurcates before making contact with the ciliary membrane. These granules and filaments probably correspond to the transitional filaments described by Gibbons and Grimstone (22) in the flagella of protozoa.

The basal body extends for about 0.35 to 0.40 μ into the cytoplasm. Its core is occupied by a clear matrix stippled with a few coarse, randomly dispersed granules (Figs. 5, 12, 13, 15, and 21). In the deep or proximal portion of the basal body, its wall of 9 peripheral doublets becomes a ring of 9 triplet fibers. The subfibers of each triplet are aligned in a straight row and each row is skewed so that subfiber A is closest to the center of the basal body (Figs. 15 and 21). The resultant pinwheel pattern is the same as that observed in protozoans (16, 17, 22, and 37).

The basal bodies of ependymal cell cilia are distinctive among mammalian cilia by virtue of their unusual filamentous appendages. Each basal body is fitted with a brush-like array of fine filaments attached to the doublet or triplet fibers of the wall and extending into the cytoplasm. The exact disposition of these filaments and their mode of attachment to the fibers are difficult to discern in our micrographs because the filaments are encompassed by a cloud of finely granular material which obscures their course. In addition, the cilia are not nicely oriented in a uniform plane and direction as they are in certain protozoa, so that sequential sections of successive cilia are not obtainable within single sections. The extent of this randomness is evident in Fig. 15. The rootlet filaments are about 100 A in diameter, and about 0.4 μ (0.30 to 0.46 μ) long. Their exact length is difficult to ascertain because the free ends of the rootlets are soon lost in the surrounding zone of dense granules. In favorable sections, the filaments appear to have a double contour as if they were tubular in structure. The rootlets often arise as two main bundles. Those emerging from the lateral aspect of the basal body wall tend to diverge; those leaving the proximal tip of the basal body converge below it in a formation resembling the pointed tip of an artist's brush (Figs. 5, 12, 13, 15, and 21). Transverse sections across this formation have a reticulate appearance, suggesting that the rootlet filaments fray at their ends and join up together (Figs. 11 and 21).

A second type of appendage is the basal foot, consisting of a short, conical collection of striated filaments attached to only one side of the basal body wall and extending into the cytoplasm at approximately 90° to its longitudinal axis (Figs. 5, 12, 13, 15, and 21). These filaments are not resolved well enough in our micrographs to permit measurement of their diameters. The striations have a repeat period of about 500 A. Occasionally, as in Figs. 5 and 14, a large striated body (sb) of filaments occurs adjacent to a basal body with which it has no obvious connections. In Fig. 14, the striations have a repeat period of 750 A which appears to be divided by a thin dense line down the middle. As in other metazoan cilia, the ependymal cilia and basal bodies are not interconnected by any structure other than the plasmalemma.

Figure 20 The basal region of an ependymal cell from the anterior portion of the iter fills the upper right half of the micrograph. Note the discrete clusters of ribosomes on the outer nuclear membrane. Two to eight sheets of glial processes separate the basal membrane of the ependymal cell from the plasmalemma of an astrocyte, the nucleus of which occupies the upper left field. The surface of this cell is joined to contiguous glial cell processes by zonulae occludentes (zo), which contain a median lamella visible at higher magnification. A large astrocytic process (g), containing filaments, partially or completely envelops neuronal elements, e.g., axons and synapses. Note the thin glial shell (s) surrounding the synaptic endings at the lower right field. Mitochondria (n) with features peculiar to those found in neurons appear in several relatively large processes which are, therefore, presumed to be neuronal. X 18,000.
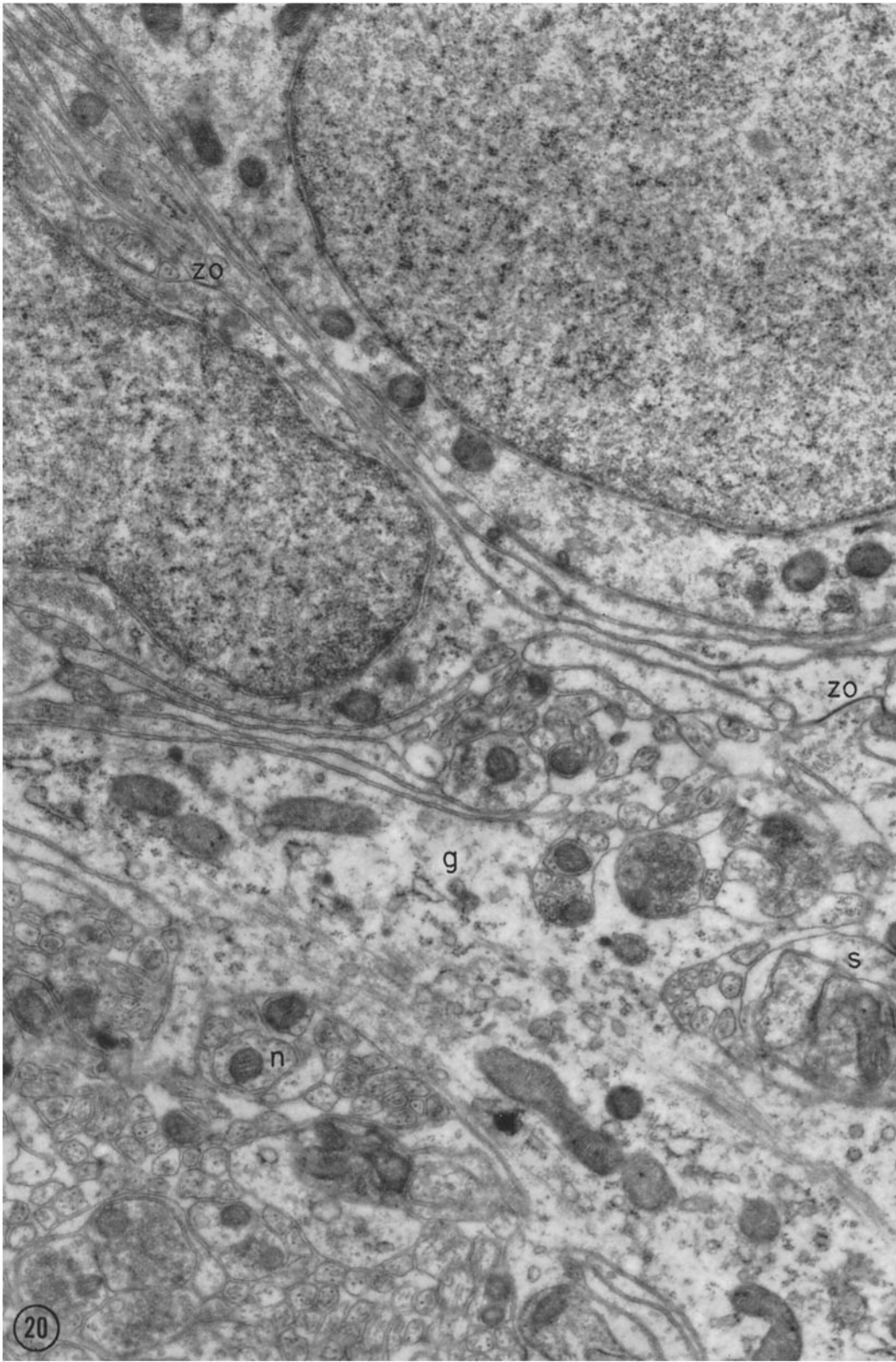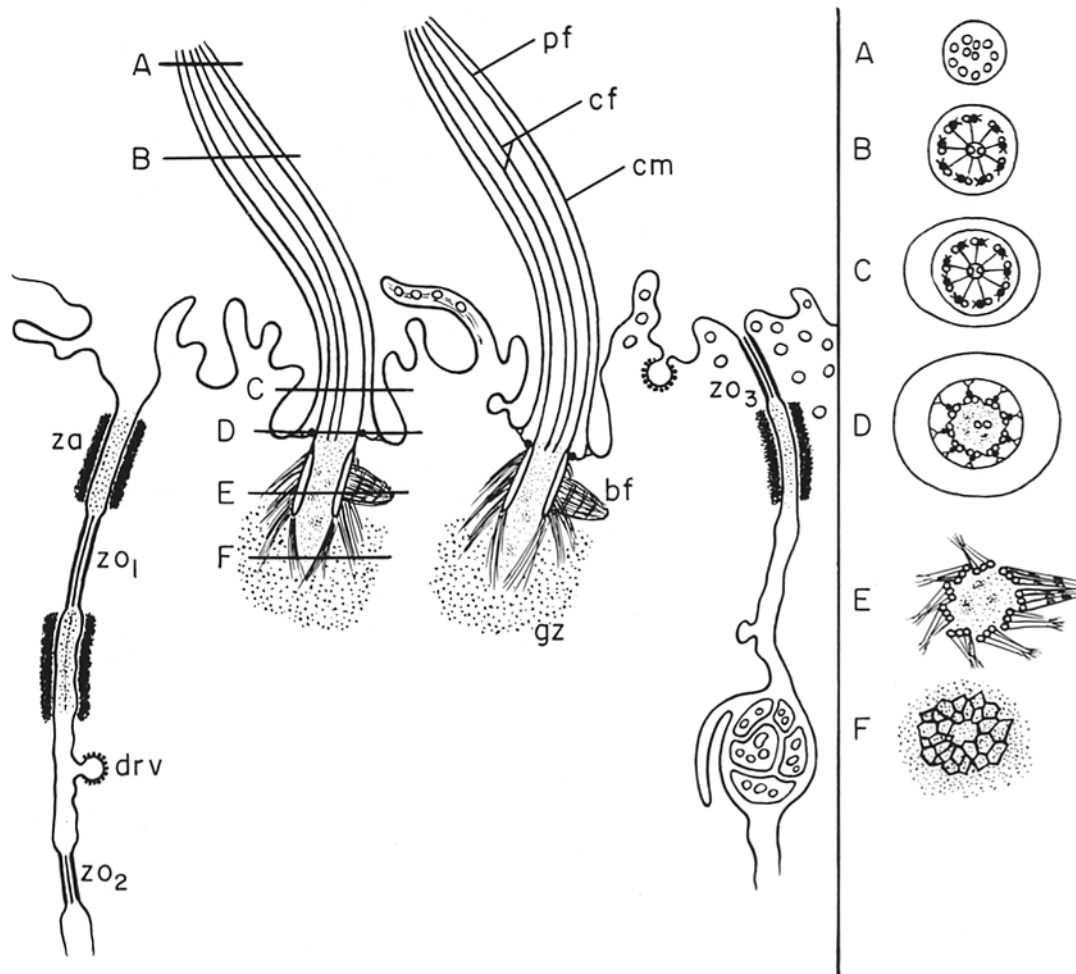
FIGURE 21 *Plasmalemma and Cilia.* Diagram summarizing the principal features of lateral and apical surfaces of the ependymal cell. The most common locations of the *zonula occludens* are at zo_1 and zo_2; the luminal junction, zo_3, is infrequent. A ciliary complex including basal body (*D* and *E*), rootlets (*E* and *F*), basal foot (*bf*), and granular zone (*gz*) is depicted in successive transverse planes designated by letters *A* to *F*. Densely rimmed vesicles (*drv*) may be continuous with either the ventricular or the lateral cell membranes. A cluster of small neuronal processes occupies a "lacuna" or local distention of the intercellular space. *cf*, central fibers; *cm*, ciliary membrane; *pf*, peripheral subfiber; *za*, *zonula adhaerens*.

DISCUSSION

When Purkinje (46) discovered the cilia of the ependyma he recognized that he was dealing with appendages of an epithelium lining the ventricles. The structure which later came to be recognized, however, as the limiting border between the ventricles and nervous tissue proper was the "internal limiting membrane" of His (28). According to His's embryological studies, this membrane was formed by the confluent apical processes of ependymal spongioblasts. The subse-

quent papers of Studnička (60) and Hardesty (26) depicted this membrane as a cuticular consdensation, which in sections appeared as a continuous, argyrophilic line representing the free border of the ependyma. This conception of the limiting border still appears in some textbooks of neuroanatomy (47).

The present study does not reveal any form of cuticular condensation at the free surface of the ependyma that could correspond to the internal limiting membrane of His (*cf.* 1, 60). Only the
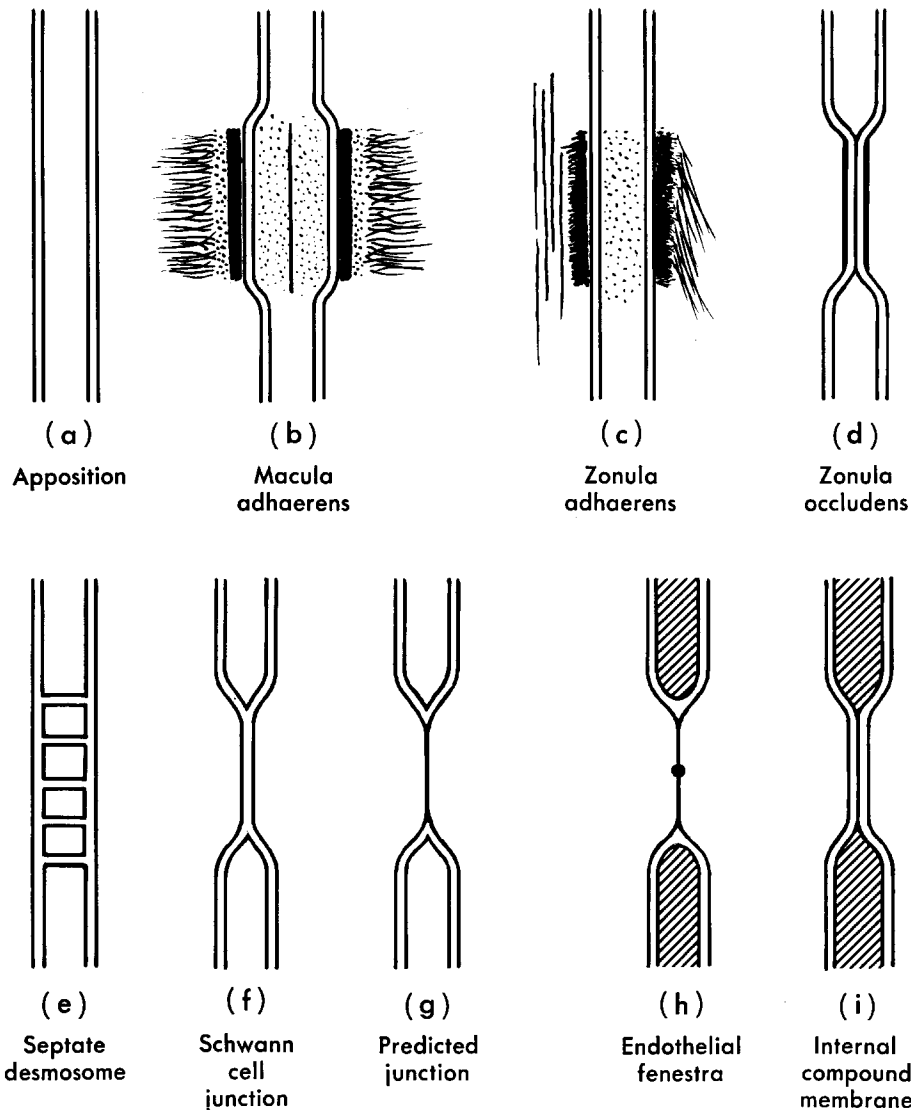
FIGURE 22 *A scheme of actual and predicted intercellular junctions.* This arrangement is not intended as a developmental or phylogenetic scheme, but serves to emphasize that the structural differences between junctions is a result of fusion or disappearance of leaflets in the constituent unit membranes.

highly folded plasmalemma delimits the apical cytoplasm. The silver-impregnated line observed in stained sections by optical microscopy probably represents a metallic coating of the numerous surface projections. The limiting structure that stands between ventricular fluid and brain parenchyma is the ependymal cell itself. It is thus more appropriate to return to the original concept of a limiting epithelium (20, 65) than it is to retain that of an independent limiting membrane.

INTERCELLULAR JUNCTIONS: The lateral surfaces of adjacent ependymal cells are fastened together by two kinds of adhesive devices, named, according to the classification of Farquhar and Palade (14), the *zonula adhaerens* and the *zonula occludens*. The structure of these junctions will be discussed below in relation to intercellular junctions in other tissues. It should be recognized that published descriptions contain many discrepancies in reported dimensions, and, in some instances, deficient information concerning the disposition

of the three leaflets of the unit membrane or plasmalemma as it enters the junction. Nevertheless, enough data have been collected to provide the basis for a tentative scheme (Fig. 22) placing the known kinds of intercellular junctions in a graded series and predicting some that may be found in the future. It must be emphasized at the outset that the progression presented in the following paragraphs is not intended to imply a developmental or evolutionary sequence, but is merely a convenient classification of the types of contact. A developmental study of the desmosome has been presented recently by Overton (38).

The simplest form of junction (Fig. 22a) is mere apposition of cells with a 150 to 200 A gap between their plasmalemmas. This is the most common form in all epithelia and in the central nervous system. The second device is the desmosome, or *macula adhaerens* (Fig. 22b), which occurs between a wide variety of epithelial and endothelial cells but not between ependymal cells. It is restricted to an oval or rectilinear area of the confronted cell surfaces and involves not only the intercellular substance but also the cytoplasm adjacent to the plasmalemma, as in the *zonula adhaerens*. The cytoplasmic plaque is very dense, directly applied to the cytoplasmic leaflet (15, Fig. 4), or slightly removed (38), and is more sharply defined than that of the *zonula adhaerens*. At these junctional points the intercellular gap can be increased to 300 or 400 A. The trilaminate structure of the plasmalemma remains discernible, even though considerable filamentous condensation occurs against its cytoplasmic face (15, 38). A median intercellular lamina distinct from two trilaminar membranes bisects the intercellular space.

The third form of intercellular junction—one which does occur between ependymal cells—is the usual *zonula adhaerens* (Figs. 4, 12, 14, 15, 18, and 22c). This type of junction differs from the *macula adhaerens* (desmosome) principally in being more extensive, in being bordered by a less highly ordered cytoplasm, and in having an intercellular space of the same width as the rest of the intercellular space (about 200 to 225 A). This third junction may form a complete apical girdle about the cell. The subjacent cytoplasm is condensed to form a plaque oriented parallel to and, as in the *macula adhaerens*, directly applied to, or slightly removed from, the cytoplasmic leaflet of the cell membrane. Unlike the *macula*

adhaerens of endothelial and epithelial cells (15, 38), the *zonula adhaerens* has no adjacent fascicle of longitudinal fibrils running perpendicularly to the plaque. Instead, tufts of cytoplasmic filaments insert directly into the plaque at an angle. It is here that the components of the terminal web (33) are attached to the cell membrane in such epithelia as the lining of the intestine. The interval between the two cell membranes may present a diffuse density which occasionally contains an indistinct, median, dense layer parallel to the cell surface.

The fourth step (Fig. 22d) in this series is a five-layered junction, the *zonula occludens*, which has received notice only recently. This structure resembles the *macula adhaerens* (desmosome) in that it consists of two confronted and parallel plasmalemmas with a dense layer midway between. But it differs from the *macula adhaerens* in three important respects: (1) The intercellular space is obliterated by fusion of the superficial leaflets of the apposed unit membranes; (2) the cytoplasmic leaflets are extremely dense and appreciably thicker than the other two leaflets of the constituent unit membranes, although this increased thickness is characteristically absent from other *zonulae occludentes* (*e.g.*, 45), and (3) the interval between the cytoplasmic leaflets is only 60 to 90 A wide whereas that of the *macula adhaerens* is 150 to 300 A wide. A fourth but variable distinction is the absence of increased density in the cytoplasm bordering the *zonula occludens* of glial, ependymal, and other epithelial cells (23, 30, 45), cardiac muscle cells (58, 59), striated muscle cells of certain blood vessels (31), and smooth muscle cells of the intestine (9, 24, 61). In contrast, the *zonulae occludentes* joining endothelial cells have a relatively dense subjacent cytoplasm along part of their extent (36). Depending upon whether permanganate or osmium tetroxide has been used as a fixative, a dense, median layer may or may not be visible between the very dense and thickened cytoplasmic leaflets. Thus, the electrical synapse of the crayfish (25) with its unusually narrow synaptic cleft (80 to 100 A) may also prove to be this type of junction. It is highly likely, as Robertson (53) has suggested, that treatment with permanganate would reveal the median layer formed by contiguity or union of the outer leaflets in this synapse.

Although five-layered configurations at intercellular junctions were first noticed in the cardiac muscle

of the guinea pig and the mouse by Sjöstrand, Andersson-Cedergren, and Dewey (58) in 1958, they were not given special consideration until 1960 when Karrer (30) described similar formations in human cervical epithelium and in the cardiac musculature of pulmonary veins in the mouse. Since then, they have been found in a wide variety of mammalian tissues including lining epithelium of the toad bladder (6, 8, 44), intestinal epithelium (14) and smooth muscle (9, 24, 61), glial cells in the optic nerve and central nervous system (23, 45), the loose myelin sheaths in the goldfish (54), renal tubular epithelium, pancreatic acinar and duct epithelium, hepatic parenchyma, bile capillaries (12, 14), and blood capillaries of many organs (36). Several investigators have pointed out the resemblance between these configurations and the external compound membrane, the mesaxon, and the lamellae of the myelin sheath (14, 30, 54). Although several names have been suggested for them, such as close appositions (44), quintuple-layered interconnections (30), quintuple-layered units (45), tight junctions (12, 13), haptomeres (5), and nexus (9), most investigators have assumed that all of these five-layered configurations are alike. Despite certain discrepancies in dimensions among them, it is highly probable that they represent a single type of specialized junction. The designations, therefore, are all synonymous.

The most careful study so far reported is that of Farquhar and Palade (14), who also examined this junction in permanganate preparations, where the three layers of both plasmalemmas can be followed without question. It is clear that here the outer leaflets of both plasmalemmas are in exact contiguity or have fused, i.e., the intercellular space is obliterated completely (Figs. 17 and 18). Whether a cleft still persists must remain undecided, because the width of this cleft, if it exists, is just at, or below the level of resolution of the best electron microscopes. It is likely, however, that the membranes are in intimate contact or are fused. Mere contiguity (with intervening cleft) would result in a median band about 50 A wide, i.e., the combined widths of two apposed outer leaflets. But in most cases, the band is only about 20 to 30 A thick, indicating an intermixing or rearrangement of membrane material. An alternative, but unlikely explanation is that the outer leaflets abruptly become narrowed as they approach each other.

According to some reports, the whole structure is 150 A wide with a 75 to 100 A gap between the dense cytoplasmic leaflets and a 20 to 30 A median line bisecting the interval (14, 31, 45). There are, however, some discrepancies here. Karrer (30) gave the dimensions of this whole structure in the human cervical epithelium as 200 A wide, with the limiting dense layers as 30 A each and the central dense layer

as 20 A thick. Without commenting on the discrepancy in dimensions, he considered this structure as identical with the five-layered junction in the venous muscle of the mouse, where the over-all width was given as 150 A. It must be pointed out that the dimensions of the junction in the cervical epithelium were taken from material fixed in permanganate, whereas those in the mouse were taken from material fixed in osmium tetroxide. Swelling in one or shrinkage in the other may explain the discrepancy. Similarly, in ependyma fixed with osmium tetroxide and stained with uranyl acetate, the entire plasmalemma (70 A thick) of the zonula occludens appears to be stained, the intercellular "gap" is about 60 to 70 A wide and the median lamella 20 A thick. After permanganate fixation, the leaflets of the plasmalemma are more readily discernible. The 45-A cytoplasmic leaflet only is the densest portion of the junction, the "gap" has increased to 90 A, and the intermediate layer is 35 A wide. The ependymal junction, therefore, has a total width, depending upon the methods of fixation and staining, of about 200 A (180 to 235 A). Another source of discrepancy occurs between stated measurements and published illustrations (e.g., 44, pp. 540–1). Yet another cause of variation is pointed out by Farquhar and Palade (14) in their study of junctional complexes, viz., the dimensions of the unit membrane vary considerably from cell type to cell type and even in different parts of the surface of a single cell. Many of the contradictions in the literature may be accounted for by these variations. For example, in material fixed in osmium tetroxide and stained with permanganate, zonulae occludentes between endothelial cells and between oligodendrocytes are about 150 A wide, whereas those involving an astrocyte are about 160 A wide (45).

If we accept the zonula occludens as the result of contiguity, with the probability that some fusion has occurred, the fifth step in the progression is complete fusion. Fusion can occur either with or without retention of the intercellular space within the region of the junction. Thus, compartmentalization of the intercellular space occurs in the septate desmosomes (Fig. 22e) described by Wood (70) in the surface epithelium of hydra, and by Tsubo and Brandt (63) in the Malpighian tubules of the grasshopper. Here the confronted outer dense leaflet and the middle light leaflet of each plasmalemma have become continuous with those of its neighbor to bridge the intercellular space. (This is not intended as a description of the mechanism of formation.) The cytoplasmic dense leaflets of the plasmalemma apparently remain independent of this fusion. The opposite extreme

is represented by the peculiar junction (Fig. 22*f*) seen in the loose myelin sheaths of the eighth nerve of the goldfish where the outer dense line disappears altogether along with the extracellular space, leaving a light layer 40 A wide between the limiting dense layers contributed by adjacent Schwann cell layers (54). Farquhar and Palade (14) indicate that a similar configuration can occur in the *zonula occludens* of some epithelial cells.

The series tends toward the prediction that the material of the light leaflets may also disappear, with consequent fusion of the cytoplasmic leaflets to form a single unit 30 to 60 A across (Fig. 22*g*). No examples of this kind of fusion have been reported between two cells, but we know that it is possible within the compass of an individual cell, as appears (Fig. 22*h*) in the membrane closing the fenestrae of renal capillaries (49), and between superimposed layers of Schwann cells in the myelin sheath. In the latter situation, the five-layered junction, which includes the internal compound membrane, involves fusion of the *cytoplasmic* leaflets (Fig. 22*i*) in contrast to union of the *outer* leaflets in the five-layered *zonula occludens*.

Although the series of intercellular junctions presented above is clearly not the sequence of stages in their development, it should be noted that different types of junction can occur at intervals on a single interface and that sometimes different types of junction can be continuous with each other. For example, simple apposition is always combined with one or more of the other types. In the myelin sheath of the eighth nerve ganglion cells in the goldfish (54), *maculae adhaerentes*, external compound membranes, and fusion all occur on the same interfaces. In certain capillary endothelia (15) the *macula adhaerens* and an extensive luminal junction are neighbors on the same interface. Although Fawcett likens these luminal junctions to miniature "terminal bars," their precise nature is not clear. In some epithelia (14), there is a characteristic arrangement of 3 types of cell junctions: the luminal *zonula occludens*, followed by an intermediate *zonula adhaerens*, and finally a *macula adhaerens*. In contrast, the sequence of junctions in the ependyma is exceptional in that the luminal junction is most commonly a *zonula adhaerens* rather than a *zonula occludens*. Furthermore, there is no *macula adhaerens* between ependymal cells.

Such variations in arrangement may represent physiological, perhaps transitory, differences in the activity of the cells and the physical state of their surface membranes.

The *zonula occludens* is interpreted as a luminal seal blocking the intercellular passage of substances across epithelia (14, 44). But it is emphasized that the luminal junction in the ependyma is often a *zonula adhaerens* rather than a *zonula occludens*. Consequently, there is generally no luminal seal between ependymal cells. When ferritin is injected into the ventricular cavities, the protein penetrates the interspace of the luminally situated *zonula adhaerens*, but does not pass through the *zonula occludens* (5).

It is still uncertain, however, whether the ependymal *zonula occludens* completely seals the intercellular space from the ventricle or merely divides the space into a labyrinth of continuous compartments. The disposition of the segments of *zonulae occludentes* that are visible in sections suggests two possible arrangements. Because of the high frequency of their occurrence in thin sections, it is likely that the *zonulae occludentes* girdle the entire cell. But only serial sections will demonstrate whether the short segments lying at different levels of the confronted cell membranes represent portions of a seal that is discontinuous or one that is continuous and undulating. The former condition suggests a system of overlapping *fasciae occludentes*, each extending for a limited distance around the cell but at a different level from the free surface. Such a disposition would compartmentalize the intercellular space but would not necessarily seal it completely.

CILIA: The ependyma first attracted the attention of early histologists because of the vigorous ciliary motion at its free surface (20, 46, 64). Purkinje recorded that the cilia were still active in the lateral ventricles, third ventricle, and iter of a full-term sheep fetus 30 hours after slaughter. He noted that the cilia are long and pointed and vibrate in a whip-like fashion. Although little has been added by subsequent light microscopists to Purkinje's simple account (see refs. 1 and 27 for reviews), beating cilia in surviving ependyma from adult human brains have now been recorded in motion pictures (71).

Electron microscopy, however, has provided new information on the organization of ependymal cilia. It is clear from the present report that the structure of the ciliary shaft is not remarkably

different from that of cilia on other cells. The distinguishing features of ependymal cilia reside, instead, in the basal body and its adnexa, especially the short, non-striated rootlets, the basal foot, the granular zones, and the striated body. To date, the only other recorded basal body rootlets in mammals are the short rootlets in cells of the rat's tracheal mucosa (50) and the well developed, striated rootlets in the cells of the choroid plexus of monkey and rabbit (67). The rootlets of the last two species resemble those within the ependymal cells of the lamprey (57) and of the goldfish (unpublished observations), where the bifid spur of striated rootlets emerges from the basal body at right angles to the longitudinal axis of the cilium. There are, however, no rootlets in the ciliated cells of the human fallopian tube and mouse's oviduct (16).

The function of the rootlets in ependymal cells remains conjectural. It may be supposed that they serve to anchor the ciliary shafts and basal bodies in the cytoplasm. But their brevity in ependymal and tracheal cells of the rat and their absence in other ciliated cells suggest that they are not essential for anchoring. It is unlikely that they are related to the mechanism coordinating the ciliary beat, because they do not form links between cilia (cf. 55). The reader is referred to the comprehensive account by Fawcett (16) for a critical review of various hypotheses that have been proposed in relation to other ciliated cells. The direction of ciliary beat, however, may be related to the position of the basal foot. In the various cilia on the gill of a fresh-water mussel, the effective stroke is toward the conical basal foot (21).

INTRAEPENDYMAL NERVE FIBERS: In the present study, certain rounded profiles were found at the free surface of the ependyma which contain small mitochondria, vesicles, and tubules, and which have been identified as tips of nerve fibers. The identification in electron micrographs is based upon the resemblance between these profiles and those of typical dendrites. The existence of nerve fibers in the ependyma has been recognized for many years and is not in itself surprising (e.g., 3). But what is of more interest for the study of fine structure is the incidental discovery of a peculiarity of neuronal mitochondria which has not been noticed before and which may be helpful in distinguishing neuronal processes from neuroglial, ependymal, and Schwann cell processes in a mixed population of profiles. It has been known for a long time that neurons contain a high proportion of mitochondria in which the cristae are longitudinally disposed (42). A second peculiarity observed in the present study is the almost total absence of dense spheroidal granules from the mitochondria of neurons. Such granules are prominent features of the mitochondrial matrix of nearly all cells except neurons. In serial sections Rhodin (48) counted an average of 7 granules per mitochondrion in the renal convoluted tubules of the mouse. The granules are also common in liver and muscle cells. In the mitochondria of ependymal, Schwann, and neuroglial cells, which are smaller than those of the kidney, liver or muscle, one or more granules can be seen in almost every section. They rarely appear, however, in neuronal mitochondria. If this observation is corroborated by similar findings in other laboratories and in other species, it may prove to be an interesting clue to the function of these granules, which, in some cells at least, appear to be binding sites for divalent cations such as magnesium and calcium (43, 66).

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