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Origin and Organization of Pigment Epithelial Apical Projections to Cones in Cat Retina

STEVEN K. FISHER AND ROY H. STEINBERG
Department of Biological Sciences, University of California Santa Barbara, Santa Barbara, California 93106 (S.K.F.) and Departments of Physiology and Ophthalmology, University of California San Francisco, San Francisco, California 94143 (R.H.S.)

ABSTRACT

The apical surface of the retinal pigment epithelial cells (RPE) in the cat extend long sheetlike membranes that wrap concentrically above and around cone outer segments forming the cone sheath. The origin and organization of these sheetlike projections were studied in serial sections by electron microscopy. The apical surface of the RPE cells was found to consist of a thin zone of anastomosing ridges, or microplicae, from which longer projections extend. The lamellar projections forming the cone sheath originate from the microplicae as small cytoplasmic tabs that rapidly expand into broader sheets. Growth of individual sheets to their final size and shape continues by lateral and longitudinal expansion, fusion, and subdivision of the membrane. The small area of connection to the cell body allows the lamellae to overlap and interdigitate in forming the complex organization of the sheath. Microfilaments but not microtubules extend into the apical processes. RPE cilia (9 + 0 microtubules) with associated basal bodies, striated rootlets, and microtubules mark the location of retinal cones. These structures may be part of a microtubule organizing center that participates in morphogenesis of the cone sheath. They also may be involved in anchoring the apical projections forming the sheath, or in the movement of apical projections during the phagocytosis of outer segment discs shed from cone tips.

Cone visual cell outer segments in cat retina are surrounded by a remarkable structure that arises from the apical surface of the retinal pigment epithelium (RPE). The epithelial cells extend long sheetlike membranes, the apical projections or processes, that ensheath the cone outer segment in multiple concentric layers (Steinberg and Wood, '74). [Similar arrangements were first observed in the retinas of rabbit (Sjöstrand and Nilsson, '64; Scullica and Tangucci, '68) and dog (Hebel, '70).] Considering the size, shape, and highly ordered structure of the cone sheath, no other vertebrate epithelial cell presents a more unique development of its surface membrane.

Following the initial description of the sheath by transmission and scanning electron microscopy many questions still remained about its structural organization, development, and functional significance (Steinberg and Wood, '74). Especially puzzling was how the sheets or lamellae that wrapped concentrically above and around the cone outer segment originated from the apical surface of the RPE cell. In the present work we studied the attachment of these apical projections to the RPE surface. During this investigation additional observations were made about the structure of the RPE cell and the cone sheath that have enhanced our understanding of sheath development and structure and their relationship to the sheath's principal known function of phagocytosis.

METHODS

The retinas of three adult domestic cats and an 18-day-old kitten were used in this study. Most of the data were taken from an animal fixed by intracardiac perfusion of

Address reprint requests to Steven K. Fisher, Department of Biological Sciences, University of California, Santa Barbara, CA 93106.

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an aldehyde fixative composed of 1% paraformaldehyde and 1% glutaraldehyde in phosphate buffer (Young, '71). This procedure resulted in the best preservation of the RPE-photoreceptor interface. The other retinas were fixed by removing the anterior structures of the eyes and immersing the remaining posterior part of the globe in 2.5% glutaraldehyde in sodium cacodylate buffer. While the latter procedure adequately preserved both the neural retina and the RPE, shrinkage was greater and there was a tendency for these two tissues to separate. This resulted in an exaggeration of the extracellular spaces that form the apical RPE surface. Although this altered the relationship between the apical projections and the outer segments of photoreceptors it also enabled us to visualize more clearly the fine structure of the apical surface, particularly the microplicae (see Fig. 6). After aldehyde fixation the retinas were washed in isosmotic buffer solution, postfixed in veronal acetate-buffered osmium tetroxide (2%), dehydrated in ethanol, and embedded in Araldite (Cargille 6005).

All of the observations were made in the tapetal region of the eye about 5–10° from the area centralis (cone density = 7,500/mm^2, rod density = 293,000/mm^2) (Steinberg et al., '73).

Sections for electron microscopy were cut on a Porter-Blum MT2B ultramicrotome and placed on formvar-covered single-hole grids for serial examination. Thin sections were stained with 1% uranyl acetate and lead citrate and examined in a Siemens 101 electron microscope.

RESULTS

Sheath structure

The structure of typical cone sheaths is shown in longitudinal section in Figure 1A–C and in tangential section in Figures 1D and E and 2A. Apical processes or projections that originate from the surface of the pigment epithelial cell fill the space above the cone outer segment—the supracone space (Fig. 1A,C). In tangential section the processes appeared sheetlike in form, circular in shape, and concentrically arranged (Fig. 2A).

These processes extended to ensheath the cone outer segment and its tip, which was often capped by a single process (Fig. 2B,C,D). The upper one-third of the cone outer segment illustrated in Figure 1E appeared surrounded by no less than five laminae at any point on its circumference and by as many as ten in some areas. At the base of the outer segment the number reduced to one to three projections that inserted or tucked between the calycal processes of the inner segment and the plasma membrane of the outer segment (Fig. 1B,D).

Some of the questions raised in the initial description of this structure now can be answered (Steinberg and Wood, '74). It was not clear, for example, whether each process was in continuity with the apical surface for its full circumference or whether it was only partially attached. We observed that in any single longitudinal section (Figs. 1A,C 2F) some processes were continuous with the apical surface (Fig. 2F, asterisks) while others were not; i.e., a typical extracellular space appeared between the process and the apical surface (Fig. 2F, arrows). In addition, several processes formed concentric caps in the center of the sheath in Figure 1C (arrows), revealing that large segments of their circumferences did not attach to the apical surface.

There also had been uncertainty about the degree of variability in length and circumference of the processes in any single sheath, as well as in the number of processes per sheath. Now, after examining many more sheaths substantial variability has been observed in all of these parameters. In a sample of 26 sheaths, for example, maximum sheath thickness (width) in the supracone space ranged from six to 14 lamellae ($\bar{x}$ = 8.7, SD 1.5). In any individual sheath some processes were relatively short, terminating in the supracone space (Fig. 1A,C) while others extended all the way to the base of the outer segment (Fig. 1A,B,D). Similarly, individual processes may be only small arcs and linear rather than circular in shape (Figs. 1E, 2A, 4C, 5C) while others can wrap concentrically (Figs. 1E, 2A) for as many as two complete turns (Fig. 5C). Branching of apical projections also has been observed (Fig. 1E, arrow). Also, we have found the cones to be more completely and thickly ensheathed than was suspected earlier. When the retina exhibited no detachment from the RPE, the cones were inserted very deeply into the sheath. At the cone tip, for example, the ensheathment tended to be asymmetrical with sheath thickness varying from a minimum of four (Rg 2–6 n = 6) to a maximum of nine (Rg 7–10, n = 7) lamellae, or considerably more than the three to four lamellae originally observed (Steinberg and Wood, '74). Similarly, although the apical processes tended toward a leaflike shape, their tips were sometimes broad enough for the outer segment base to be completely ensheathed by at least one lamella. More often, the sheath at the outer segment base also was asymmetrical with gaps appearing in some regions, while at other points on the circumference the thickness could reach a maximum of three to seven lamellae (n = 7, Fig. 1D).

It was not clear from the earlier observations if the electron-dense profiles observed as a single row of "dots" within each sheath process were microtubules of microfilaments. Our observations indicate that each process contains a single row of 6–7-nM-diameter filaments (Fig. 2E). We have not found microtubules within the apical processes although numerous microtubules occurred in the apical cytoplasm of these cells (Figs. 9I, 9J). This is consistent with other observations of actin filaments but not of microtubules in the apical projections of mammalian RPE (Burnside, '76; Burnside and Laties, '76; Anderson and Fisher, '79).

Two additional observations have been made. First, in the center of most sheaths, there is an expanded process, filled with cytoplasm, which may or may not reach the tip of the outer segment. This relatively enlarged process or central "post" of cytoplasm often contained more organelles than the other processes, including ribosomes and smooth endoplasmic reticulum (Figs. 1A, 5B,D). Secondly, special vacuoles often appeared in the processes, which were distinctive both for their large size and their dense flocculent material, resembling that seen in the subretinal space. A series of four of these vacuoles, decreasing in size in the vitreal-scleral direction, are shown in Figure 1A. Another appears as a "hole" in the central post of cytoplasm in Figure 2A. These vacuoles can also appear in the apical cytoplasm of the RPE (Fig. 2F). Some of these vacuoles (e.g., Figs. 2A, 5E) were shown in serial sections to be intracellular; others, however, could be connected to the extracellular space and be analogous to large, coated pits.

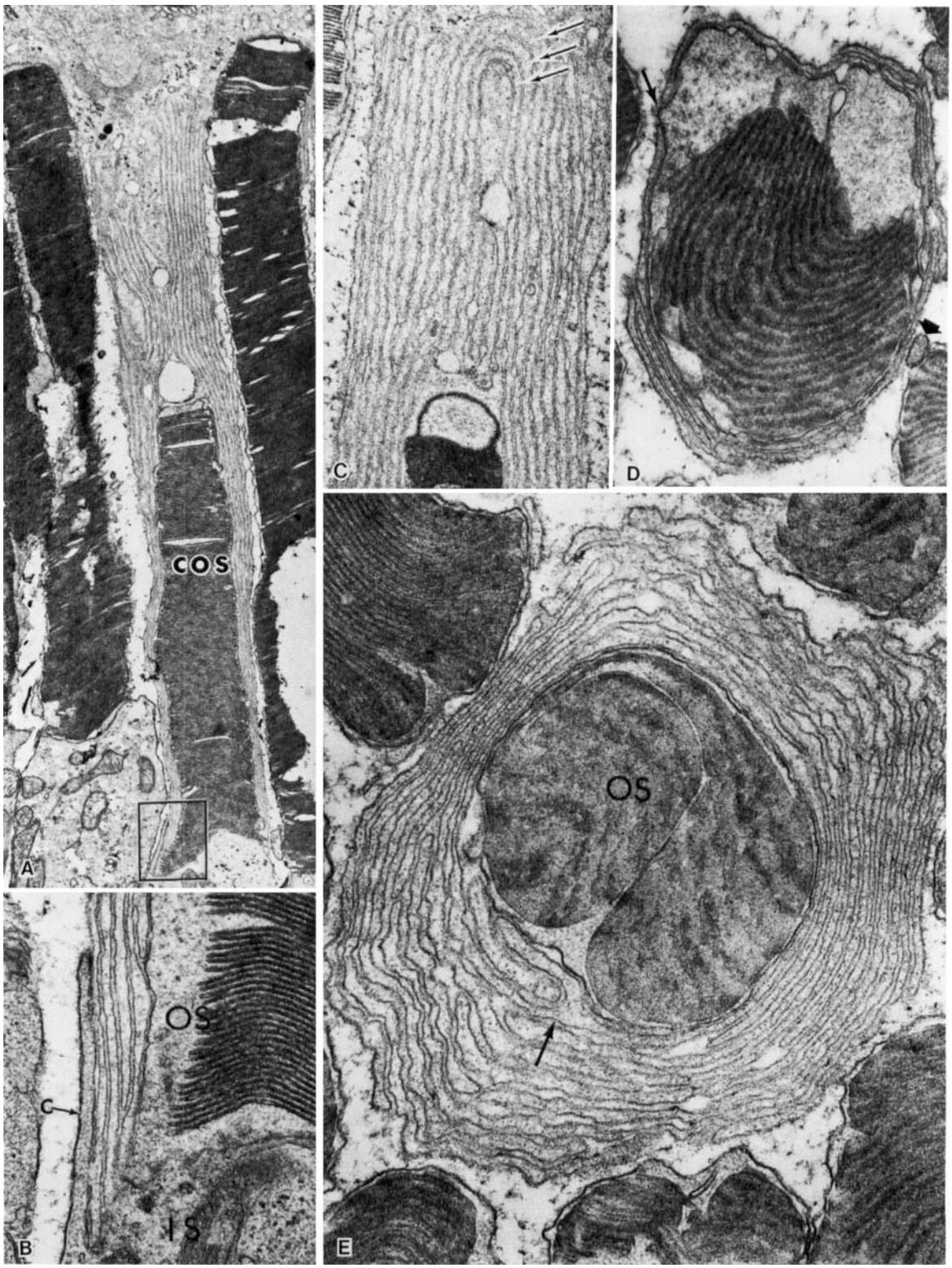

Fig. 1. A. Electron micrograph of a longitudinal section through a cone sheath and the cone outer segment. Retinal pigment epithelial apical projections (processes) extend through the supracone space to surround the cone outer segment (COS). An enlarged process containing ribosomes and smooth endoplasmic reticulum appears in the center of the sheath near the apical surface. There is also a series of vacuoles in the sheath that decrease in size in the vitreal to scleral direction. The area in the rectangle is shown at a higher magnification from an adjacent section in Figure 1B. B. Electron micrograph of a longitudinal section through the base of a cone outer segment. Four lamellae of the cone sheath insert between the outer segment (OS) and a calycal process (c) from the inner segment (IS). Two of the processes reach the inner segment. C. Electron micrograph of a longitudinal section through the supracone space. Three processes in the center of this sheath form complete loops near the apical border (arrows). In this plane of section the majority of sheath processes do not connect to the cell body. D. Electron micrograph of a tangential section through the base of a cone outer segment. At this level the number of ensheathing processes has decreased to one to three and there are gaps in the ensheathment (thin arrow). The thick arrow indicates a calycal process. E. Electron micrograph of a tangential section through the upper one-third of a cone outer segment showing the outer segment (OS) and the processes forming the sheath. One lamellae of the sheath is branched (arrow). A, × 10,700; B, × 34,400; C, × 22,500; D, × 32,500; E, × 41,000.

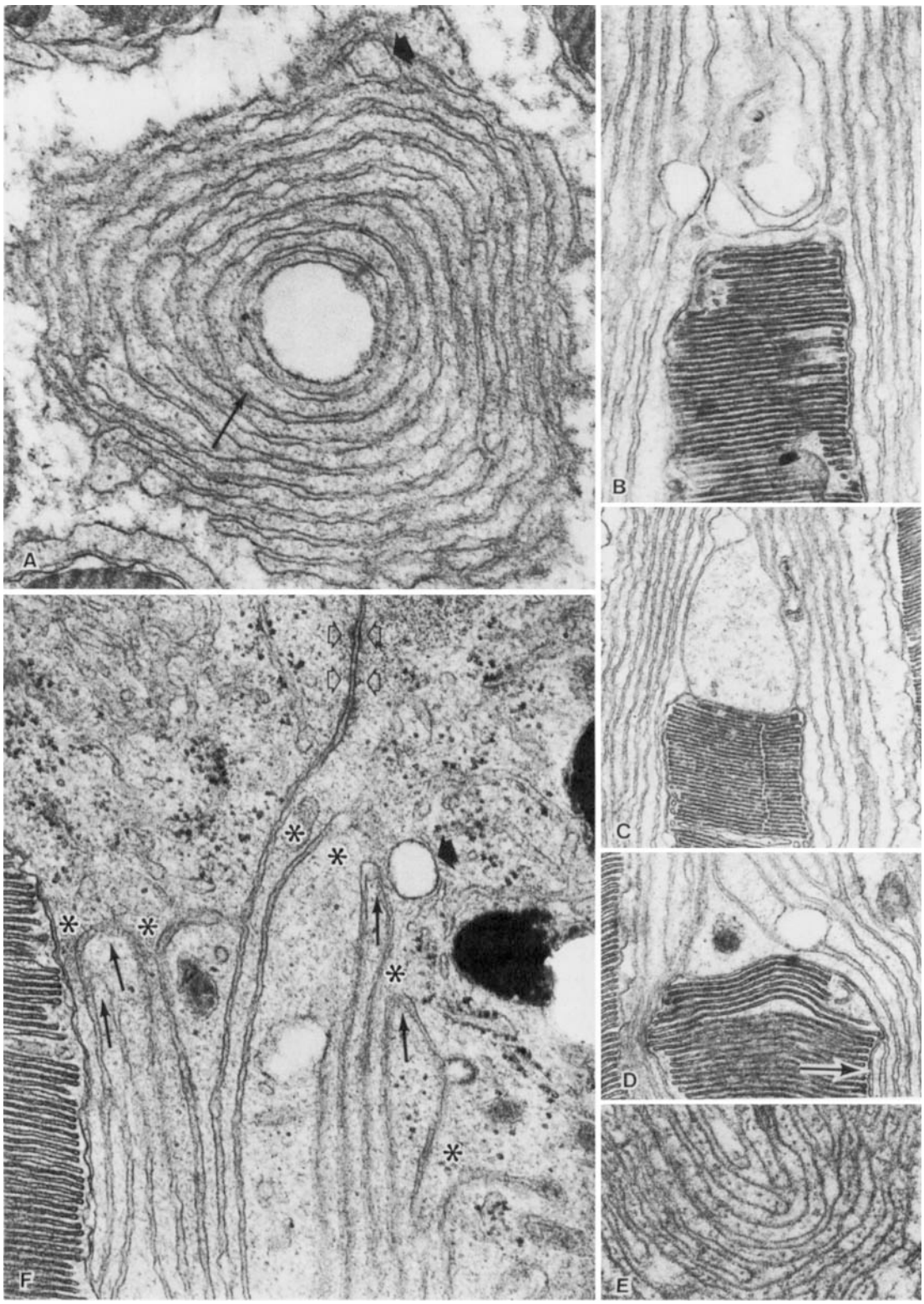

Fig. 2. A. Electron micrograph of a tangential section through the supracone space. The sheath's apical projections occur either as complete annuli (thin arrow) or as segments of varying lengths of arc, some quite small (large arrow). The central process is annular in this section probably because it contains a large vacuole. Profiles of rod outer segments appear in the periphery of the figure. B–D. Electron micrographs of longitudinal sections through the tips of three cone outer segments showing their capping by retinal pigment epithelial cell (RPE) apical lamellae. In *C*, capping is by an enlarged central process. The arrow in *D* indicates a sheath lamella that is not connected to the apical surface in this plane of section. E. Electron micrograph of a tangential section through some cone sheath processes showing the single row of 6–7-nM-diameter filaments that occurs in the center of each process. F. Electron micrograph of a longitudinal section through the supracone space at the apical border of the RPE. Processes that do not connect to the cell body in the plane of section are indicated by arrows; those that do connect, by asterisks. Observe that this cone sheath is formed by apical projections from two adjacent cells. Open arrows indicate a portion of the junctional complex between the two cells. A small vacuole, similar to those seen in the sheaths of Figures 1A and 2A, appears at the thick arrow. A, × 52,000; B, × 34,000; C, × 25,000; D, × 34,000; E, × 67,000; F, × 48,800.

Origin of apical processes from the surface

Surface structure. Tangential sections through the apical surface show that it is formed by small interconnecting ridges that range between 0.05 and 0.1 μM in width and can be estimated to be 1–1.5 μM in height. They are consistent in size and appearance with the microplicae previously described for the surfaces of other cells (Andrews, '76). These ridges were not in the same plane in any one section, appearing to lie on larger hills of cytoplasm that protruded into the subretinal space, as well as on the valleys between. In mature retina this was best observed in a specimen where shrinkage had opened the extracellular spaces between ridges (Fig. 3A). The ridges were well visualized also in developing retina (18-day kitten, Fig. 3B) where they were more prominent.

Origin from surface. From observations already presented it is clear that the apical processes are not continuous with the cell surface for their entire circumference. The way they are attached, that is, their origin at the apical surface, was studied by serial sectioning of individual sheaths from the level of the supracone space back toward the apical surface. Figure 4A–C, for example, show sections through a sheath in the upper one-third of the supracone space (Fig. 4C) and at the apical surface (Fig. 4A,B). This sheath happened to originate from two RPE cells (diagram, Fig. 7), as did the sheath in Figure 2F. (Since RPE cells are hexagonal, as many as three cells, at their juncture, could contribute apical projections to one sheath).

Examples of the different forms taken by the apical projections where they are continuous with the surface are shown in the electron micrographs of Figure 4A–F and summarized in the diagram of Figure 4G. At its origin an apical projection may be connected to the surface by a thin tab (Fig. 4A, open arrow) or the connection may be broader than the width of the projection (Fig. 4A,F, thin arrow). Also, the projection at this point may be only a small, circular profile (Fig. 4A,D, thick arrow). Other forms of origin are a post, or a ridge, both surrounded by extracellular space (Fig. 4A,D,E). The origins, therefore, are outgrowths of the microplicae on their apical or apicolateral surfaces or, in some instances, simply a growth and expansion of the microplicae, themselves.

In sections very close to the apical surface the projections can already assume a sheetlike form. Figure 5 shows such sheets. In 5A and B the sheets already have formed a parallel array but the origin of several of the projections from the apicolateral surface can still be observed (asterisks in Fig. 5B). Further vitreally (about 1–2 μM) the projections have expanded further and begun to align concentrically, but even here connections to the microplicae still can be found (asterisks in Fig. 5D). These figures demonstrate, therefore, that the projections assume their sheetlike form generally within about 0.1–0.2 μM from their origin at the apical surface. This would explain the profiles in longitudinal sections (e.g., Fig. 2F) where sheetlike projections abut the apical surface of the RPE cell.

As shown previously in the longitudinal section of Figure 1A, a post of cytoplasm can appear in the center of the sheath. These can be observed in tangential section in Figure 5B and D. Figure 5D and E also demonstrate that this large extension of cytoplasm can serve as the origin of the circular sheets that lie in the center of the sheath. In the 0.5 μM between Figure 5D and E the central post (dark arrow) has been remodeled to form a circular sheet.

Figures 6–8 are diagrams drawn from tracings of tangential sections through the cone sheaths of Figures 4A–C and 5D and E. By shading individual projections and indicating, with symbols, how they change through different sections their evolution to concentric sheets becomes more apparent. Figure 6, for example, presents four sections from the sheath of Figure 5D and E. Closest to the cone (Fig. 6D) the apical projections clearly vary in form and circumference. A circular sheet in the center surrounds a smaller sheet that includes a cytoplasmic vacuole. The evolution of the circular sheet from a large cytoplasmic post can be traced sclerally in Figures 6A–C. The surrounding projections are either circular or open loops, or smaller circular and linear sheets. The darkly shaded profiles all originate from a single root in Figure 6A; it can be observed to form a sheet in B that makes 1½ turns, and to further subdivide into four sheets in C.

Figure 8 summarizes some of the growth and modeling events that lead to the formation of individual apical projections (note, Fig. 7 diagrams how this sheath was formed from two separate RPE cells). The origin from the surface membrane can occur in a variety of ways—for example, as illustrated here, either as tablike outgrowths or as posts or ridges surrounded by extracellular space (Fig. 8A). They then rapidly expand into sheets (Fig. 8A,D,F,G—straight arrows). Since the apical membrane does not form a flat surface, the projections have their origins at different levels along the sheath (Fig. 8A,D,F,G) but all within about 2–2.5 μM. As the projections become sheetlike they rapidly lose their attachment to the apical surface and then hang freely in the subretinal space (Fig. 8B–G, darkly shaded profile).

Figure 8 also illustrates the importance of the central cytoplasmic mass in sheath formation. In this example the three stippled sheets in Figure 8G all originated from one central root. The central "U"-shaped process in Figure 8G was formed by narrowing and then loss of its connection to the two concentric stippled sheets and by a break (Fig. 8F) that opened the "U."

RPE cilia

An additional finding, which we consider to be significant for sheath development (see Discussion), was the frequent appearance of cilia and basal bodies with associated structures in the RPE cytoplasm, scleral to cones, and in association with the apical projections of the cone sheath in the retinas of cat, rhesus monkey and human. Especially noteworthy was the reliability with which cilia "marked" the location of cones in the contiguous neural retina. In cat, for example, where the majority of our observations were made, all but one of 72 photographed cilia were situated above (scleral to) individual cones.

In the periphery of cat retina, in the region studied most thoroughly, there were approximately two cones for each RPE cell. Here it was not unusual to find two basal bodies or cilia within the cytoplasm of single RPE cells (Fig. 3A). More centrally, at a higher cone density, cilia were also regularly observed but sufficient observations were not made to indicate how ciliary density varied with cone density. (In the area centralis of one cat retina and the fovea of one monkey retina there was not the increase in ciliary density that would be anticipated were each area centralis and foveal cone associated with an RPE cilium.)

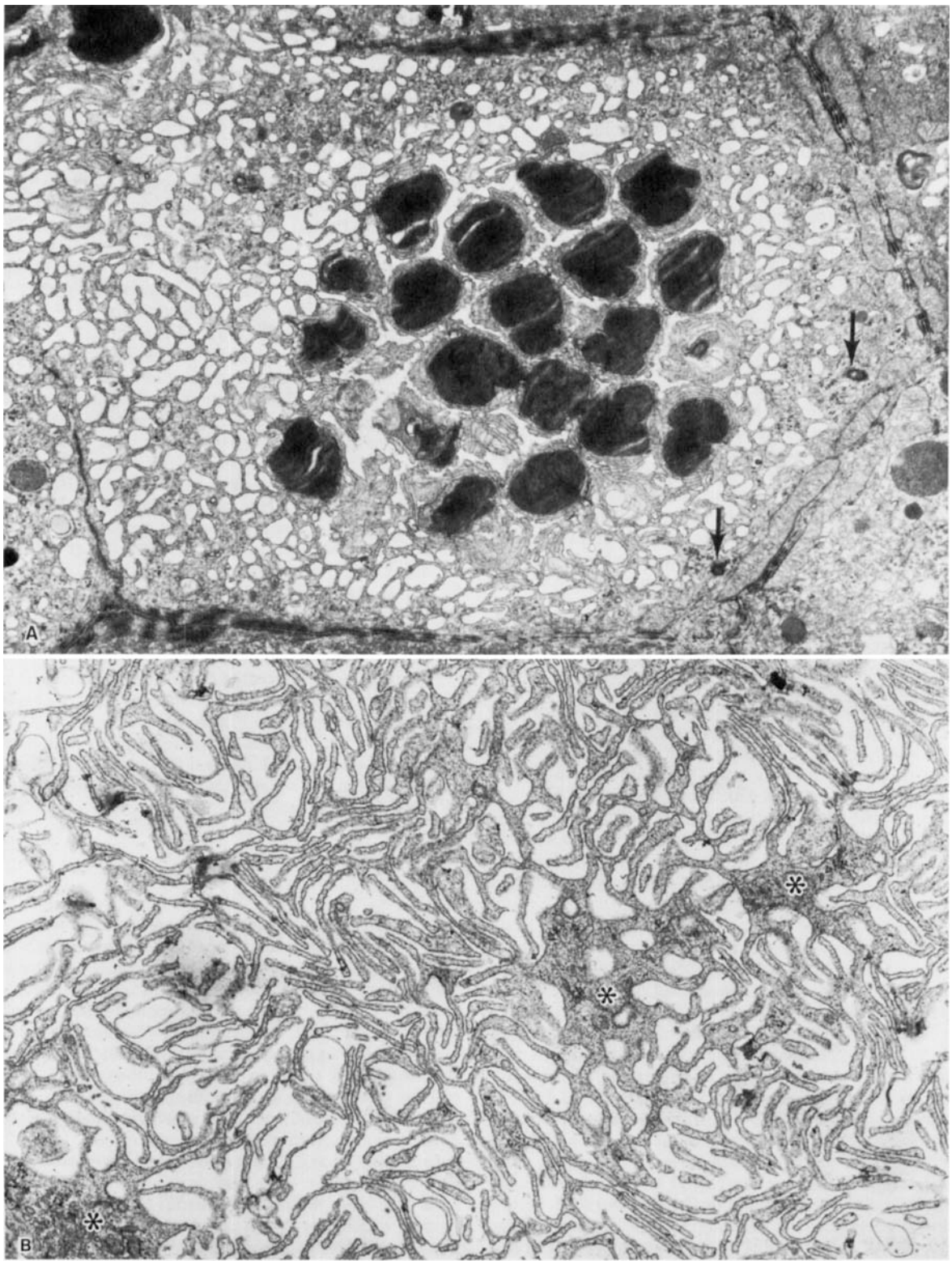

Fig. 3. A. Electron micrograph of a tangential section through the apical portion of an RPE cell showing the cytoplasmic ridges (microplicae) that form the apical surface. Two basal bodies are indicated by arrows. The tips of several rod outer segments appear in the center of the figure. B. Electron micrograph of a tangential section through the apical surface of the RPE in an 18-day-old kitten showing numerous branching cytoplasmic ridges. Some of these ridges can be seen extending from mounds of cytoplasm (asterisks), reflecting the overall appearance of the apical surface—a series of gently rolling hills with microplicae extending from the surfaces of the "hills" and from the "valleys," as well. A, × 44,400; B, × 21,000.

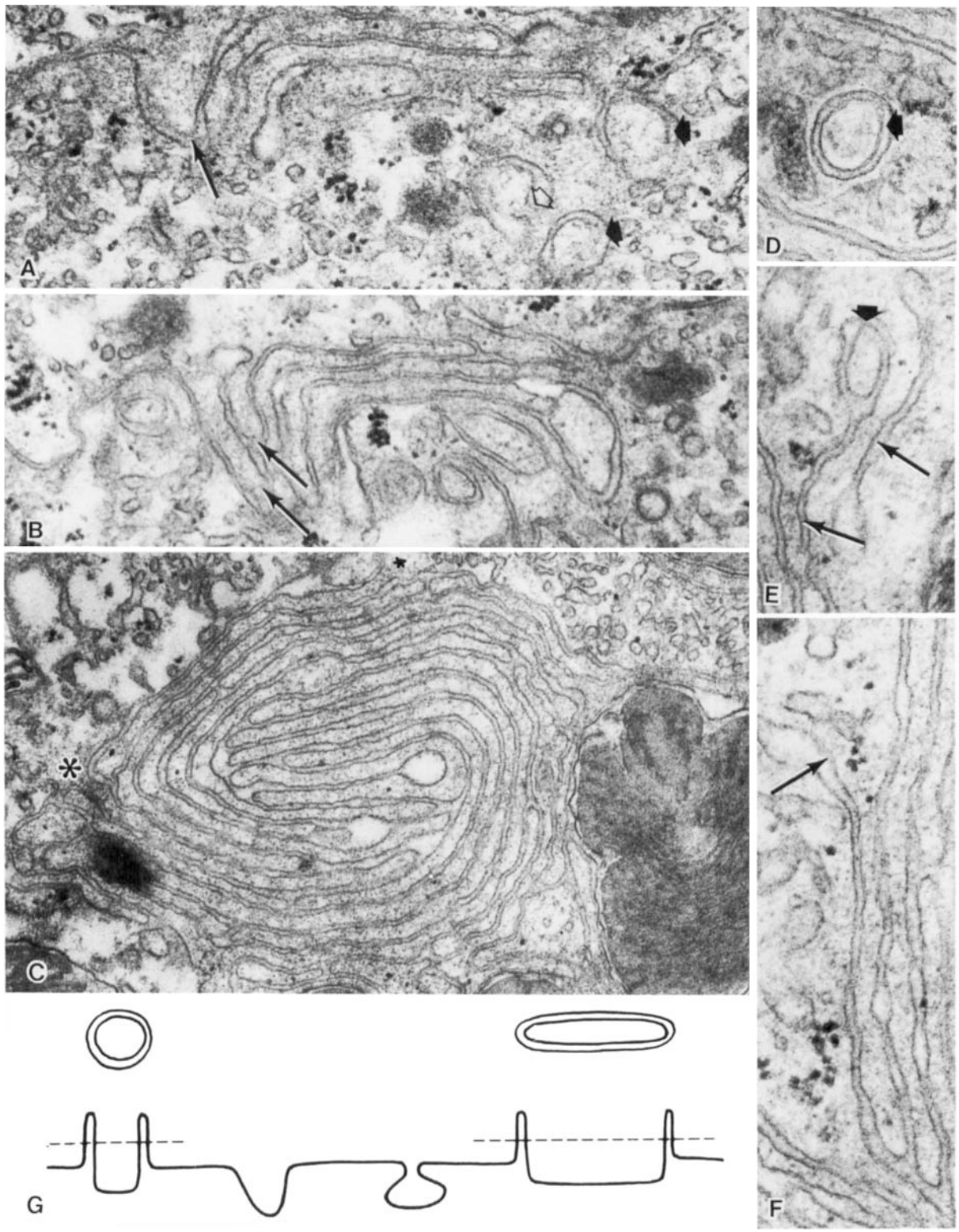

Fig. 4. A–C. Three electron micrographs taken from a series of tangential sections through a cone sheath. The first section is taken at the sheath's origin near the apical surface. At its origin (A) the apical projections assume various forms including posts of cytoplasm and short sheetlike projections. The thin arrow in *A* indicates a broad projection that subdivides into two lamellae in *B* (arrows). The projections change and expand rapidly as seen in *B*, which is only the second section of the series. A near final form of the sheath, much further vitreally, appears in *C*. The section is sufficiently close to the cell body that some lamellae are still attached to it (asterisks). D–G. Different forms taken by lamellae of the cone sheath at their origin are also shown in these electron micrographs and diagrams (G). In *F* a long, thin neck of cytoplasm extends out from a thicker, short ridge (arrow) while in *A*, *D*, and *E* the sheets originate as circular profiles or small posts of cytoplasm (thick arrows) surrounded by extracellular space. The open arrow in *A* shows a thin connection between a small tab and the RPE cell's cytoplasm. The relatively broad outgrowth in *A* (thin arrow) gave rise to two cytoplasmic sheets in the cone sheath shown in *B* (thin arrows). Two narrow ridges of cytoplasm (thin arrows), partially surrounded by extracellular space, are shown in *E*. On the left of the diagram in *G* a post of cytoplasm extends from the folded apical membrane. When cut tangentially at the dashed line this forms the circular posts shown diagrammatically at the upper left of *G* and in the micrographs of *A*, *D*, and *E*. A larger post appears as a ridge when cut tangentially, as shown on the right of *G*. A relatively broad outgrowth (as in *A*, thin arrow) and a thin connection to a tab of cytoplasm (as in *A*, open arrow) are shown in the center of the diagram. A, × 62,000; B, × 52,200; C, × 46,800; D, × 77,400; E, × 72,000; F, × 77,400.

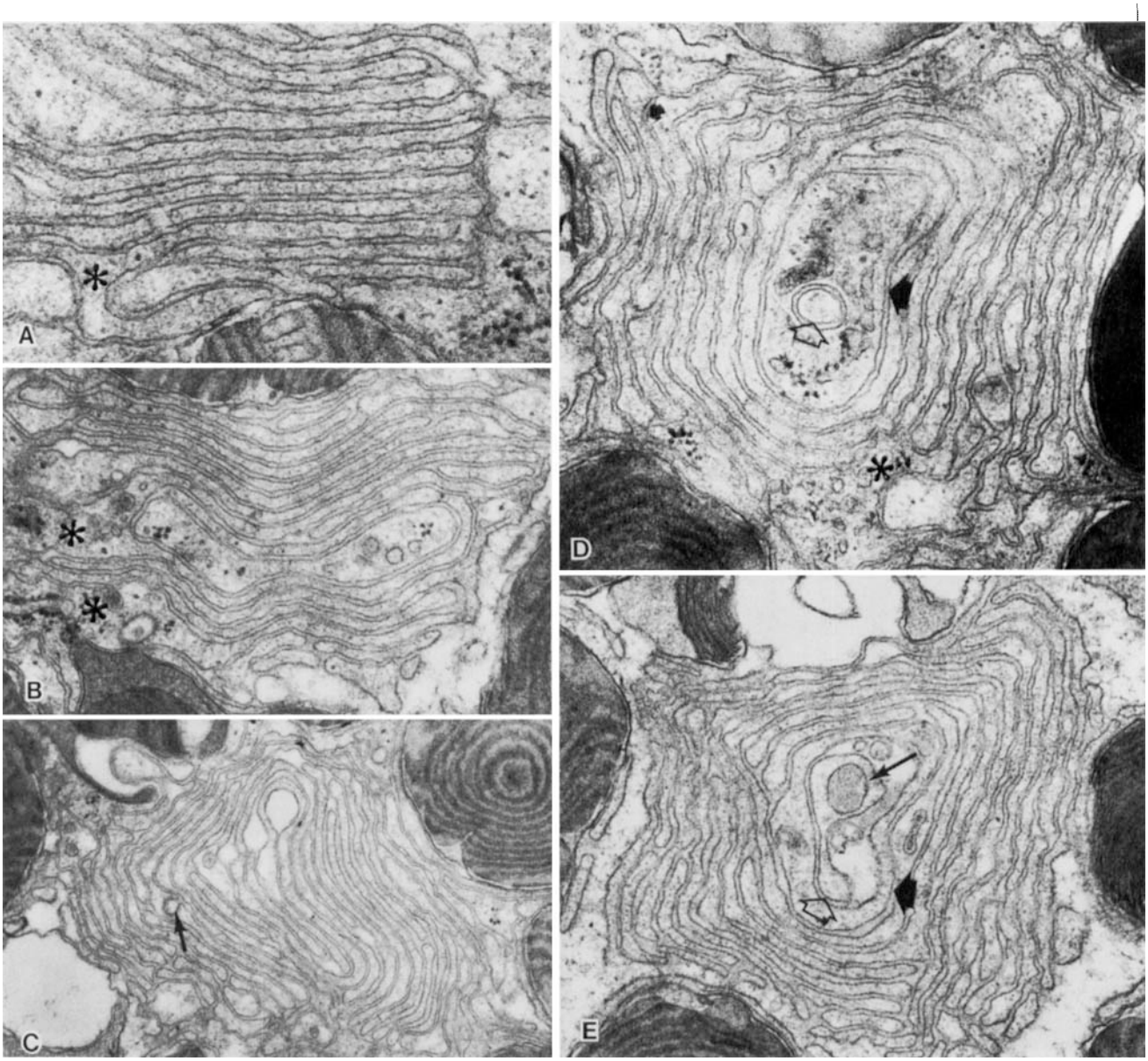

Fig. 5. A–C. Electron micrographs of tangential sections through the cone sheath lamellae near their origin at the apical surface (A and B) and several micrometers further vitreally (C). In *A* and *B* the sheath lamellae are arranged in parallel, but they become concentric further vitreally in the supracone space (C). In *B* an enlarged central process contains ribosomes and small vesicles. A central lamella in *C* makes two complete turns. Another process in *C* has a coated vesicle (arrow). Branching of the processes can be seen (asterisk in *A*). Lamellae marked with asterisks in *B* are still attached to the ridges of cytoplasm that extend from the apical surface. D, E. Two electron micrographs selected from a series of tangential sections through the supracone space. A large central post in *D* (dark arrow) transforms into an circular sheet in *E* (dark arrow), while the smaller central post of cytoplasm in *D* (open arrow) has expanded linearly in *E* (open arrow) and contains a vacuole filled with flocculent material (thin arrow). The asterisk in *D* indicates a connection between a sheath lamella and a ridge of cytoplasm extending from the apical surface. A, × 56,400; B, × 37,300; C, × 25,000; D, E, × 40,000.

Figure 9 presents examples of cilia, basal bodies, and associated rootlet structures from the three species studied. The ciliary structures were always located in the apical region (vitreal one-third) of the RPE cytoplasm. This regularity of location is also illustrated in Figure 3A by the presence of two basal bodies in the same tangential section through the apical region of one RPE cell. It was also common to find these structures at the bases of the apical projections (Fig. 9A,G); occasionally the cilium projected alongside the cone sheath in the subretinal space (Fig. 9D).

Although the orientation of these cilia and associated structures within the RPE cell varied, one most frequent configuration was observed: A short cilium of the 9 + 0 microtubular pattern (Satir, '77) and average length of 1.6 μM (Rg 1.25–2.3, n = 9) projected into an intracellular

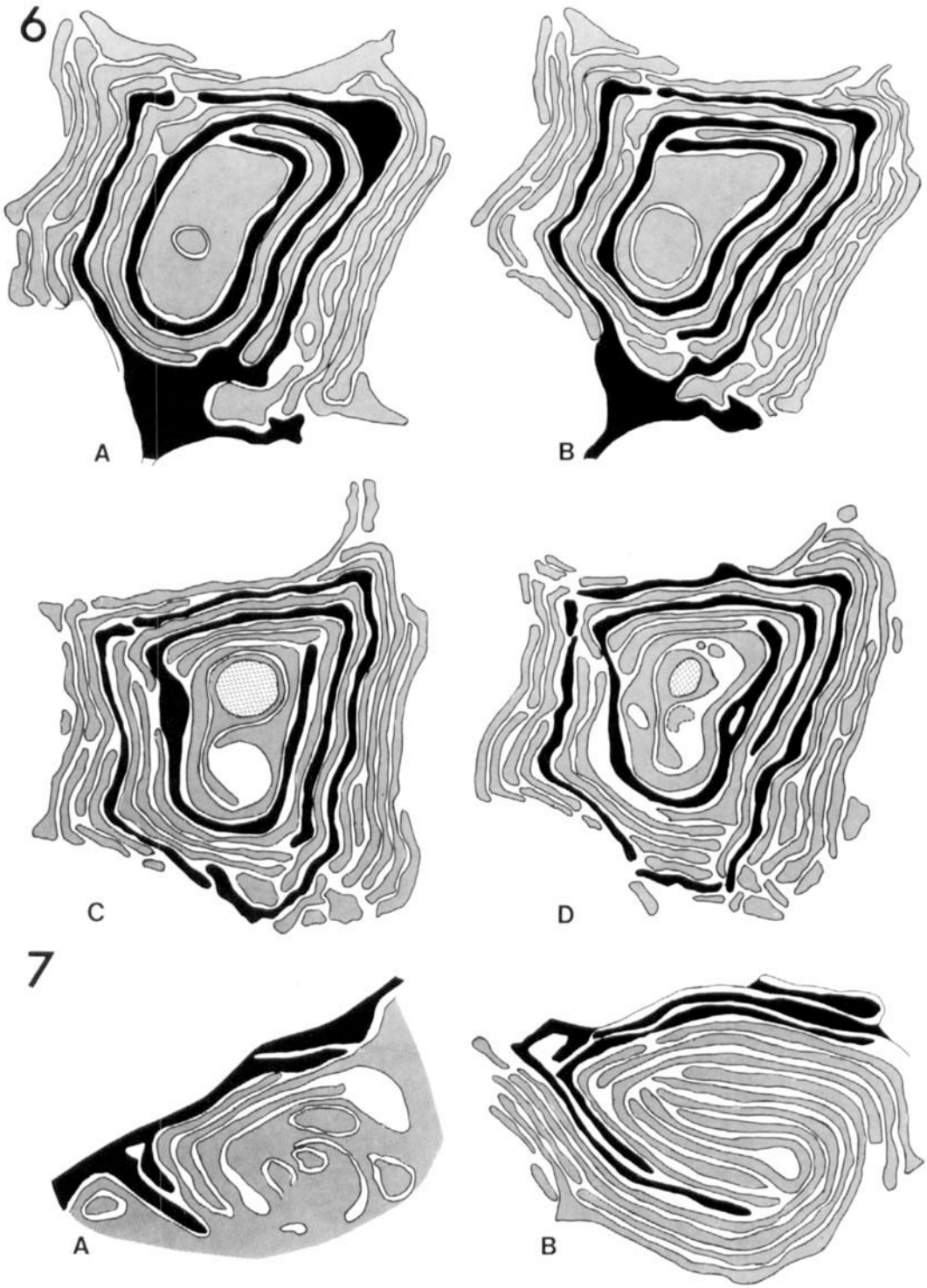

Fig. 6. Tracings made from a consecutive series of tangential sections through a cone sheath. The three processes shaded black in *C* have formed from the one more complex process shaded black in *A*, which is still attached to a column of cytoplasm extending from the apical surface (also in B). This process divides twice, once between *A* and *B*, and again between *B* and *C* to form the three processes. The variety of shapes taken by the cone sheath lamellae are also illustrated. Electron micrographs of *A* and *D* appear in Figure 5D and E.

Fig. 7. Tracings made from two sections of the series taken through the cone sheath in Figures 4A–C to show the origin of sheath processes from two RPE cells. The dark and light shading indicate cytoplasm of the two RPE cells and their contributions to sheath lamellae.

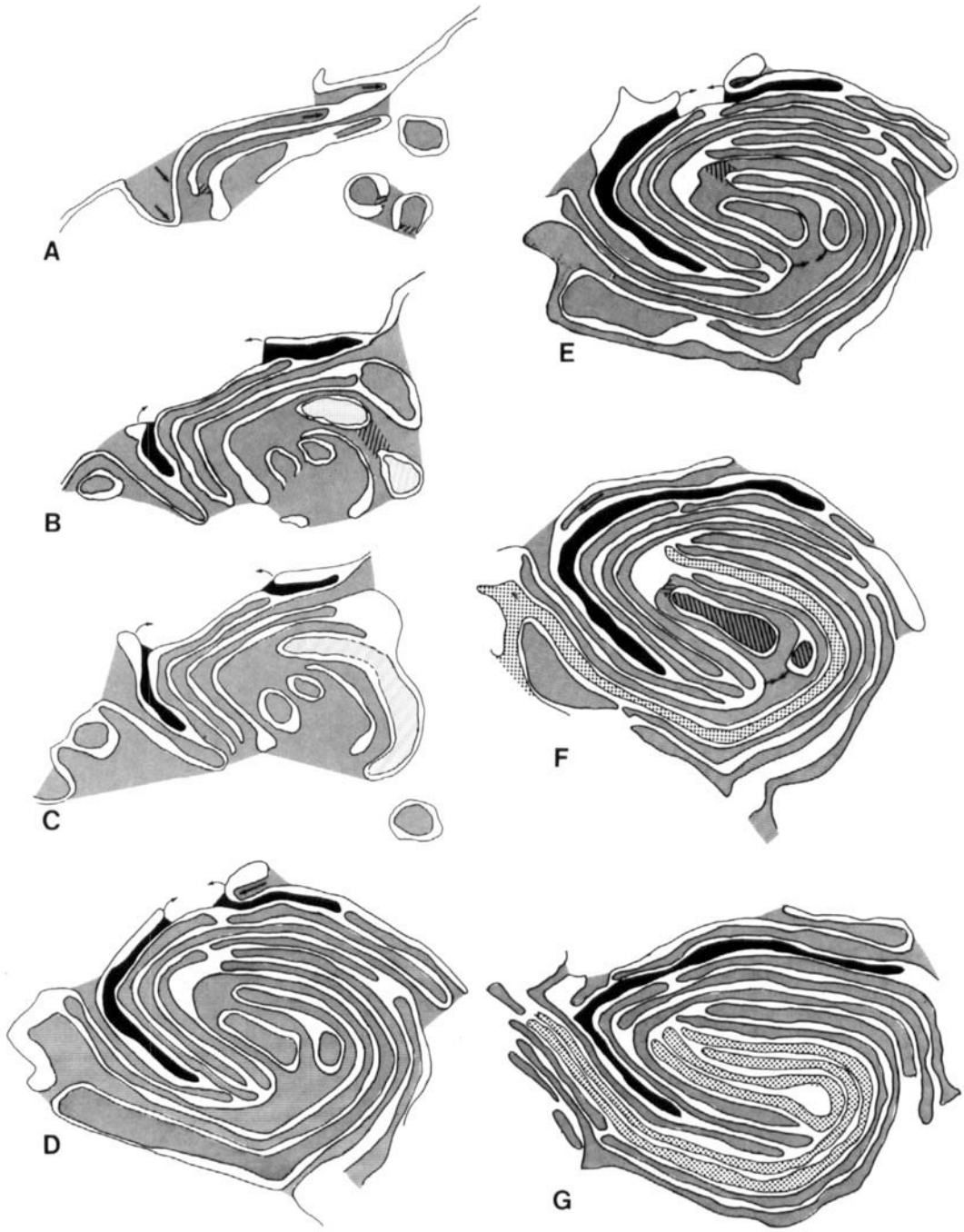

Fig. 8. Seven tracings selected from a series of 25 tangential sections that follow the cone sheath from the supracone space to its origin at the apical surface. In this series the formation of sheetlike projections by lateral growth, fusion, and subdivision of processes is illustrated. In *A*, arrows show points of growth of lamellae while the cross-hatching indicates regions where processes will break free. The two small processes in the lower right of *A* form the lightly stippled processes in *B*, which then fuse in *C*. One process in *A* forms a ridge that breaks free at the cross-hatching and grows laterally in *B*. The two black processes in *B* form by lateral growth at the top left and right arrows in *A*. These processes eventually form one free process (in F) as they detach from the cell body (shown by curved arrows in B–E). Growth of a process begins at the straight arrow in *D* and continues through the series. The heavily stippled processes in *G* are formed in two stages. The crosshatched area in *E* disappears to isolate the stippled process in *F* and the crosshatched area in *F* disappears to form the central "U" and the additional free lamella in *G*. The other two crosshatched processes in *F* disappear in the next section.

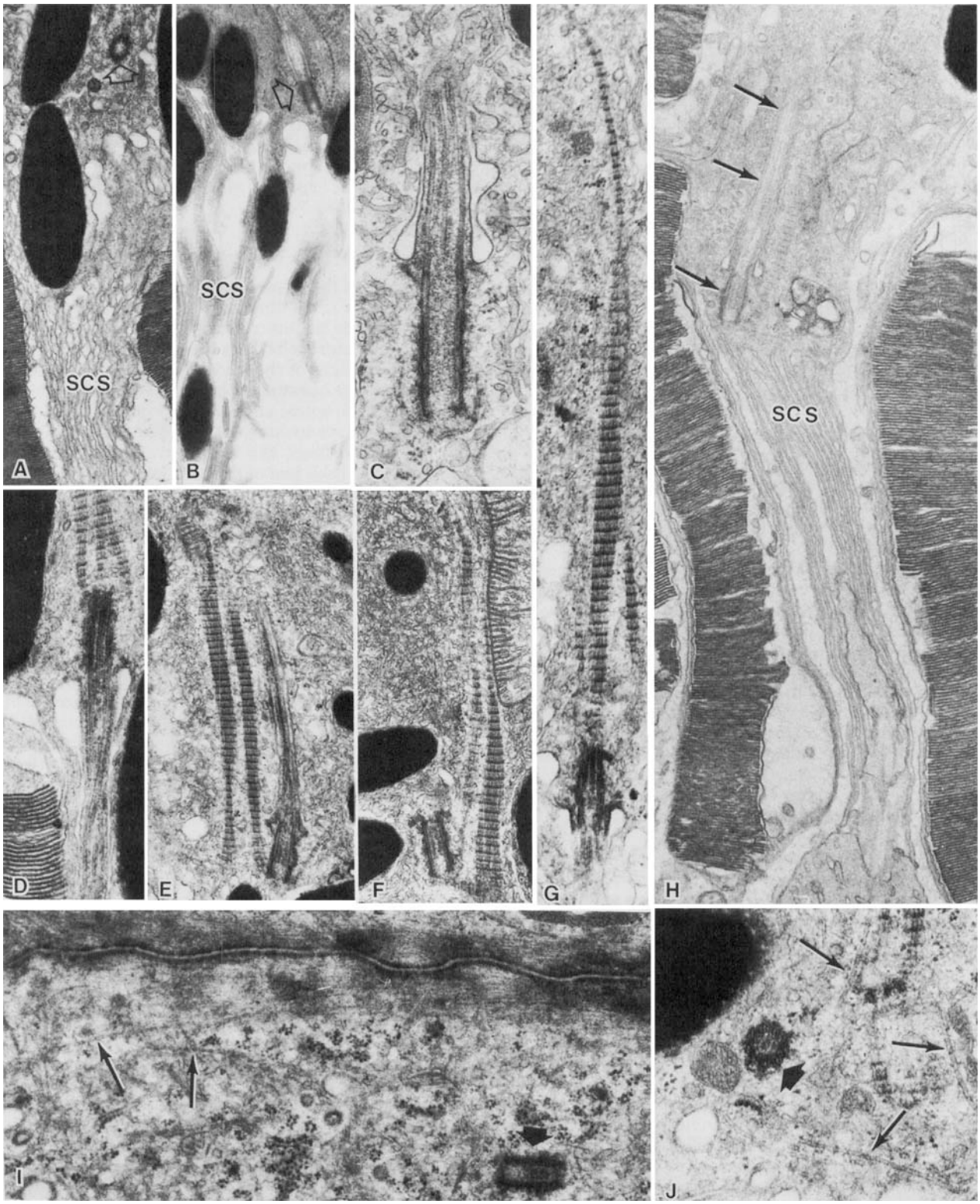

Fig. 9. Electron micrographs of RPE cilia from cat, human, and monkey retinas. A. A basal body (arrow) located directly above a cone sheath (cat). SCS, supracone space. B. A cilium (arrow) adjacent to the processes forming the cone sheath. The cilium points into the cell body and the ciliary rootlet loops around it (human). SCS, supracone space. C. An RPE cilium from rhesus monkey that extends into an intracellular vesicle. D. In this example the cilium extends into the supracone space parallel to the projections of the cone sheath (cat). E. Cilium and rootlets in cat RPE. The cilium extends into a vesicle in the RPE cell and the rootlet loops around the basal body to parallel the cilium. F. Basal body and striated rootlet of an RPE cilium. The striations of the rootlet are in register with cristae in the mitochondria (cat). G. Part of a cilium and its extensive striated rootlet (cat). H. An electron micrograph of a section through a regenerated cone sheath in a cat retina that had been detached for 7 days and reattached for 7 months. SCS, supracone space. I, J. Electron micrographs of the RPE apical cytoplasm (cat) showing microtubules (thin arrows) and basal bodies (thick arrows). The adhering junctions between two cells are shown at the top of *I*. A, × 15,000; B, × 8,400; C, × 36,800; D, × 30,000, E, × 17,500; F, × 20,000; G, × 28,000; H, × 15,000; I, × 30,400; J, × 42,000.

vesicle (Fig. 9B,C,E). (It was not ascertained whether or not individual vesicles were continuous with the apical membrane, thus representing invaginations of this membrane; Sorokin, '62; Dahl, '67). Sometimes a centriole was found orthogonal to the basal body of the cilium. The most prominent and frequently observed appendages were striated rootlets. These rootlets usually looped alongside the cilium and projected into the cell away from the apical membrane (Fig. 9E,F). They had a typical main band periodicity of 60–70 nM and microfilament diameter of 5–8 nM (Wolfe, '72). In all three species mitochondria (Fig. 9F) occurred alongside the rootlets, and, as has been observed previously in other cells, the cristae of the mitochondria were aligned with the main bands of the striated rootlets (Olsson, '62; Welsch and Storch, '69; von Boletsky, '73). Microtubules were also observed in the vicinity of the basal bodies (Fig. 9I,J).

DISCUSSION

Microplicae of the apical surface

The RPE apical membrane is generally considered to be organized into only two types of cell surface structures: cylindrical projections usually referred to as microvilli, and much more flattened or sheetlike projections (Steinberg and Wood, '74). We now have observed that the surface membrane underlying these projections is formed into a thin zone of anastamosing ridges or microplicae. These structures most closely resemble the microplicae that appear by SEM on the surfaces of stratified squamous epithelial cells and, in the eye, on the exposed surfaces of the cornea and conjunctiva (Andrews, '76; Motta et al., 1977). Although not confined to squamous epithelia they are most often observed on surfaces subjected to repeated abrasion and are usually the major structural feature of such surfaces (Andrews, '76). In form, they are somewhat different structures in the RPE, where they appear as a delicate and thin spongy region that has the potential to wrap photoreceptor outer segments with large expansions of membrane.

There has been, to our knowledge, only one previous reference to microplicae on the RPE surface (rat retina, Motta et al., '77: Plate 69c). That they have not been more frequently described by SEM may be because the apical projections obscure and dominate the surface (Pfeffer and Fisher, '81), and in TEM longitudinal section, the tendency would be to describe them as short microvilli (Andrews, '76). In the present work they were most clearly observed in tangential sections through the apical surface of cat RPE and this was also the case for the foveal RPE of monkey (Anderson and Fisher, '79). It is possible, therefore, that microplicae, underlying the apical projections, are a general feature of many RPE apical surfaces.

Such structures would be better visualized, of course, in the absence of apical projections. Thus, microplicae were observed to dominate the apical surface of kitten RPE prior to the extension of the apical projections (Pfeffer and Fisher, '81). Here, these sheetlike ridges can be thought of as embryonic structures, the source of the varied types of apical projections needed by the apposed rods or cones. (The microvilli, which also were observed in kitten RPE, could also contribute to the projections; Pfeffer and Fisher, '81). A closely related observation has been made in the cave dwelling amphibian, *Proteus anguinas,* where the photoreceptors and RPE exist in a rather immature state (Nguyen-Legros and Durand, '74; Nguyen-Legros, '78). In this retina the photoreceptors have short, poorly developed, outer segments and the RPE appears embryonic both in intracellular content and surface structure. Instead of well-developed apical projections the surface has been described (longitudinal TEM sections) as consisting of "convoluted and anastomotic slender microvilli" (Nguyen-Legros, '78), which look very much like the microplicae that we have described.

Origin of the cone sheath

The origin of the cone sheath and also its form are summarized in the three-dimensional drawings of Figure 10. It is clear in the mature retina that the apical projections originate as outgrowths from the microplicae or as expansions of the ridges of the microplicae (Fig. 10A). We can now answer the question of how the separate lamellae of the cone sheath spiral and wrap concentrically in the supracone space, even where they are directly apposed to the apical surface. Thus, the explanation presented earlier that, perhaps, each leaf of the cone sheath is connected to the apical surface by a "tab-like extension of cytoplasm" appears to be supported (Steinberg and Wood, '74). These connections, although varied in configuration, are always small relative to the circumference of the leaves. Thus, the sheath achieves its multilaminated concentric configuration very close to the apical surface because each projection rapidly widens into a broad sheet within a very short distance of its tablike origin. Each sheet, therefore, hangs in the subretinal space by its small tab and is free to curl and wrap concentrically around its neighbors (Fig. 10A). This wrapping often seems to be organized around a central root, the same one that forms the central post of cytoplasm and the circular U-shaped processes in the center of the sheath. Possibly this central structure functions in a specialized way in the growth and organization of the concentric laminated pattern of the sheath.

Sheath form and phagocytosis

One known sheath function is to phagocytose the shed portions of cone outer segments. Phagosomes now have been described in the long apical projections that reach cone outer segments in a number of mammalian species, including cat (Steinberg et al., '77; Anderson et al., '78; Anderson and Fisher, '79). While functions other than phagocytosis can be imagined (Steinberg and Wood, '74), some structural characteristics of the sheath can be understood in terms of phagocytosis alone.

Although the apical projections tend to be shaped like tapering leaves (Steinberg and Wood, '74), they vary considerably in length, width, and shapes (see also Pfeffer and Fisher, '81); and some leaves are even interconnected (Fig. 10B). These varied forms imply that the sheath is probably not a static structure. Regression and regrowth of processes may be imagined as well as a continuous reshaping related to the ultilization of membrane during phagocytosis. This would be most prominent for the central leaves that directly participate in phagocytosis, and especially for the membrane that caps the outer segment tip. These central processes may be short, barely reaching the cone; lateral extensions of longer processes; or large central posts of cytoplasm, all of which can cap the outer-segment tip (Fig. 10B).

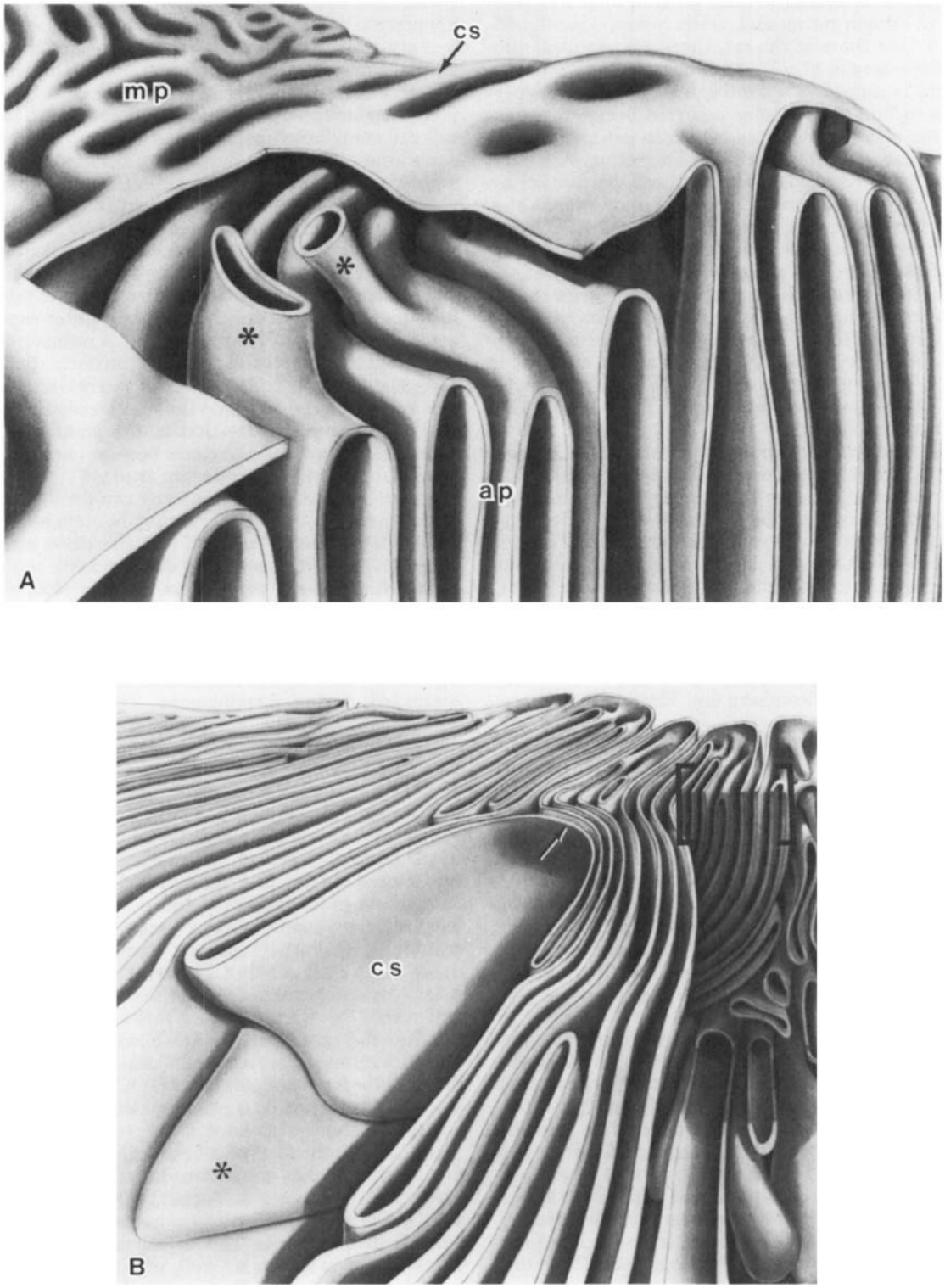

Fig. 10. Three-dimensional drawings showing the form of the cone sheath and its origin from the apical surface of an RPE cell. The view in 10A looks down from inside the cell at the cytoplasmic surface of the apical membrane (cs). This surface is formed into microplicae (mp) which appear as interconnecting ridges and valleys. The microplicae are the origin of the tablike connections that grow out to form the apical projections. These projections (ap) appear in longitudinal section toward the front of the drawing. A portion of the apical membrane has been cut away on the left to show the origins of two apical projections (asterisks) from the apical membrane. Connections made by other apical projections to the apical membrane also are shown. Observe how the connections form concentric lamellae very close to their origin at the apical surface. In 10B we view a cone sheath (cs) in longitudinal section. The RPE cell has been tilted back to show some of the sheath's concentric apical projections (brackets, upper right) tangentially sectioned close to the apical surface. Adjoining these processes are some more villouslike projections. The central region of the sheath contains a process (arrow) that caps the tip of the outer segment. It overlies a leaflike process (asterisk). Drawings by M. Pederson.

The sequence of events in cone phagocytosis has been described in human retina and, while human sheaths differ in form from those of the cat, there are essential similarities (Steinberg et al., '77). In human retina, the outer segment tip is capped in a similar way prior to phagocytosis. Also in human, vacuoles occurred between phagosomes in the apical projections, which in cat appeared to decrease in size in a vitreal to scleral direction. Although not observed between phagosomes in cat, we conjecture that they were formed similarly to those in human. Thus, each vacuole represents the incorporation by RPE membrane of the extracellular space that exists between the membrane that has just phagocytosed the cone and the membrane that recaps the tip of the outer segment. A space appears because the phagosome has begun to ascend toward the soma of the cell away from the cone (for further details see Steinberg et al., '77). In the cat, in contrast to human, the vacuoles seem to remain behind in the cytoplasm of the apical projections after the phagosomes have moved into the RPE soma.

However, it is probably wrong to assume that the multilaminar nature of the sheath in cat was developed solely or even principally to serve the phagocytic function. We do not actually know why the sheath is so highly ordered and thick in the supracone space and around the cone or why it reaches all the way to the base of the outer segment. In human and monkey peripheral cones (Steinberg et al., '77; Anderson and Fisher, '79), which are similar to cat cones by not reaching the apical surface, the phagocytic function appears adequately provided by a considerably thinner, shorter, and generally, less-ordered sheath (for further discussion see Steinberg and Wood, '74).

Sheath development and cilia

The presence of 9 + 0 RPE cilia is yet another example of nonmotile or "rudimentary" cilia in cells whose surfaces are generally nonciliated (Sorokin, '62; Dubois and Girod, '70; Satir, '77). In spite of the ubiquity of such cilia and associated structures—virtually all cells may exhibit them occasionally—their functional significance remains unknown. Although the identification of cilia in the RPE is not a new finding (Allen, '65; Braekevelt and Hollenberg, '70), their observed association with cones and the membrane specializations of the cone sheath might represent a clue to their function in the RPE and, perhaps, elsewhere.

This association was first reported by Hebel ('70), who noted that centrioles appeared above cones with regularity in the dog RPE. Bülow ('75) also observed cilia above monkey cones *(Ceropithelus aethiopus)* but did not further explore the frequency or anatomical characteristics of this association. What seems particularly significant now is our finding that in three mammalian species—cat, monkey, and human—RPE cilia and basal bodies are almost invariably associated with retinal cones and, furthermore, that they are always apically located, often in close proximity to the apical projections of the cone sheath. To our knowledge this is the first time that nonmotile cilia and basal bodies have been shown to be related to noncilial specializations of surface membrane.

The association between basal bodies, cilia, and cones also has been observed in developing kitten retina (Pfeffer and Fisher, unpublished observations). Although the timing of the appearance of basal bodies relative to the cone sheath has not yet been ascertained, it is clear that they are present at an early stage in sheath development. Since it is probable that the photoreceptor mosaic is specified in the developing neural retina and not in the RPE, a cone sheath will form wherever a developing cone appears. This is consistent with our observation that more than one RPE cell can contribute apical projections to the sheath of the same cone. Thus, the developing cone would appear to stimulate the RPE at that location to form a sheath and either cause development of a basal body or cause migration of an already formed basal body.

The developmental situation can be duplicated to some extent in the mature cat retina that has undergone an experimental retinal detachment and reattachment. Following detachment, the photoreceptor outer segments degenerate and the RPE cells undergo a number of changes that include cell division and the transformation of apical projections into a fringe of short microvilli. Upon reattachment both the RPE and the photoreceptors can return to a more normal state—that is, the photoreceptor outer segments regrow and the cones become ensheathed again by apical projections (Anderson et al., '81). During recovery there is little chance that any cone will be reapposed to the place on the RPE cell that is occupied before the retinal detachment. The RPE cell, therefore, must be provoked anew, as it was during development, to extend the ensheathing apical projections. In this situation, basal bodies and cilia again appear in close association with cone sheaths (see Fig. 9H) and are probably new to these locations. This experimental evidence further supports our contention that cilium and sheath are functionally interconnected during development. The question remains whether the presence of a developing cone stimulates the development of two functionally related or unrelated structures, the cone sheath and the cilium-basal body complex.

One possibility is that the cilium marks the location of a microtubule-organizing-center (MTOC) that participates in the morphogenesis of the cone sheath. [Hebel ('70) had conjectured earlier that the centrioles of dog RPE might be involved in the growth of apical processes.] MTOCs are localized cytoplasmic regions that nucleate arrays of microtubules (Pickett-Heaps, '69; Tucker, '79). There is evidence that a class of MTOCs is structurally associated with centrioles and basal bodies (see discussion and references in Tucker, '79). Significant for this hypothesis is the finding that the basal body, which forms a MTOC, may also extend a cilium (Pearson and Tucker, '77). In tissue-cultured cells, for example, the MTOC of the centrosphere also can be marked by a primary cilium (Osborn and Weber, '76; Weber and Osborn, '79).

While microtubules have been implicated in the growth process that pushes out extensions of cell membranes and cytoplasm (references in Tucker, '79) the skeletal framework of the apical projections of mammalian RPE consists only of microfilaments. This appears to be the case during development in kitten, as well, where microfilaments but not microtubules were observed in the projections (Pfeffer and Fisher, '81). As in other cell extensions that contain only actin filaments (Burnside, '76), it would appear that only they are directly involved in the outgrowth of the apical projections (Tilney, '75; Edds, '77). Microtubules, however, have been postulated to interact with microfilaments during development, providing support and direc-

tionality for microfilament growth (references in Roberts, '74; Tucker, '79). There is evidence, for example, that blood leukocyte microtubules regulate microfilament distribution (Berlin et al., 1979). Both microfilaments and microtubules occur in the apical region of RPE cells (Burnside, '76; Nguyen-Legros, '78; Crawford, '79; Anderson and Fisher, '79) and either or both of these organelles could be involved in sheath development. Pitelka ('74) has emphasized that the basal body itself can organize not only the ciliary axoneme, but also can serve as an organizing center for a variety of both microtubular and fibrillar elements. Perhaps, therefore, the MTOCs or the basal bodies themselves organize the development of a microtubular and/or microfilament substructure that is related to development of the long apical projections of the cone sheath.

Finally, it is possible that such elements function in the mature RPE to provide structural support for the sheath. This could be in association with the well-developed ciliary rootlets, often observed at the bases of the cone sheaths. The rootlets might anchor the microfilaments of the apical projections (Pitelka, '74). Movement of the apical projections, perhaps related to their phagocytic function (Burnside, '76), might be associated with a contractile function of the rootlets or filaments associated with the basal body complex (Sleigh, '79; Gordon et al., '80).

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