Mammalian cones: disc shedding, phagocytosis, and renewal

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During the past several years we have examined a variety of different mammalian retinas for ultrastructural evidence of cone disc shedding and RPE phagocytosis. In this paper we review our previously published evidence from squirrel and human retinas as well as present new evidence of cone disc shedding in rhesus monkey and cat. All these species show definitive evidence for the shedding of discs from cone outer segments and the phagocytosis of shed discs by apical processes of the RPE; both of these events closely resemble those described for mammalian rods. The occurrence of cone disc shedding leads directly to the conclusion that new membrane must be added to the cone outer segment in order to maintain its length. The successive evaginations, which are observed at the bases of cone outer segments, we consider to be indirect evidence for the addition of new discs. Finally, we propose a model for the structural organization of mammalian cone outer segments.

Key words: cones, phagocytosis, renewal, pigment epithelium, retina

In 1963, Bairati and Orzalesi[1] hypothesized that the discs of photoreceptor outer segments are in a state of balanced addition and loss. Autoradiographic studies by Young and his collaborators provided direct evidence that rods continually renew the membranes that comprise the disc stack. When ^{3}H-amino acids are injected into various vertebrates, a transverse band of newly-formed, radioactive protein appears at the base of rod outer segments within hours.[2] With the passage of time, the band of labeled protein can be traced as it ascends toward the outer segment tip, coincident with the continuing formation of new discs at the outer segment base. Once the band leaves the rod tip, it can be identified within pigment epithelial inclusions called phagosomes.[3] In cones, however, the radioactive protein is diffusely distributed throughout the outer segment,[8] as is a small amount in rod outer segments.[4] This difference in the pattern of protein incorporation has since been verified in a wide range of both mammalian and nonmammalian photoreceptors—in both developing[5–7] and adult[3, 8–13] animals.

The absence of a discrete band or concentration of radioactive protein in cone outer segments led Young[8] to hypothesize that mature cones neither assemble new discs nor shed old ones, replacing instead only some of their molecular constituents. This, in turn, led to a proposed distinction between rods and cones based upon whether the outer segments are renewed by membrane replacement or by molecular replacement.[14] The idea received some preliminary support

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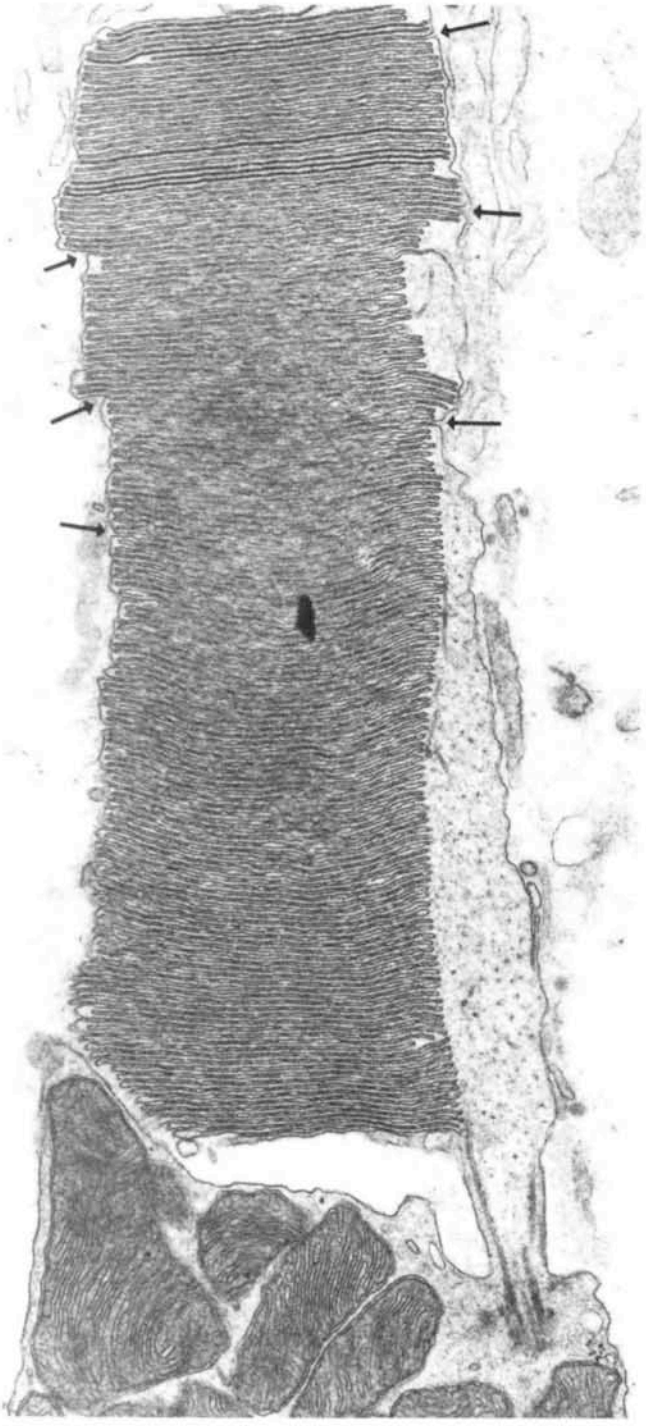

Fig. 1. Cone outer segment from the retina of the 13-lined ground squirrel. The disc membranes at the base form one uninterrupted network and are continuous with the membrane bordering the inner margin of the connecting cilium. At distal sites, disc–outer membrane continuities (arrows) are present, even near the outer segment tip. (×33,000.) (From Anderson, D. H., and Fisher, S. K.: J. Ultrastruct. Res. 55:119, 1976.)

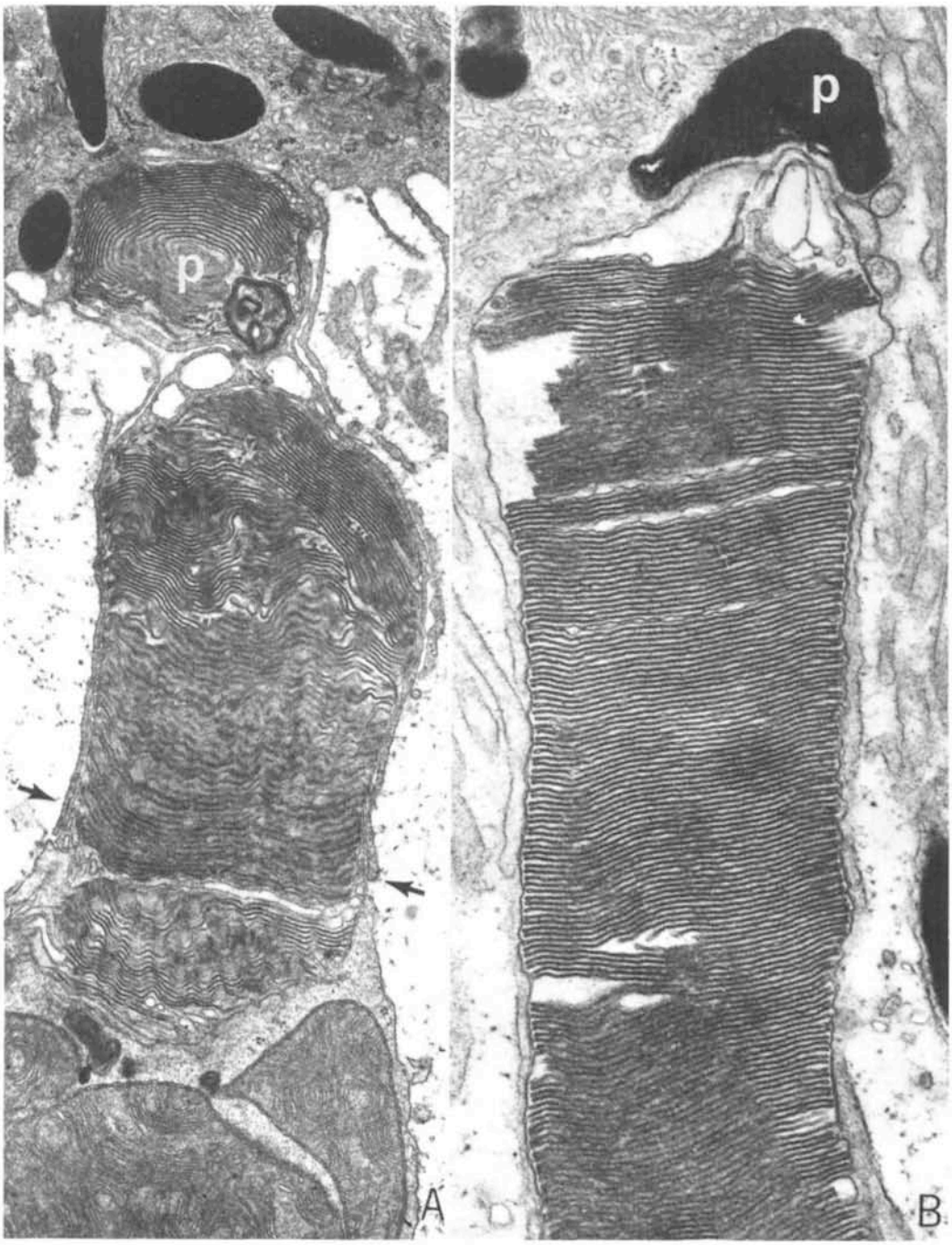

Fig. 2. Cone disc shedding and phagocytosis of squirrel cones. **A**, Cone outer segment with detaching disc packet *(p)* in the retina of the Western gray squirrel. Slender RPE processes ensheath the outer segment down to the level of the calyceal processes (arrows) and phagocytize packets of terminal discs. **B**, Cone outer segment and cone phagosome *(p)* in the retina of the California ground squirrel. Cone phagosomes immediately above cone outer segments often appear electron dense and compressed. Note that the outer segment tip has resealed. (×20,000.)

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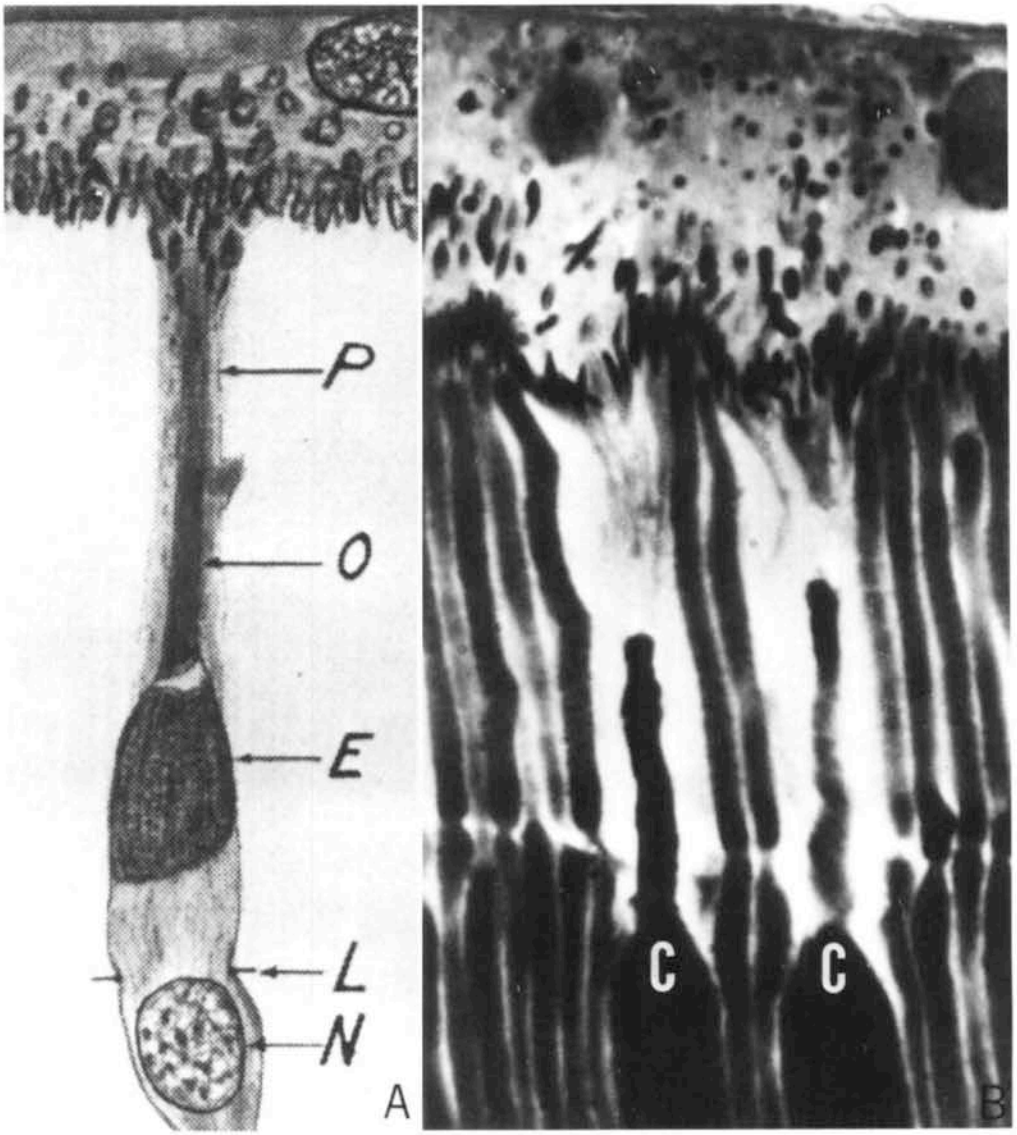

Fig. 3. Relationship between primate cones and the RPE. **A,** Drawing of an extrafoveal human cone. *P* is termed the "tubular process of the pigment cell"; *O*, the outer segment; *E*, the elipsoid; *L*, the external limiting membrane; *N*, the nucleus. (Original magnification, ×1,000; printed magnification, ×2,100.) **B,** Light micrograph of the rhesus monkey retina. Two cones *(C)* are shown. Note the apical processes that descend from the RPE toward the cone outer segments. Rod outer segments terminate quite close to the pigment epithelial somas. (×2,500.) (A from Walls, G. L.: Arch. Ophthalmol. 12:914, 1934. Copyright 1934, American Medical Association.)

from findings showing an absence of phagosomes within the retinal pigment epithelium (RPE) of several cone-dominant species.[15] Thus much of the available evidence at the time was consistent with the notion that rods, but not cones, continually replace their outer segment discs.

There were two early studies, however, which suggested that mature cones retain the capacity to assemble new discs and to dispose

of old ones. Kroll and Machemer[16] showed that cone outer segments in the rhesus monkey retina, which degenerate after experimental retinal detachment, later regenerate following reattachment surgery. These results did not indicate that new discs are assembled as a normal event in the intact retina. But Hogan's report[17] showing phagosomes within the pigment epithelium overlying the rod-free human foveola intimated that foveal cones shed discs, although no evidence of shedding or phagocytosis by the pigment epithelium (RPE phagocytosis) was actually presented in his study. More recent studies provided definitive evidence for disc shedding and RPE phagocytosis for mammalian cones. Hogan et al.[18, 21] showed that human extrafoveal cones shed their terminal discs in a way similar to the shedding process in primate rods,[19, 20] and Anderson and Fisher[10, 11] reported that disc shedding occurs in various species of diurnal squirrels.

In squirrels, cone shedding was found to occur despite the fact that the outer segments show the usual, diffuse pattern of protein renewal.[10, 11] Furthermore, protein renewal studies in developing salamander cones, in which the outer segments are known to be increasing their length and thereby adding new membrane, failed to show any sign of a discrete band or concentration of labeled molecules.[5] Taken together, these results *strongly* suggested that the absence of a band of radioactive protein in cone outer segments indicates neither the absence of continuing disc synthesis nor disposal. Thus we recently proposed[11] that at least some mammalian cones, like their rod counterparts, continue to assemble as well as shed their discs as a normal ongoing process.

We have now examined a variety of mammalian cones for evidence of membrane assembly, disc shedding, and RPE phagocytosis. All the examined species, including arboreal and terrestrial squirrels,[10, 11] man,[18, 21] rhesus monkeys, and cats, show convincing evidence of disc shedding and indirect evidence of new membrane assembly. In addition, we find that the shedding and assembly processes are similar to those in rods. The similarity also extends to the involvement of the RPE. Apical processes of the RPE cap or ensheath the tips of *both* rods and cones, and it is these processes that phagocytize the shed disc packets of both. Ensheathing processes exist even for those mammalian cones that fall far short of reaching the apical surface of the RPE, and in some species, the ensheathment by the RPE forms an especially elaborate arrangement.[22] In this paper we review our previously published findings of disc shedding from squirrel and human cones. The significance of these observations for mammals, in general, is strongly supported by the addition of new and similar observations in the domestic cat and rhesus monkey.

Disc shedding from squirrel cones.

Unlike the retinas of most mammals, diurnal squirrel retinas are strongly cone-dominated. In arboreal squirrels, cones outnumber rods by about 5:4,[23] and in the ground-dwelling species the ratio is 10:1 or greater.[12, 24, 25] The preponderance of cones makes the squirrel a desirable candidate for studying the interactions between cone outer segments and the RPE.

For the most part, squirrel cones resemble the cones of other mammals. The outer segment consists of a stack of disc membranes that in single thin sections frequently exhibit continuities with the outer plasma membrane in the basal one third of the outer segment and, to a lesser degree, at more distal locations[11, 23, 26] (Fig. 1). The cylindrical outer segments are connected by a cilium to larger-diameter inner segments filled with mitochondria. The cone terminates in a pedicle ending in which the synaptic organization is similar to that observed in primate cones.[27] There are two features of squirrel cones that differentiate them from others. First, squirrel cone outer segments are short (7 to 8 μm), whereas primate cone outer segments, for example, may reach 30 μm. Second, the outer segments of squirrel cones associate quite simply with the RPE. Their tips abut the apical surface and are capped to the tips of the calyceal processes by slender

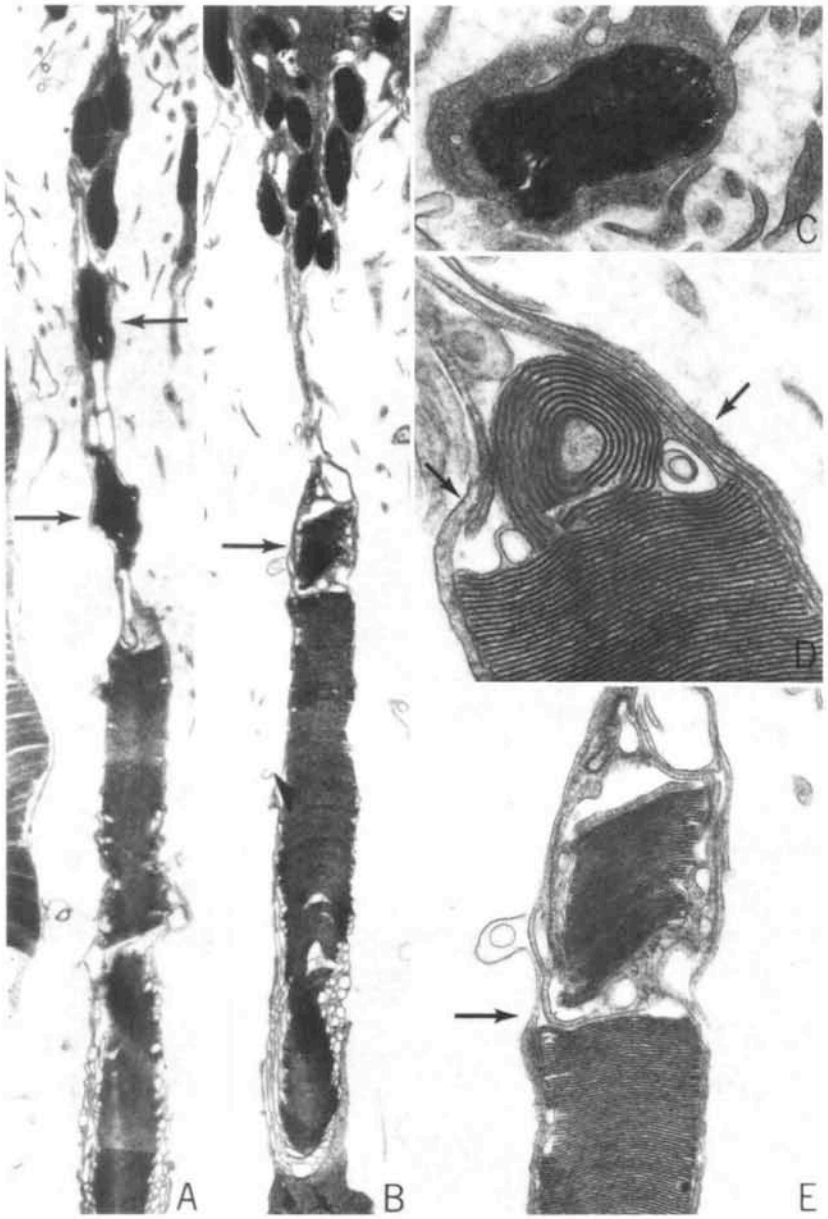

Fig. 4. Disc shedding and RPE phagocytosis of human extrafoveal cones. **A** and **B**, Electron micrograph montages of two cone outer segments and their associated RPE apical processes. Arrows indicate groups of terminal discs or detached packets of discs in various stages of the phagocytic process. **C**, Transverse section through the supracone space in the human retina. An electron-dense phagosome occurs in the center of this space surrounded by RPE apical processes. **D**, Cone outer segment tip. RPE apical processes (arrows) extend along both sides of the outer segment. The curled discs remain enclosed within the outer segment, but the outer membrane on the left side appears to be pinching inward. **E**, Cone outer segment tip. RPE processes (arrow) appear between a packet of some 40 discs and the remainder of the outer segment. (**A** ×4,700; **B** ×4,800; **C** ×48,000; **D** ×27,500; **E** ×12,000.) (From Steinberg, R. H., Wood, I., and Hogan, M. H.: Philos. Trans. R. Soc. Lond. [Biol.] 277:459, 1977.)

apical processes that extend down from the RPE (Fig. 2, *A*). These processes are different from those occupying the interreceptor space that are filled with pigment granules.[11] The processes that cap the outer segments participate in the phagocytosis, and possibly in the detachment, of terminal disc packets (Fig. 2, *A*).

The shedding process in squirrel cones is qualitatively similar to that described for primate rods.[19, 20] The initial event is usually a curling of a small packet of terminal discs, from one or both sides of the outer segment, toward the RPE surface. Often the outer plasma membrane follows the contour of the curled packet, forming a close-fitting envelope around it. In the detachment phase, the invaginating outer membrane separates the packet from the remainder of the outer segment and, in an event rarely seen, fuses with the outer membrane at the opposite margin. Thus the shed packet is enclosed by a membrane derived from the outer plasma membrane, and the outer segment itself is resealed at the tip (Fig. 2, *B*).[11] Whether or not the pigment epithelial processes participate in the actual detachment of the packets is uncertain. In the squirrel retina, there are instances where these processes seem to penetrate inward in a pseudopod-like fashion,[11] but such observations are the exception rather than the rule. Steinberg et al.[21] have proposed that both kinds of events may be responses to an even earlier, but not yet visualized, initiating signal. In rods, O'Brien[28] has presented some biochemical evidence for such a signal, suggesting that the addition of sugar residues to the outer segment tip may provide recognition sites for the initiation of phagocytosis.

Disc shedding and phagocytosis

Human cones. Cone outer segments of most mammalian species resemble human extrafoveal cones in that the outer segment tips do not reach the apical surface of the RPE—as they do in rods. Early evidence that the RPE extends long processes that reach the cone tips was obtained by Walls[29] (Fig. 3, *A*). He cites H. D. Judd as being the first to observe this intimate relationship in preparations of human retina stained with Kolmer's fluid. Walls described tubular processes protruding out from the apical surface to ensheath the cone outer segment. He says that the processes were "so conspicuous that in low power magnification the cones may be counted by looking only at the pigment epithelium."[29]

This early description by Walls was generally forgotten as was a similar observation by Eichner.[30] Confirmation was later obtained with the electron microscope,[31, 32] and most recently the ultrastructure of this relationship was described in detail for human extrafoveal cones.[21]

These recent electron microscopic observations indicate that the processes which descend from the apical surface are actually fingerlike or villous-type projections that become tubular when they reach the outer segment tip (Fig. 4, *D*). The process expands to cover the tip and continues down around the outer segment for up to one-third its length. The tip of the outer segment is therefore ensheathed by the processes, and several may be concentrically arranged to form a multilayered sheath around the cone. The villouslike processes usually contain little in the way of organelles except for pigment granules located close to the apical surface, vesicles, and endoplasmic reticulum. Some unusually large processes are filled with a rich variety of organelles, including mitochondria and small, dense, membrane-bounded vesicles that could be primary lysosomes.[33] In these large processes, the appearance is as if pigment epithelial cytoplasm poured out of the soma into the processes all the way down to the ensheathing region at the cone tip.[21]

For human extrafoveal cones, the processes of disc shedding and RPE phagocytosis are also similar to those described for primate rods. Two different events could be identified as the earliest stages in this process. In one, a group of terminal discs curls at its lateral edges and begins to separate from the

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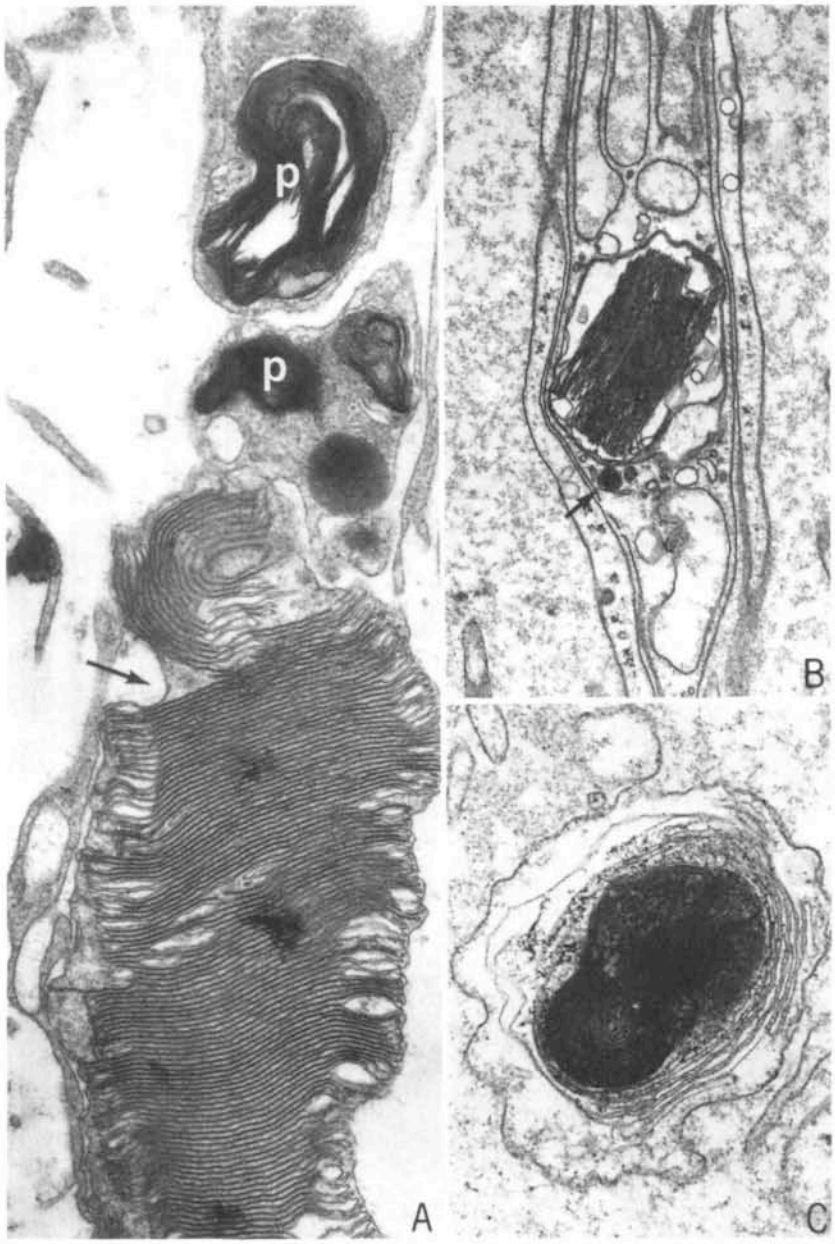

Fig. 5. Disc shedding and RPE phagocytosis of rhesus monkey perifoveal cones. **A,** Cone outer segment tip. A group of terminal discs curl from one side of the outer segment, and the outer membrane invaginates inward (arrow). This is one of the earliest identifiable stages in the disc shedding process. Two phagosomes *(p)* and the remnants of a possible third are enclosed within the RPE processes that border the outer segment tip. **B,** Cone phagosomes within the RPE apical processes. A vesicle with a dense core (arrow) lies close to the phagosome. **C,** Transverse section through the supracone space. An electron-dense phagosome occurs in the center of this space and is surrounded by RPE apical processes. (**A** ×29,000; **B** ×15,000; **C** ×48,000.)

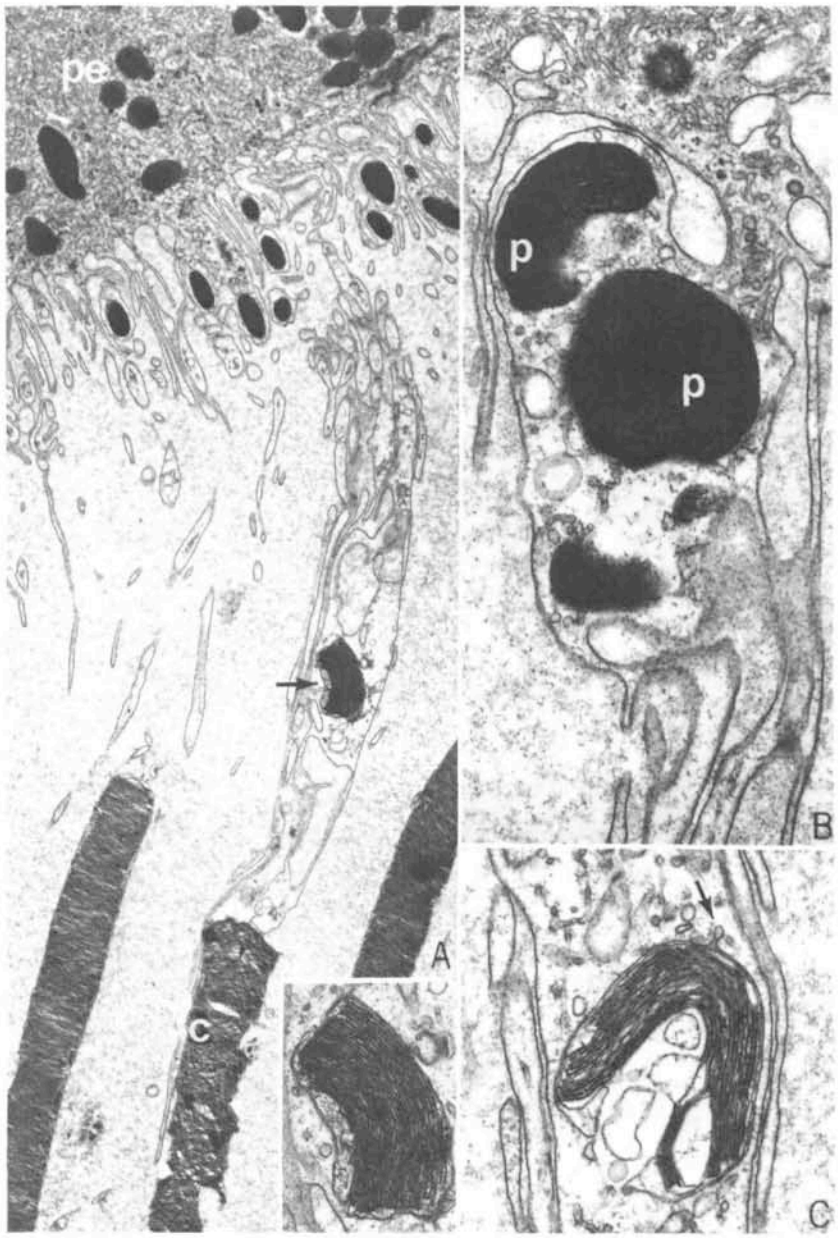

Fig. 6. Disc shedding and RPE phagocytosis of rhesus monkey perifoveal cones. **A**, Distal portion of a monkey cone outer segment *(c)* and apical processes that protrude from the RPE. The arrow indicates a packet of shed cone discs en route to the RPE interior. The phagosome at the arrow is shown at higher magnification in the inset. **B**, Longitudinal section of the RPE apical processes associated with a cone outer segment. Two electron-dense phagosomes *(p)* lie just vitread to the apical surface of the RPE. Their lamellar structure is just barely discernable. **C**, Phagosome within the RPE apical processes above a cone outer segment. The arrow indicates a small vesicle fused with the membrane enclosing the phagosome. (**A** ×9,000; inset ×38,700; **B** ×30,000; **C** ×30,000.)

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outer segment before there is any sign of phagocytosis by the apical processes (Fig. 4, *D*). In the other, apical processes are seen surrounding a terminal packet of discs that shows no morphological change from its normal orientation (Fig. 4,*B* and *E*). This latter event also has been described for human rods where an RPE pseudopod appears to penetrate between the terminal packet and the remainder of the outer segment.[19]

In the next stage, the terminal portion of the outer segment appears within the cytoplasm of the apical processes, surrounded by RPE plasma membrane, and positioned just above the tip of the cone (Fig. 4, *A*). The disc membranes of the phagosome at this stage are seen as more electron dense than those of the outer segment. The phagocytized packet can assume different orientations with respect to the outer segment. Usually it rotates 90 degrees so that the disc edges face into the lumen, thus providing a narrower profile for passage up the process.

Phagosomes occur at different levels in the processes on their way to the RPE interior (Fig. 4, *A*, and *C*). As the phagosomes ascend, there is a progressive decrease in normal disc structure. Distortion and compression of the disc membranes, blurring of the disc edges, and shrinkage and irregularity of the enclosing outer membrane are seen. This strongly implies that enzymatic digestion begins while the phagosomes are still within the apical processes. Observations showing small vesicles resembling lysosomes fusing with phagosomes inside the ensheathing processes (Fig. 6, *C*) suggest that this is indeed the case. In parafoveal cones, the outer segments terminate closer to the apical surface of the RPE; hence the apical processes are somewhat shorter. Phagosomes within these processes show less evidence of deterioration, as do phagosomes just above the processes within the interior of the cell.

Monkey perifoveal cones. In rhesus monkey perifoveal cones, approximately 2 mm from the foveal center, the outer segments are slightly tapered from base to apex and measure about 30 μm in length (Fig. 3, *B*). Apical processes from the RPE extend down, just as they do in the human retina, to ensheath the cone tips (Fig. 5, *A*). At the apical border a meshwork of processes, some of which contain pigment granules, protrudes from the soma and tapers as it reaches the cone tip. By contrast, the tips of rod outer segments extend very close to the apical surface (Fig. 3, *B*). As is the case in squirrel and human cones, terminal disc packets are intermittently shed from cone outer segments in the rhesus monkey. Once more, the curling and detachment of the packets appear to proceed as they do in the other examined species (Fig. 5, *A*). In some instances, however, packets of cone discs in the monkey retina rotate 90 degrees rather than curl as a prelude to detachment.

Phagosomes within the ensheathing processes some times contain cone discs that are not altered in membrane density or integrity (Figs. 5, *B*, and 6, *A* and *C*). But in most cases the discs within the phagosomes are more electron dense, distorted, and compressed (Figs. 5, *A* and *C*, and 6, *B*)—suggesting that as in the human retina, digestion is initiated within the apical processes. Cone phagosomes come in various sizes and shapes (Figs. 5 and 6), and they typically contain anywhere from one-half dozen to several dozen discs. Along with phagosomes within the processes, we were able to identify several different types of vesicles (Figs. 5, *B*, and 6, *C*), as well as microfilaments, free ribosomes, and endoplasmic reticulum. Multiple cone phagosomes occurring within the ensheathing processes are commonly seen (Figs. 5, *A*, and 6, *B*), suggesting that their transit to the RPE interior may not be rapid[21] or that several packets of discs may have been shed over a short time period. Dense phagosomes that lie just vitread to the apical surface of the RPE (Fig. 6, *B*) are still distinguishable from pigment granules, which always show a circular or elliptical profile in thin sections.

Thus far, we have been unable to discern any significant differences between monkey and man in the shedding or phagocytosis of

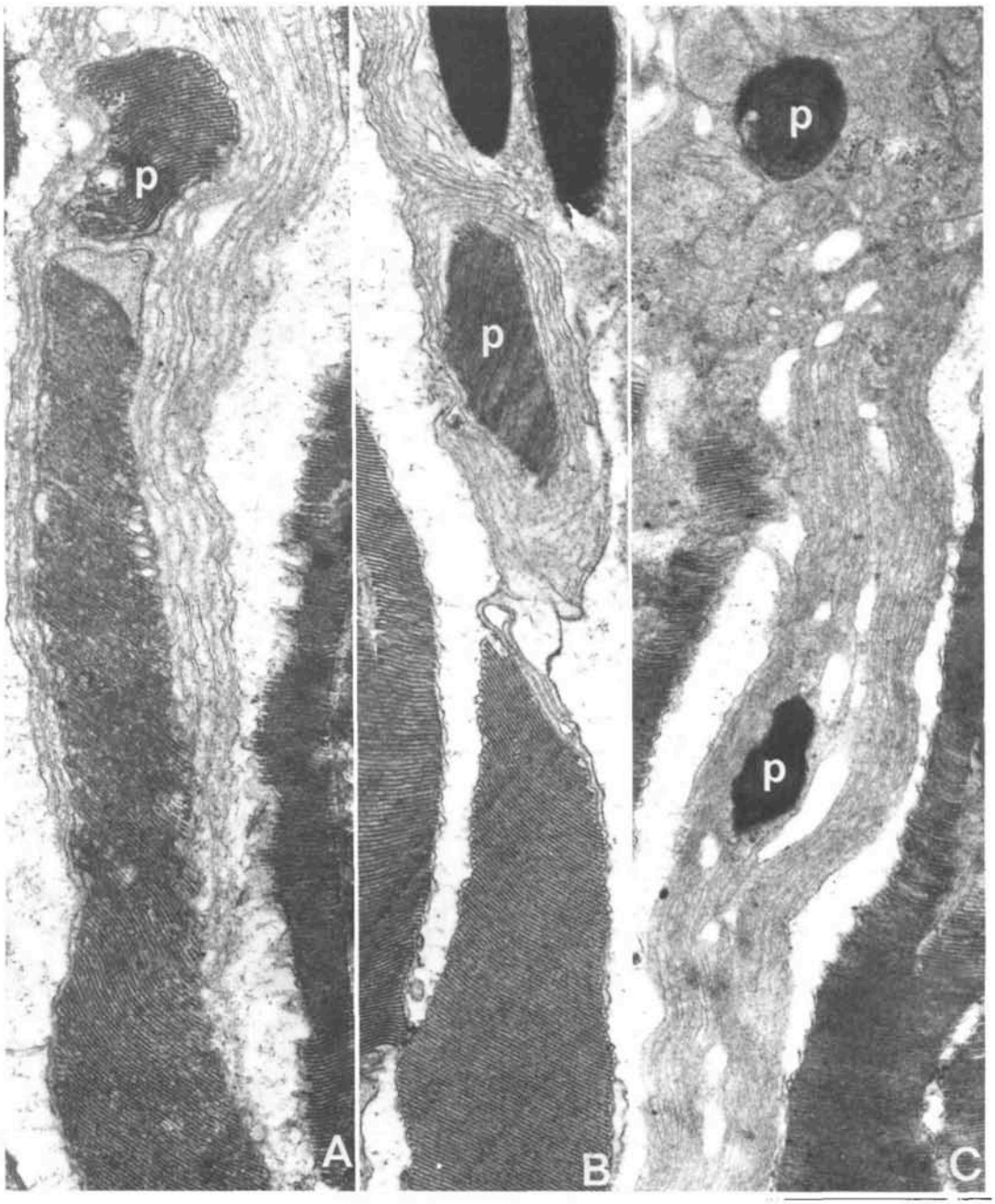

Fig. 7. Phagosomes within the cone sheath of the cat retina. Three of four phagosomes *(p)* in these micrographs occur within the RPE apical processes which make up the elaborate cone sheath in the cat. Each cone sheath contacts only one cone outer segment and no rods; thus phagosomes within the sheaths must have a cone origin. The lamellar structure of the cone phagosomes is clear in **A**, partially obscured in **B**, and no longer evident in **C**. The phagosome seen in the main body of the RPE in **C** could be of either rod or cone origin. (**A** ×60,000; **B** ×27,000; **C** ×28,000.)

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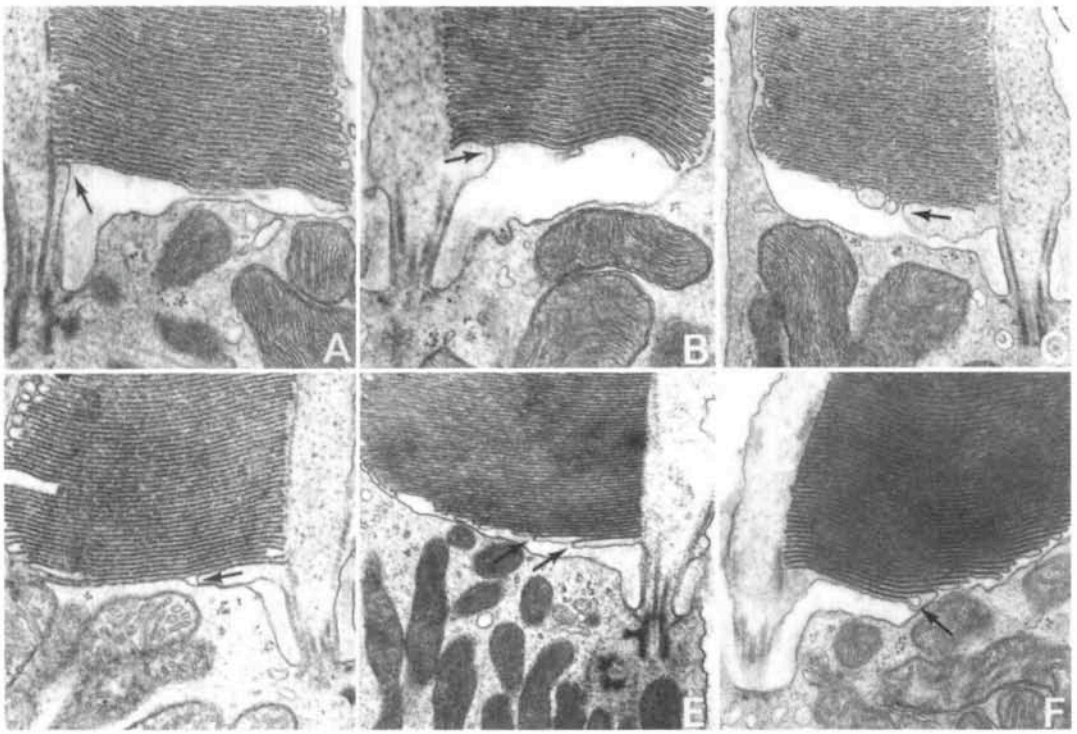

Fig. 8. Cone outer segment bases in the 13-lined ground squirrel (**A** to **C**), the California ground squirrel (**D**), the rhesus monkey (**E**), and human (**F**) retinas. A proposed sequence of new disc assembly is suggested by viewing **A** to **F** in succession. The membrane bordering the inner margin of the connecting cilium may elongate, tracing a path from the inside border of the disc stack to the disc periphery. The arrows indicate the leading edge of newly-forming membrane. (**A** ×36,000; **B** ×37,000; **C** ×28,000; **D** ×35,000; **E** ×23,000; **F** ×28,000.) (**C** and **D** from Anderson, D. H., and Fisher, S. K.: J. Ultrastruct. Res. 55:119, 1976.)

cone discs. Before any firm conclusions can be drawn, however, detailed comparisons between central and peripheral cones in each species are required.

Cat retina cones. In the cat retina, the ensheathing processes that cap the cone outer segments are arranged in multiple, concentric cytoplasmic sheets.[22] They appear to be more highly ordered than in either the human or rhesus monkey retinas. In our survey, we have found numerous examples of cone disc packets embedded within these ensheathing processes en route to the RPE interior (Fig. 7). These observations confirm the hypothesis of Steinberg and Wood[22] that one of the functions of the elaborate cone sheath in cat may be to phagocytize shed cone discs. Cone disc shedding and RPE phagocytosis in cat is similar to what we have described above for primates.

Disc assembly in mature cones

The presence of disc shedding from mature mammalian cones leads directly to the inference that new discs must be added in order to make up for their intermittent loss. Were this not so, cone outer segments would progressively shorten over time. It has already been demonstrated that cones do synthesize new discs under certain extraordinary conditions, i.e., following retinal reattachment surgery,[16] after arousal of the animal from hibernation,[34] or following light-induced damage.[35] It remains to be determined precisely *how* the new membrane is added under these conditions, under normal conditions, and at what rate.

As mentioned before, no band of labeled protein molecules appears in developing cone outer segments,[5, 7] nor do ground squirrel cones show a band during their repair

after arousal from hibernation.[34] One explanation for this apparent paradox is that labeled molecules simply diffuse throughout some or all of the interconnected network of cone discs,[36] whereas a significant proportion of such molecules is confined within the independent rod discs.[14, 37] The different labeling patterns in rods and cones, according to this interpretation, result from differences in the structural organization of their outer segments and do not indicate qualitatively different renewal systems. Some support for this view comes from studies showing that radioactive protein can longitudinally diffuse throughout the plasma membrane of frog rod outer segments.[38] The diffusion is made possible by the continuity of the plasma membrane with only the most basal discs—the few that are not yet independent units in the stack. In independent discs, the radioactive molecules are apparently trapped and capable only of lateral diffusion within the disc membrane.[37, 39]

How are new discs formed in mature cones? One possibility is that they are formed in an analogous way to the formation of rod discs.[11, 40] Earlier studies have concluded that rod discs are formed at the outer segment base by an *infolding* or *invagination* of the enveloping outer membrane.[41, 42] In developing photoreceptors, new rod and cone discs both have been described as being formed by invagination.[40] In mammalian rods, up to a dozen or so of the most basal discs retain some continuity with the outer membrane,[43] indicating that they are incompletely formed. The buttonlike disc edges may be absent or indistinct, the paired membranes may not be closely apposed, or the invaginations may not run the full width of the outer segment.[11] One recent study in frog rods suggests that the number of such partially formed discs may be taken as an index of the assembly rate at various times in the lighting cycle.[44]

At the base of mature cone outer segments a similar type of assembly process is suggested by electron micrographs. In human, monkey, squirrel, and cat cones the most basal discs consist of a series of successive, interconnected foldings (Fig. 9, *B*). The membrane at the very base of the disc stack is a continuation of the membrane that borders the connecting cilium. Electron micrographs taken at the level of the cone cilium show stages of what could be the assembly process (Fig. 8). The hypothetical sequence of assembly is suggested by viewing Fig. 8, *A* to *F*, in succession. The ciliary membrane appears to elongate, pushing outward from a point close to the inside edge of the disc stack toward the disc periphery. A sequence of this kind should be termed an *evagination*, rather than invagination, to emphasize the "inside-out" aspect to this process. Through repeated evaginations, new cone discs could be continually added to the base of the stack, thereby displacing those above them toward the outer segment tip.

Cone structure and disc displacement

Rod outer segments are cylindrically shaped, and their discs have been described as being like "coins in a bell jar."[45] Since each rod disc becomes independent after its initial formation at the outer segment base, the continual displacement of discs toward the outer segment tip is easy to visualize in three dimensions. If we presume that cone discs are also displaced, the process must be somewhat more complex because many, and perhaps all, discs retain some connection with the outer plasma membrane—much like the rungs of a ladder.

In nonmammalian cones the outer segments are composed of a stack of discs, all of which remain continuous with each other and with the outer membrane adjacent to the connecting cilium.[46] In mammalian cones, only the basal part of the outer segment seems to show this organization in single thin sections.[43] Cohen[47] found that precipitates of lanthanum salts infiltrate into the infoldings at the base of monkey cone outer segments, indicating that this region is not bounded by an enveloping outer membrane. At more distal locations only occasional discs infiltrate with the precipitate. These results suggest that although many discs do remain continuous, some distal discs could be independent units. Other evidence by Laties et al.[48] shows that the fluorescent dye Procion yellow

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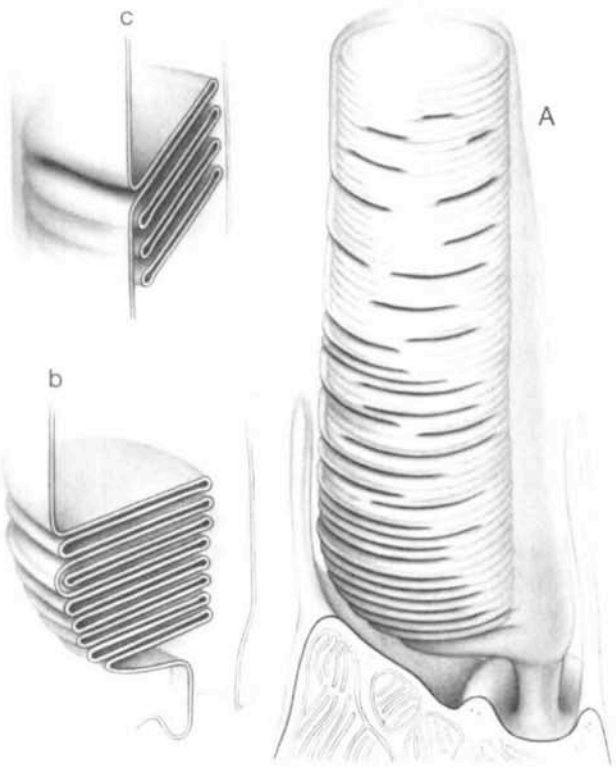

Fig. 9. Proposed model for the structure of mammalian cone outer segments. The drawing is based on the cone outer segment shown in Fig. 1, but the scale has been altered for the sake of clarity. **A,** Entire outer segment and the distal portion of a cone inner segment are shown. Single incisures that may be present in some cone discs[11, 22] and the details of the connecting cilium have been omitted. The inner segment and its associated calyceal processes are connected to the outer segment by the tubelike cilium that borders the disc stack. The extent of continuity around the perimeter of each disc is increasingly restricted from base to outer segment apex. The regions of continuity are not in register. Near the tip, independent discs may occur, or each disc may retain a discrete point(s) of continuity, so that discs which appear isolated from the outer membrane in the drawing might have an area of continuity in a region of the outer segment not visible. **B,** Longitudinal section through a cone outer segment base. The basal discs form one interconnected network. The membrane at the very base of the stack is a continuation of the membrane that borders the connecting cilium. **C,** Longitudinal section through a region of the drawing where one disc is continuous with the outer membrane and several others appear to be separate.

infiltrates the entire outer segment in mammalian cones, implying that more than a few discs are open along the length of the outer segment. But it is not possible from these findings to say how many discs are open or if all discs are open to the extracellular space.

Fig. 9 presents a model for the organization of a mammalian cone outer segment. As first suggested by Cohen,[43, 47] the extent of continuity around the perimeter of each disc becomes increasingly restricted from the base to outer segment apex, and the regions of continuity among adjacent discs are not in register. Thus what is seen in a single, longitudinal plane of section is a reflection of both these features. At the base, the continuous region around each disc's perimeter is complete or nearly so; hence, at this level,

there is no indication of an enclosing membrane (Fig. 9, *B*). At more distal locations, the region of continuity becomes more restricted, so that the chances of seeing it in any single section become less likely (Fig. 9, *A*). Because the region of continuity shifts from disc to disc, adjacent discs may appear as continuous or discontinuous in single thin sections (e.g., see Fig. 1), depending upon the particular plane. In the distal third of the outer segment, the region(s) of continuity may be limited to a discrete point (or points) or it may disappear altogether, leaving a disc isolated from surrounding membrane (Fig. 9, *A*).

This kind of organization requires that the displacement of cone discs from the base to the outer segment tip must be accompanied by a similar displacement of the outer membrane. Otherwise, the connections between the discs and the outer membrane would have to be continually made and broken to allow for disc displacement, a much less likely alternative. This, in turn, indicates that the outer membrane must also be continually replaced in conjunction with the discs. That this occurs for *both* rods and cones follows directly from observations showing that shed disc packets from both photoreceptor types are surrounded by shed outer plasma membrane.[11, 20, 21]

Conclusion

Our findings on cone disc shedding and their RPE phagocytosis in several different groups of mammals—squirrels, primates, and cats—indicate that these events are almost certainly as universal for mammalian cones as they are for rods. The processes of disc shedding in both rods and cones are qualitatively similar, and it seems highly likely that the similarity extends to the assembly process as well. Earlier concerns about the effects of excess light exposure to the human retinas during preoperative ophthalmoscopic examination[21] can now be put to rest. Nearly identical observations in the monkey retinas, which had no prior history of excess light exposure, confirm that cone shedding and RPE phagocytosis are part of a *normal,* ongoing process in the primate retina. Thus the possibility that disc shedding from cones is merely an anomalous event or represents a case of deteriorative change unbalanced by new disc assembly is no longer tenable.

In rods, certain metabolic and environmental factors that affect the *rate* of assembly and/or shedding are just starting to be identified. The availability of polyunsaturated fatty acids,[49, 50] increases in ambient temperature[51] and the onset of light[52–55] have all been shown to affect either the assembly or phagocytic rate or both. Because both rods and cones are now thought to share the same system of outer segment renewal, it is appropriate to inquire whether the factors governing the control of the renewal process are also shared. That is, do the same factors that affect the rate of assembly or shedding in rods play a similar role in cones? Results recently obtained on the relationship of cone shedding to cyclic light suggest an affirmative answer to this question. In goldfish, chicks, and lizards maintained on a 12 hr light/12 hr dark cycle, an increase in cone shedding is detected during the early part of the dark period.[56–59] These findings introduce a complementary aspect to the regulation of disc shedding by cyclic light, and they support the conclusion that the continual turnover of outer segment discs is a general characteristic of vertebrate cones. It is not possible from the studies presented above to assess the influence of darkness on the rate of mammalian cone shedding. We must point out, however, that all these retinas were fixed during the light period, and for the squirrels we know that the animals were thoroughly adapted to a 12 hr light/12 hr dark schedule. If the rate of cone shedding had been negligible during the light period, then it would have gone undetected.

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