

The Relationship of Primate Foveal Cones to the Pigment Epithelium¹

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Processes of the retinal pigment epithelium that drape or ensheath the tips of cone outer segments in the rhesus monkey fovea were examined by electron microscopy. These processes emerge from the apical surface of the pigment epithelium (RPE) and form a multilaminar sheath enclosing the outer segment tips. Some of the processes extend down along the lateral border of cone outer segments for up to 30 μm . The relationship of the RPE apical processes to foveal cones is a modified version of their relationship to extrafoveal cones, in that the supracone space is reduced in size and the extent of apical RPE surface devoted to each cone is substantially less.

The pigment epithelium (RPE) of the vertebrate retina consists of a monolayer of hexagonally shaped cells that border the photoreceptors. A complex array of processes protrudes from the apical surface of each RPE cell, draping or enveloping the tips of the rod and cone outer segments. It was formerly thought that the function of these apical processes might be limited to that of mechanical support or metabolic exchange. However, it is now known that they are responsible for the engulfment of disc packets shed from both rod and cone outer segments (Young, 1976; Anderson *et al.*, 1978), and for their transport from the outer segment tips to the RPE cell interior (Spitznas and Hogan, 1970; Young, 1971; Steinberg *et al.*, 1977; Anderson and Fisher, 1976.).

In most mammalian retinas the tips of rod outer segments terminate at the apical border of the RPE cells, sometimes actually indenting their surface. By contrast, the cones of most mammals do not reach the apical RPE border. Instead, processes originating from the apical RPE surface descend between the rod outer segments to contact the cone tips (Steinberg and Wood, 1974; Steinberg *et al.*, 1977; Carter and LaVail, 1977; Anderson *et al.*, 1978).

Steinberg and Wood (1979) recently observed that the relationship of the apical processes to human macular cones, outside of the fovea, is a modified version of their relationship to peripheral cones. As the cone outer segments become longer and more slender in the central retina, the apical RPE processes shorten. There have been no observations reported on the association between foveal cones and the RPE. Thus, we decided to examine the relationship of rhesus monkey cones lying within the foveal pit to the overlying RPE, and to compare the organization of the apical processes in the fovea with that from more peripheral locations.

MATERIALS AND METHODS

Animals. The foveal regions from four retinas of three adult rhesus monkeys (*Macaca mullatta*) were examined histologically. Each of the animals was maintained on a light-dark cycle consisting of 12 hr light and 12 hr darkness. Electron microscopic observations were made on both foveas from one animal that was sacrificed at midday, approximately 5 hr following the onset of light in the morning.

Electron microscopy. Animals were anesthetized initially with an intramuscular injection of ketamine hydrochloride (Bristol, 50 mg/ml). Anesthesia was maintained thereafter by intravenous injection of sodium pentobarbital (Abbott, 50 mg/ml). The animals were fixed by intracardiac perfusion of an aldehyde fixative composed of 1% paraformaldehyde and 1% glutaraldehyde in phosphate buffer (Young, 1971). After perfusion of approximately 800 ml of fixative, the eyes were carefully enucleated and the anterior

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one-half of the globe was cut away using tenotomy scissors and a double-edged razor blade. At this stage as much of the vitreous humor as possible was teased away from the chamber. The remaining posterior portion of the globe was transferred to fresh fixative for several hours. When removed from the fixative, the tissue was washed in the phosphate buffer (pH 7.1) plus sucrose (40 mg/ml) and cut into wedge-shaped pieces with double-edged razor blades. The optic nerve head and the fovea were positioned at the apex of one of the wedges. This piece was segregated from the rest of the tissue and processed separately.

After several washes in buffer solution, the specimens were postfixed in Veronal acetate-buffered osmium tetroxide (2%) for 1.5 hr, washed in distilled H₂O, then dehydrated in a graded ethanol-H₂O series and embedded in Araldite (Cargille 6005).

In order to locate the foveal region, 1- μ m sections were cut from the tip of the wedge-shaped foveal specimens on an LKB Ultratome III and examined in a Zeiss Universal Research microscope. When the optic nerve head was identified, sections were surveyed at 100- μ m intervals until a slight thinning of the ganglion cell layer could be recognized. The boundaries at the edge of the fovea could be distinguished because fibers from Henle's layer tended to course away from both sides of the foveal region. At the outer boundary of the fovea, the retina was oriented so that the outer segments were aligned along their longitudinal axes. As we approached the center of the fovea, we found that the outer segments had to be constantly reoriented to preserve the longitudinal alignment.

Thin sections (550–600 Å) from the central fovea were placed on Formvar-coated slot grids, stained with 1% uranyl acetate and lead citrate, and then examined in a Siemens 1A or 101 electron microscope.

RESULTS

In the rhesus monkey retina, the fovea is a hemispherical indentation on the vitreal surface several millimeters temporal to the optic disc. It is about 1.0 mm in diameter

from edge to edge and 0.2 mm deep at its center. In the central fovea, the retina is reduced in thickness from 375 to just under 200 μ m because the ganglion cell, inner plexiform, and inner nuclear layers are absent (Fig. 1).

Our estimates of the receptor density inside the fovea indicate that there are about 130 000 cones/mm². The outer segments of foveal cones are very slender (1.0–2.0 μ m wide), minimally tapered, and measure 40–45 μ m in length (Fig. 2). The inner segments are 3–4 μ m in diameter at their widest point; in transverse section, they form a tightly packed and regularly spaced mosaic (Fig. 5). Outer segment structure in foveal cones departs little from that of extrafoveal cones, or from the cones of other mammals. In single thin sections, most of the discs at the outer segment base are continuous with the outer membrane. Most of the distal discs, on the other hand, appear as separate from the cell membrane. However, there are clearly cases where such disc-outer membrane continuities are present even at the distal tip of the outer segments (Fig. 3). In transverse sections, single incisures can be seen in some of the distal discs (Fig. 10).

In the animal sacrificed at 5 hr after the onset of light, the foveal pigment epithelium shows evidence of recent phagocytic activity. Phagosomes similar in size to the outer segment diameter were present at or close to the cone tips (Fig. 3), and also within the mid- and basal zones of the RPE.

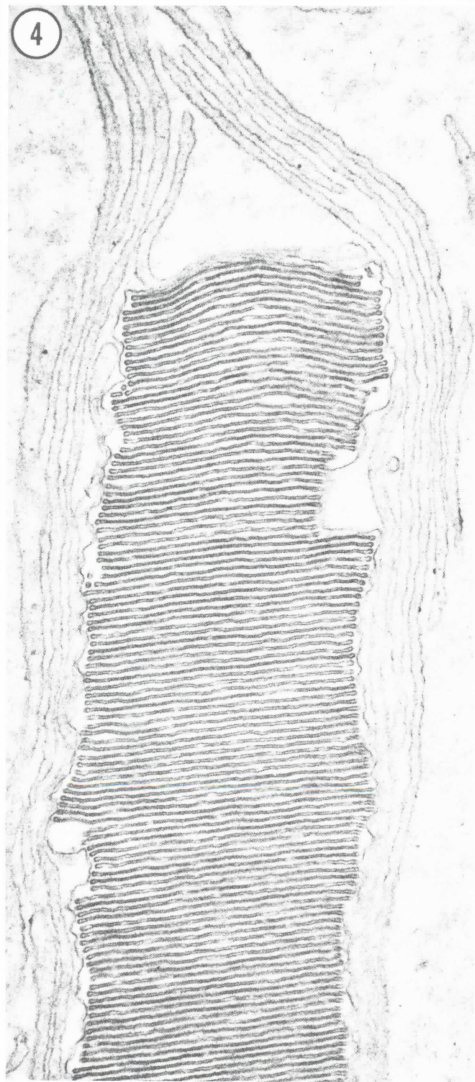
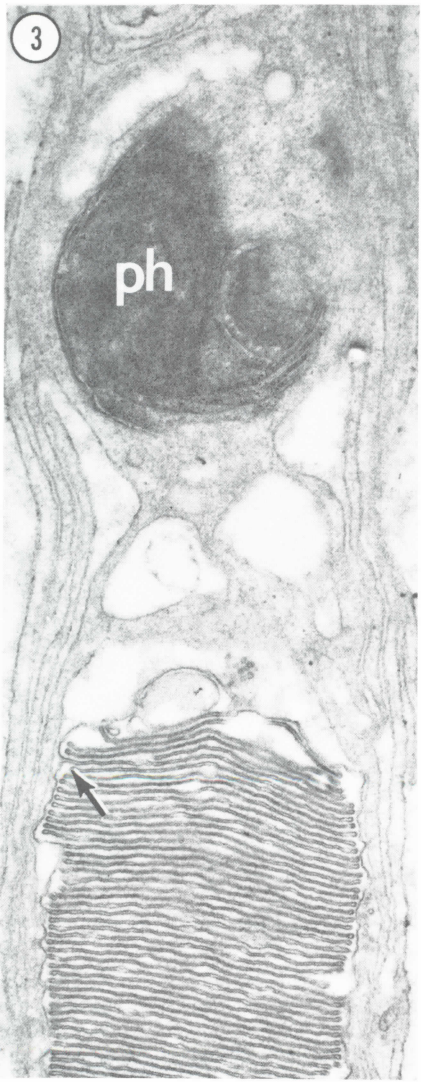
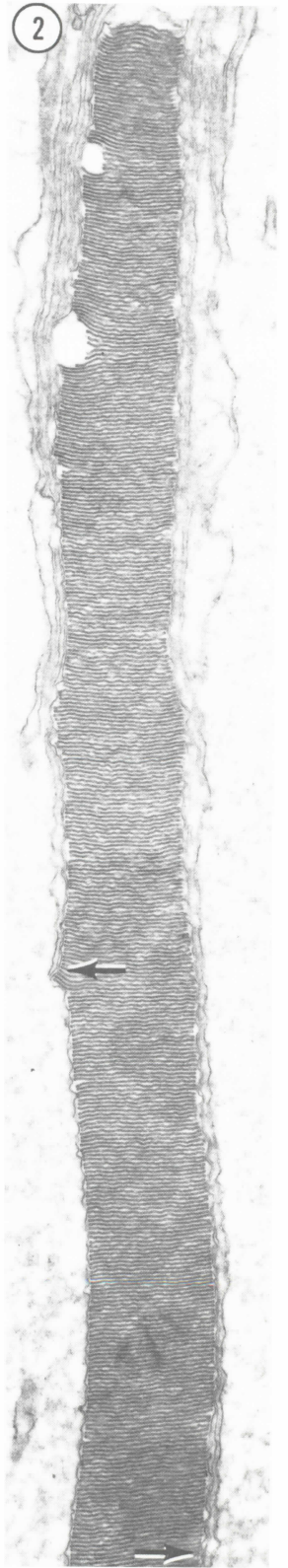
Unlike the cones at extrafoveal locations, foveal cone outer segments terminate very

FIG. 1. A low-power light micrograph close to the center of the monkey fovea. The ganglion cell (g) and inner plexiform layers are absent; the inner nuclear layer (i) has only a few scattered nuclei remaining at the center of the pit. $\times 170$.

FIG. 2. The distal part of a foveal cone outer segment. The top of the outer segment is draped by multiple RPE processes. Some of these processes can extend down along the outer segment (arrows) for up to 30 μ m. $\times 7000$.

FIG. 3. The tip of a foveal cone outer segment. RPE processes envelope the tip of the outer segment. A recently shed cone phagosome (Ph) lies immediately above the tip. In foveal cones, disc-outer membrane continuities (arrow) occur even at the extreme top of the outer segment. $\times 30\ 000$.

FIG. 4. The tip of a foveal cone outer segment showing how the apical RPE processes form a multilayered arrangement around the outer segment tip. $\times 30\ 000$.



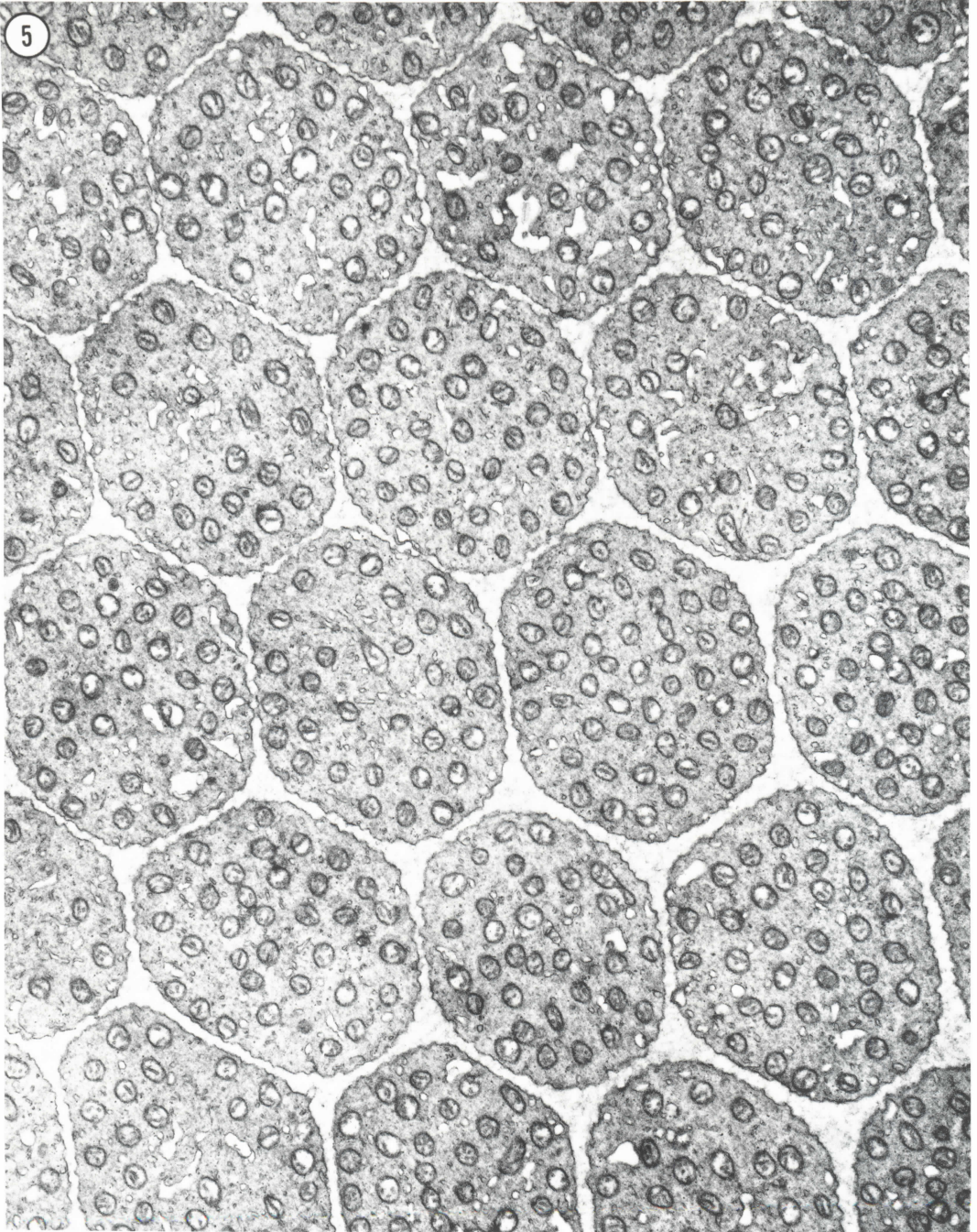


FIG. 5. Transverse section through foveal cone inner segments. Within the all-cone area of the fovea, the inner segments form a densely packed and regularly spaced mosaic. $\times 12\ 000$.

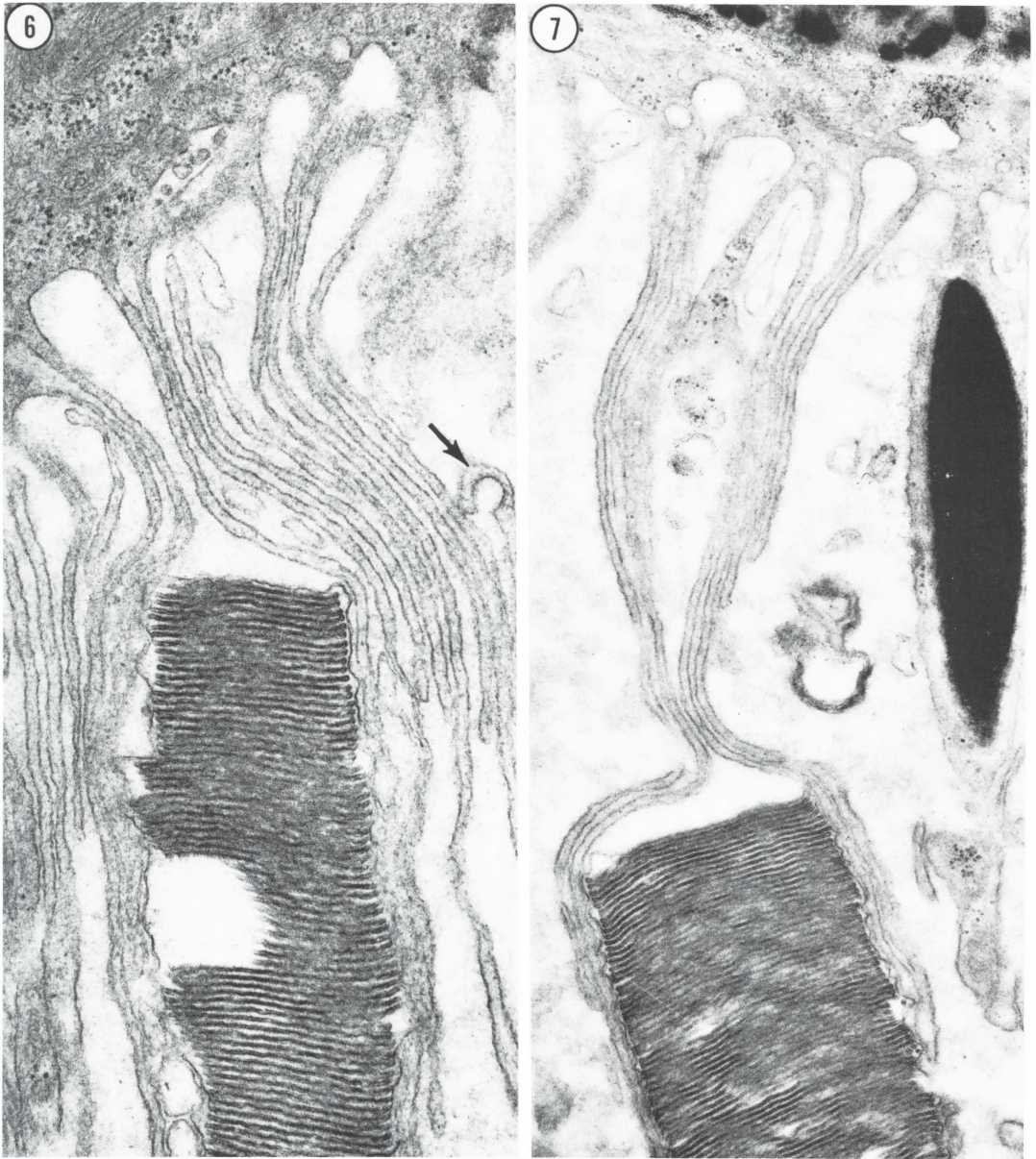


FIG. 6. Foveal cone outer segment and its relationship to the apical RPE. The tip of the outer segment fits into a socket created by the innermost RPE processes. The outer processes follow their contour, resulting in a series of laminae. Coated vesicles, about 1000 \AA in diameter (arrow), are frequently observed in apparent fusion with the outer membranes of the apical RPE processes. $\times 28\ 000$.

FIG. 7. Apical RPE processes descend about $3 \mu\text{m}$ before they encounter the outer segment tips of foveal cones. The processes converge as they leave the apical surface, and then diverge again, draping themselves over the lateral margins of the outer segments. $\times 35\ 000$.

close to the apical RPE surface. Measurements of the distance between the cone tips and the RPE surface show a range of from less than 0.5 to a little over $5.0 \mu\text{m}$. Conse-

quently, the processes from the RPE extend down only a short distance before they encounter the cone tips. Usually, this distance is $2\text{--}3 \mu\text{m}$ (Figs. 6 and 7), but occa-

sionally some cones nearly abut the apical RPE surface (Fig. 8) in a manner strongly reminiscent of rod outer segments.

In longitudinal view, the cone tips are bordered by several layers of RPE processes (Fig. 4). The processes first converge in the supracone space, then diverge as they approach the cone tip and, finally, course down along the lateral border of the outer segment where they terminate (Fig. 2). Some processes extend down along the outer segment border for up to 30 μm . Thus, the total length the ensheathing processes can reach is 35 μm in the fovea. The innermost processes enclose a small lacuna of extracellular space into which the outer segment tip fits. The outer processes follow the inner processes' contour, resulting in a multilayered appearance (Figs. 4, 6, and 7). Coated vesicles slightly larger than 0.1 μm in diameter are frequently found within the RPE adjacent to both apical and basal surface, and also within the apical processes themselves (Figs. 6 and 14). It is not known whether they are secretory vesicles, or whether they are endocytotic in nature.

In transverse section, the ensheathing properties of the apical processes are more apparent. As shown in Fig. 14, the processes emerge from the apical RPE surface in two different forms: as branching sheets of cy-

toplasm and as small tubular profiles. Both types can also be found immediately above the cone tips as part of the cone sheath (Figs. 12 and 13). The tubular processes lie just peripheral to the sheetlike processes, and in the interreceptor space between the outer segments. Except for an occasional pigment granule and a few ribosomes, the processes are filled with only an amorphous ground substance at the level of the photoreceptor tips. Closer to the apical surface, some endoplasmic reticulum is evident, as are microtubules 200–250 \AA in diameter (Fig. 14). Cross sections of microfilaments (90–100 \AA in diameter) are also seen in both types of processes (Fig. 14, inset).

Directly above the tips of the cone outer segments, the sheet-like processes are often folded to form a "U"-shaped profile or that of a complete oval. These folded sheets envelop the space that will be occupied by the outer segment (Fig. 13). When they reach the outer segment tip, the processes completely encircle the outer segment (Fig. 11). One process can extend around the entire outer segment perimeter (Fig. 11); but, more often, they extend only part of the way around. In this respect, the ensheathment of the cone tips is quite similar to that found for monkey rod outer segments (Fig. 9).

FIG. 8. Occasionally, foveal cone outer segments actually abut the apical RPE surface in a manner highly similar to rod outer segments. In this figure, the cone outer segment tip appears to have compressed a few processes against the cell surface. $\times 36\ 000$.

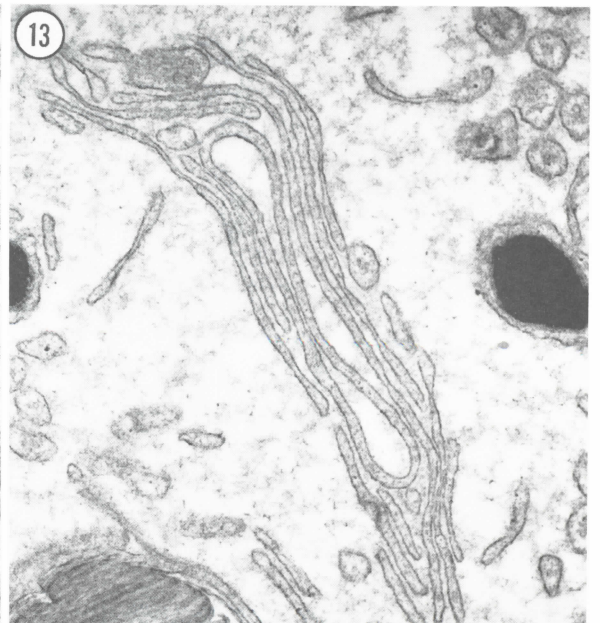
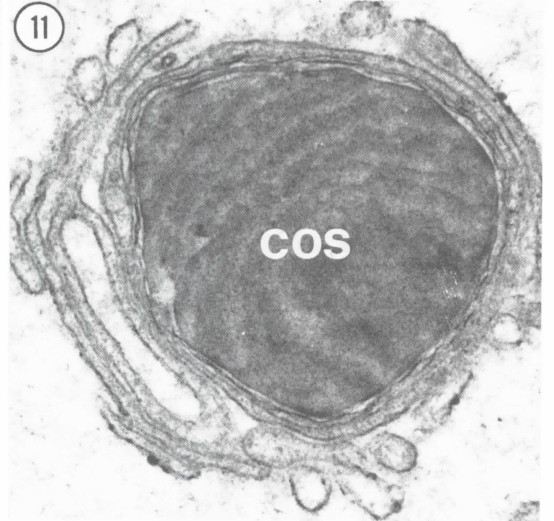
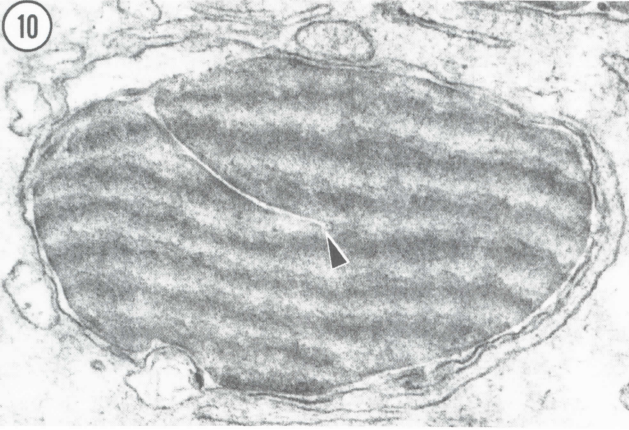
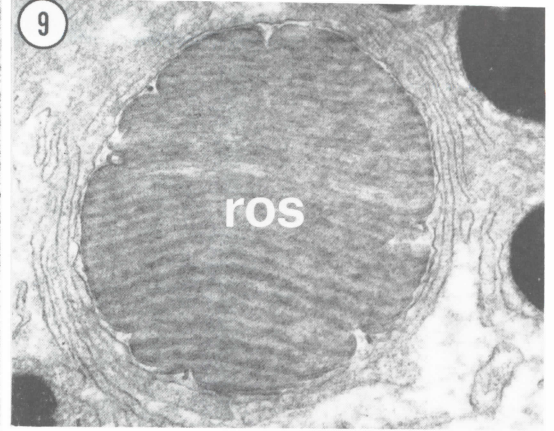
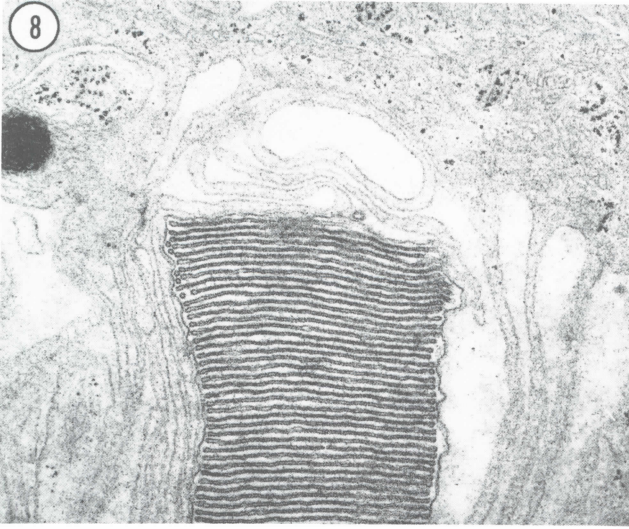
FIG. 9. Transverse section of the tip of a monkey rod outer segment (ros). Rod outer segments abut the apical RPE surface, and are also ensheathed by RPE processes at their tips. The outer segment discs possess a scalloped margin that can be used as a distinguishing characteristic. $\times 30\ 000$.

FIG. 10. Transverse section of a foveal cone outer segment about two-thirds of the way from the distal tip. At this level, the ensheathment by RPE processes does not extend around the entire outer segment perimeter. Several tubular processes lie adjacent to the outer segment. There is no identifiable continuity between the discs and the outer membrane. A single incisure (arrowhead) extends from the disc margin to the disc center. $\times 45\ 000$.

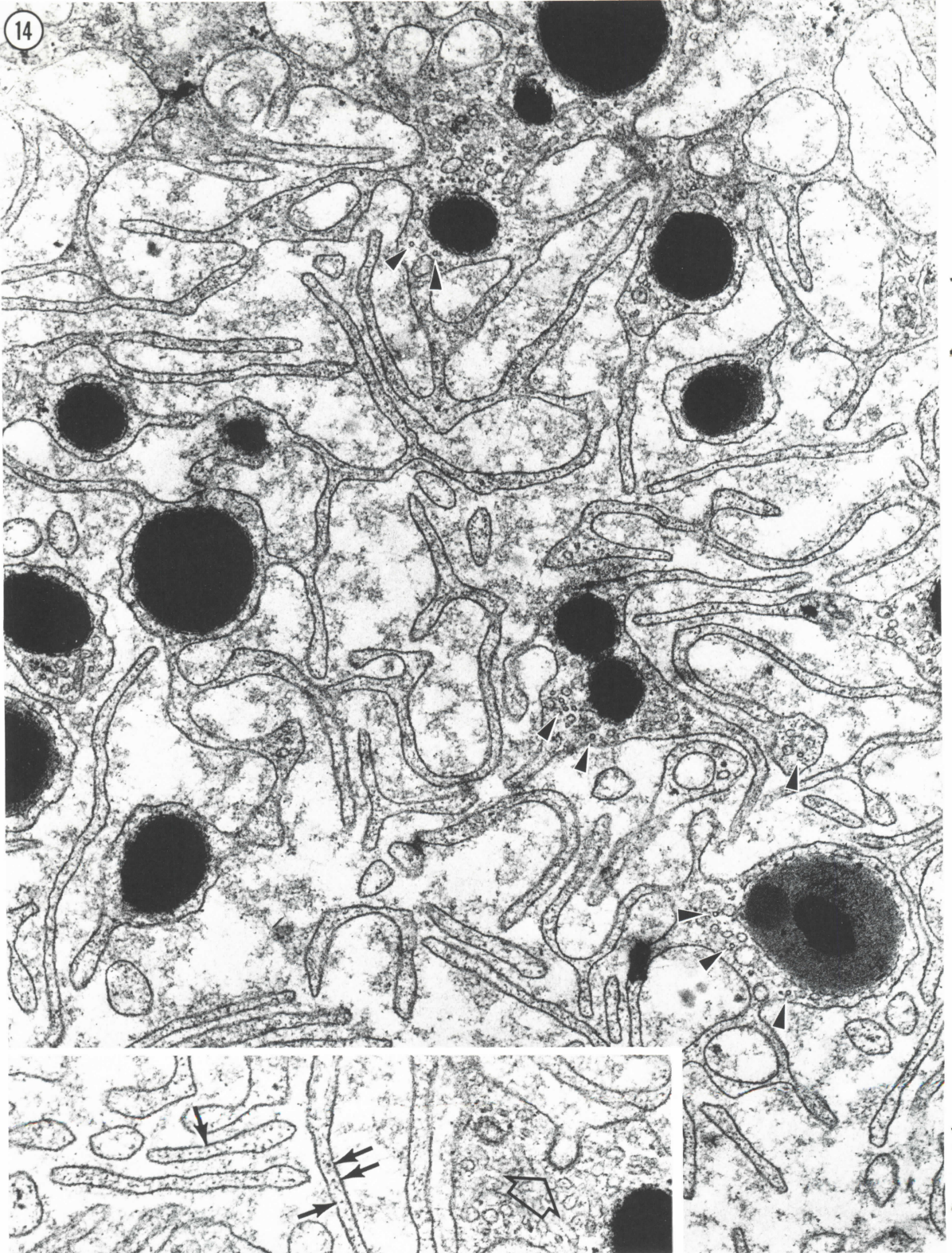
FIG. 11. Transverse section of a foveal cone outer segment (cos) close to the distal tip. At this level, the ensheathment is more extensive. A single process appears to completely encircle the outer segment. Other processes wrap around a portion of the outer segment. $\times 35\ 000$.

FIG. 12. A set of apical RPE processes, a cone sheath, lying immediately above the tip of a foveal cone outer segment. In transverse section, the processes are both tubular and sheet-like. The sheet-like processes are often folded, while the tubular processes are usually peripheral to the sheet-like processes. $\times 35\ 000$.

FIG. 13. RPE processes above a foveal cone outer segment. The innermost process can be seen, in transverse section, to form a continuous and elongated oval. The space within the oval is the socket into which the foveal cone tip will be inserted. $\times 30\ 000$.



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DISCUSSION

The morphological relationship between monkey foveal cones and the RPE is not as simple as for most mammalian rods, nor is it as highly organized as for other mammalian cones—such as those in the cat (Steinberg and Wood, 1974) and rabbit (Bunt, 1978) retinas. With respect to primate extrafoveal cones, the main differences are in the dimensions of the apical processes and in the quantity of organelles that they contain.

In both human and rhesus monkey extrafoveal retina, the processes that ensheath the cones traverse a distance of 10–20 μm between the apical RPE surface and the outer segment tips (Steinberg *et al.*, 1977; Anderson *et al.*, 1978). This region has been termed the *supracone space* (Steinberg and Wood, 1974). The processes in the supracone space occupy an area up to 8 μm in diameter in the monkey RPE as they emerge from the apical surface (Anderson and Fisher, unpublished). Like the processes described here for the fovea (Figs. 6 and 7), those outside of the fovea also taper and converge as they leave the apical surface, then diverge to ensheath the cone tip. Scanning electron micrographs of the RPE surface in the rhesus monkey are consistent with these observations (Sakuragawa and Kuwubara, 1976). The extent of RPE apical surface devoted to each foveal cone is reduced to an area about 2 μm in diameter, and, because of the increased length of foveal cone outer segments, the size of the supracone space is reduced to only a few microns. However, the *total* length of the ensheathing processes, i.e., the distance from the apical RPE surface to their termination alongside the outer segment, for both foveal and extrafoveal cones is not

greatly different. Endoplasmic reticulum, pigment granules, ribosomes, and several types of vesicles can be identified within the processes bordering extrafoveal cones (Steinberg *et al.*, 1977; Anderson *et al.*, 1978). These organelles are usually congregated toward the apical RPE surface, but occasionally can appear close to the cone tips. RPE processes associated with foveal cones contain few of these organelles.

By comparison to cat (Steinberg and Wood, 1974; Fisher and Steinberg, in preparation) or to rabbit (Bunt, 1978) cones, the ensheathment of foveal cones appears less complex. In these species, the number of laminae bordering the cone outer segment may be twice that in rhesus foveal cones; multiple processes may ensheath the cone outer segments all the way down to their base; and one apical process may wrap the outer segment more than once.

Apical RPE processes are also concentrically arranged around the outer segment tips of primate rods. In the case of both human (Hogan, 1971) and monkey rods (Young, 1971), the processes form a “loose-fitting” cap into which the outer segment is inserted. In the monkey, the processes may extend to the rod outer segment midpoint, making them approximately 12–17 μm long. In the human, they may be somewhat shorter (Hogan, 1971). Spitznas and Hogan (1970) describe the distal portion of human rod outer segments as being “surrounded by a single solid cytoplasmic sheet.” They also describe a second type of tubular process that is probably identical to the tubular processes identified here in association with foveal cones. Thus, many of the features of the foveal cone–RPE relationship are also present in the association between rods and the RPE.

A few years ago, it was believed that rods,

FIG. 14. Transverse section of the apical surface of the foveal RPE. The surface consists of a network of branching sheets and smaller tubular profiles. Pigment granules are relatively common. Microtubules (arrowheads) and microfilaments (inset, small arrows) are present, as are coated vesicles (inset, large arrow). $\times 35\ 000$; inset, $\times 48\ 000$.

but not cones, shed packets of disc membranes from their tips. We are now aware that both cell types regularly shed discs, and that one of the major functions of the apical RPE processes is the engulfment and transport of these shed packets [see Anderson *et al.* (1978) for review]. Therefore, in this respect, an intimate anatomical association between cones and the RPE is just as important as it is for rods. In the primate retina, the structural relationship between rods and cones and the RPE is quite similar. Since the relationship between foveal cones and the RPE does not vary substantially from what is found outside of the fovea where rods greatly outnumber cones, it seems less likely that one function of the more elaborate ensheathing processes of other species is the isolation of cones from surrounding rods. This interpretation is consistent with the finding that horseradish peroxidase diffuses freely between the laminae of the cone sheath in the rabbit retina (Bunt, 1978). However, this does not rule out the possibility that the ensheathing processes serve to modify the exchange of ions or molecules between the cone and neighboring photoreceptors. Indeed, it may be that the only purpose served by the *multilaminar* ensheathment of photore-

ceptor outer segments by the RPE is to increase the surface area over which the molecular, metabolic, or electrolytic interactions between these cells occur (see Steinberg and Wood, 1974).

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