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Development of Retinal Pigment Epithelial Surface Structures Ensheathing Cone Outer Segments in the Cat[1]

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Specialized sheet-like projections elaborated from the apical surface of the pigment epithelium (RPE) ensheath cone outer segments of the adult cat retina. The postnatal development of this sheath in 10- to 40-day-old kittens was studied using scanning electron microscopy (SEM) stereoscopic pairs and transmission electron microscopy (TEM). The course of development of the sheath can be divided into five stages. The surface of the RPE at around 10–12 days is dominated by microvilli. Neither these nor the developing photoreceptor outer segments are oriented in parallel as they are in the adult retina. At 2 weeks the developing cone sheath can be seen by TEM as a compact array of membranous lamellae oriented in parallel. The presence of vesicles within processes forming the growing sheath may be indicative of accelerated membrane addition. Between 16 and 21 days the processes comprising the cone sheath are seen by SEM to overlap each other and appear more highly organized. As the developing rod outer segments elongate and the interphotoreceptor space narrows during the fourth postnatal week, the basal portion of the sheath becomes visible by SEM. The degree of overlap of the ensheathing processes increases gradually until about 1 month of age when the sheath in most respects attains adult morphology. The cellular mechanisms of sheath morphogenesis as well as the functional morphology of the adult cone sheath are discussed. Related physiological, anatomical, and behavioral events occurring during this period are enumerated.

The apical surface of the vertebrate retinal pigment epithelium (RPE) is characterized by cytoplasmic projections that interdigitate between the photoreceptor outer segments. Yet the detailed morphology of the RPE apical processes and their anatomical relation to the photoreceptors differ widely between species. In some vertebrate classes, notably Mammalia, there are even distinct differences between the cone- and rod-associated RPE processes. These differences are extreme in dogs (Hebel, 1971), cats (Steinberg and Wood, 1974), and albino rabbits (Scullica and Tangucci, 1968) where specialized, sheet-like apical processes wrap around the length of the cone outer segments (COS) forming a multilamellar sheath. In contrast, only a single layer of RPE apical cytoplasm caps the distal end of the rod outer segments (ROS). It is also especially interesting to us that rods and cones, two unique cell types, elicit the formation of different cytoplasmic structures from the surface of a single cell type (RPE). In this regard Steinberg and Wood (1974), in their ultrastructural characterization of the adult cat cone sheath, have proposed that the study of its morphogenesis might contribute to our understanding of its complex adult organization.

In this study, conventional transmission electron microscopy (TEM) alone was inadequate to describe the development of the apical RPE surface in kittens, either because kitten outer segments are not oriented in parallel until sometime after birth (Vogel, 1978a,b) or because they are inad-

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equately preserved. Therefore, we used data from both scanning electron microscopy (SEM) and TEM to describe the development of the apical RPE processes forming the cone sheath.

MATERIALS AND METHODS

Animals

Domestic shorthair cats were obtained commercially and litters from these animals were reared under cyclic lighting conditions. Kittens ranging from 1 to 40 days postnatal age, as well as an adult cat, were sacrificed during midday.

TEM

Eyes were removed from kittens anesthetized by an intraperitoneal, lethal dose of sodium pentobarbitol (Abbott, 50 mg/ml). The cornea, iris, lens, and vitreous body were carefully removed to insure that the neural retina-RPE attachment remained intact. Eye cups were then fixed overnight at 4°C in 1% paraformaldehyde and 1% glutaraldehyde in 0.086 (*M*) sodium phosphate buffer (pH 7.2) with 20 m*M* $CaCl_2$ added (Karnofsky, 1965; Young, 1971). The tissue was then washed in buffer containing 4.5% sucrose and 20 m*M* $CaCl_2$. During the rinse, the eye cups were sliced transversely to separate the superior, tapetal half from the inferior, pigmented half. Tissues were postfixed for 2 hr in veronal acetate-buffered 2% OsO_4 with 4.5% sucrose and 20 m*M* $CaCl_2$. Tissues were then processed through a cold graded ethanol : water series up to 50% ethanol (v/v), at which point the material was stained *en bloc* in 2% uranyl acetate in 70% ethanol solution for 2 hr. Dehydration was followed by embedment in Araldite 6005. Thin sections were taken from the tapetal region, aligned either longitudinally (along the axis of the photoreceptor outer segments) or tangentially (in cross section to the outer segments and parallel to the apical border of the RPE). Sections were stained in 1% aqueous uranyl acetate and lead citrate.

SEM

Eyes were dissected from kittens and adult cats, placed in sodium phosphate buffer (room temperature, see above) and their anterior portions removed. Next, the posterior portion, containing the retina, choroid, and sclera, was pinned flat in a dissecting dish containing buffer solution and neural retina was gently peeled away from the underlying RPE. Buffer was gradually replaced with several changes of aldehyde fixative (see above). It was important that the surfaces to be examined were kept submerged and not exposed to the liquid–air interface since this resulted in deformation of fine cytoplasmic structures. The protocol below, modified from Sweney and Shapiro (1977), was used for most of the material.

Specimens were placed in cold (4°C) fixative overnight and then washed in cold phosphate buffer (see above) for three 30-min changes. After an additional night in cold, filtered, 2% tannic acid (low-molecular-weight galloyl glucose, Perfect Parts Co., Baltimore, Md.) buffered by 0.134 *M* sodium cacodylate–HCl with 1% sucrose (pH 7.0), the tissue was briefly rinsed in 1% Na_2SO_4 in 0.134 *M* sodium cacodylate–HCl buffer with 0.2% sucrose (Simoniescu and Simoniescu, 1976). Next were several rinses in 0.067 *M* sodium cacodylate–HCl buffer with 4.5% sucrose and 20 m*M* $CaCl_2$ (until the rinse solution cleared of brown color). Specimens were then transferred to 1% OsO_4 (room temperature, veronal acetate buffer) for 1 hr followed by six rinses in distilled water over 1 hr. This was followed by immersion for 1 hr in freshly filtered 5% tannic acid solution and six more rinses in distilled water. Following an additional 1 hr in 0.5% aqueous OsO_4, specimens were rinsed in distilled water until the rinsate was cleared of brown color, and then were dehydrated through a graded tertiary butyl alcohol–ethanol–water series, ending in absolute tertiary butyl alcohol (solutions containing a high proportion of tertiary butyl alcohol were kept at 45°C to keep the alcohol liquid). Tissues were next transferred through several changes of isoamyl acetate and left in this solvent overnight before being critical-point dried in a Bomar critical-point dryer, using liquid CO_2 as the transition fluid.

Dried samples, affixed to aluminum stubs with colloidal carbon or silver paint, were coated in a Ladd vacuum evaporator with 5–15 nm of gold–palladium (60–40%) and then were examined in a Jeolco JSM-2 SEM at an accelerating voltage of 25 kV. Stereopairs of scanning electron micrographs were recorded photographically using a relative tilt angle difference of 8°.

Unless otherwise indicated in figure captions the material was prepared as described above. Some specimens were prepared with the following techniques:

(1) Dehydration in an acetone : water, or an ethanol : water series, and critical-point drying from acetone or ethanol with liquid CO_2 as the transition fluid.

(2) "Freeze drying" using camphene (Eastman No. P2653, mp 40–45°C) (Watters and Buck, 1971; Robertson and McKalen, 1975).

(3) Substitution of 1% aqueous *p*-phenylenediamine for tannic acid (Estable-Puig *et al.*, 1975).

(4) Sputter-coating in a Denton sputter-coater apparatus or

(5) examination at 20 or 25 kV without metallic coating.

RESULTS

Adult Cat Cone Sheath

In order to more fully understand the developmental sequences of the adult cone sheath, and because our preparative tech-

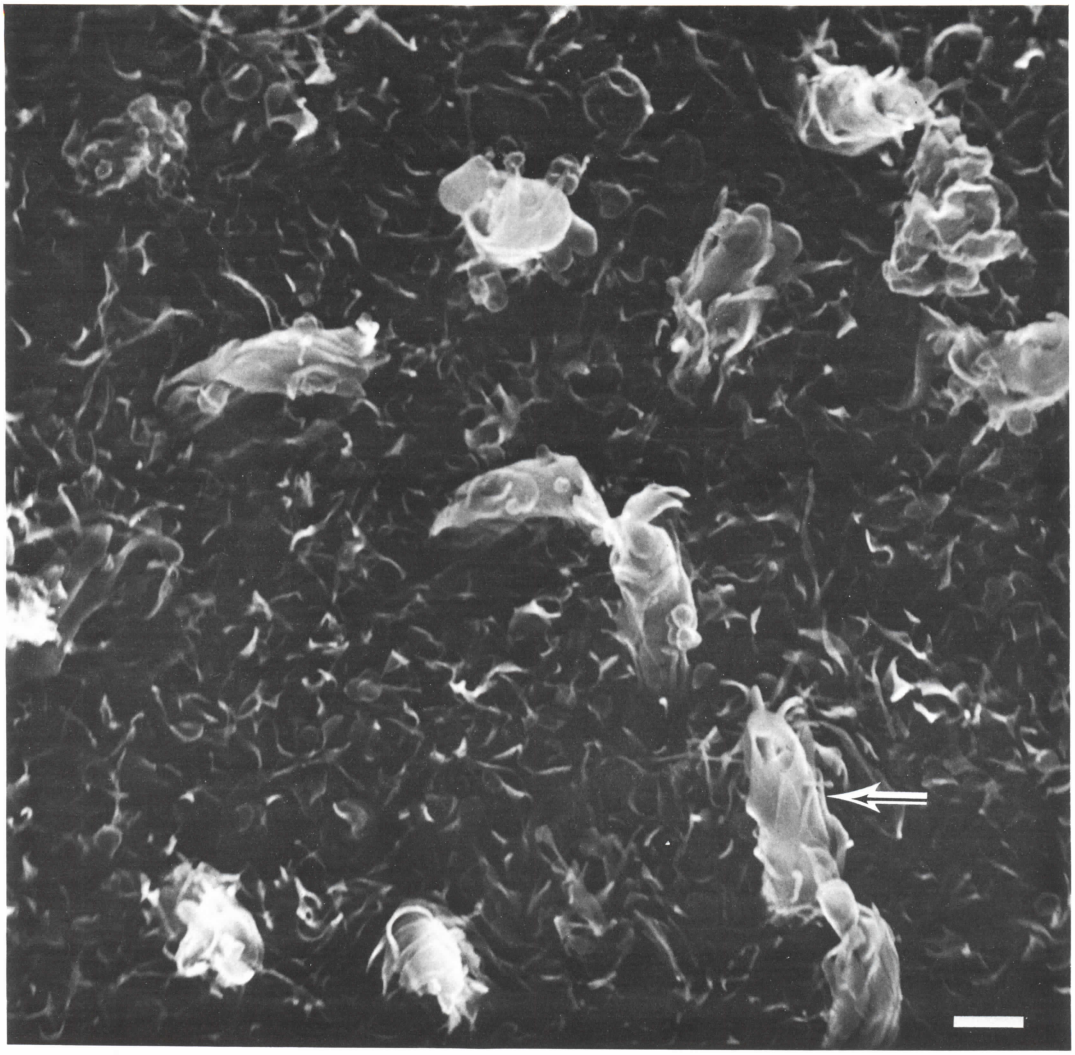

FIG. 1. Adult cat. SEM survey of apical RPE surface. The arrow indicates one of several cone sheaths extending from the apical surface. The surface itself is made up of extensive short cytoplasmic sheets and slender microvilli. The faint outline of a single RPE cell's borders can be seen in the center of the figure. Bar = 2 μm.

niques for SEM differ considerably from those used by Steinberg and Wood (1974), our first step was to study adult RPE prepared for SEM.

All the cone sheaths we observed consist of whorls of overlapping cytoplasmic sheets which envelop the outer segment, as described by Steinberg and Wood (1974). There is, however, a good deal more variability in the sheaths' forms as viewed by

FIG. 2. Adult cat. SEM stereopair. The cone sheath in the center of the field narrows at its base representing the supracone space. The tips of the leaflets forming the sheath have folded inward sealing off its interior. Shorter lamellae associated with ROS are in the background. One of these has a cup-like shape (long arrow) while another bifurcates (short arrow). Bar = 2 μm.

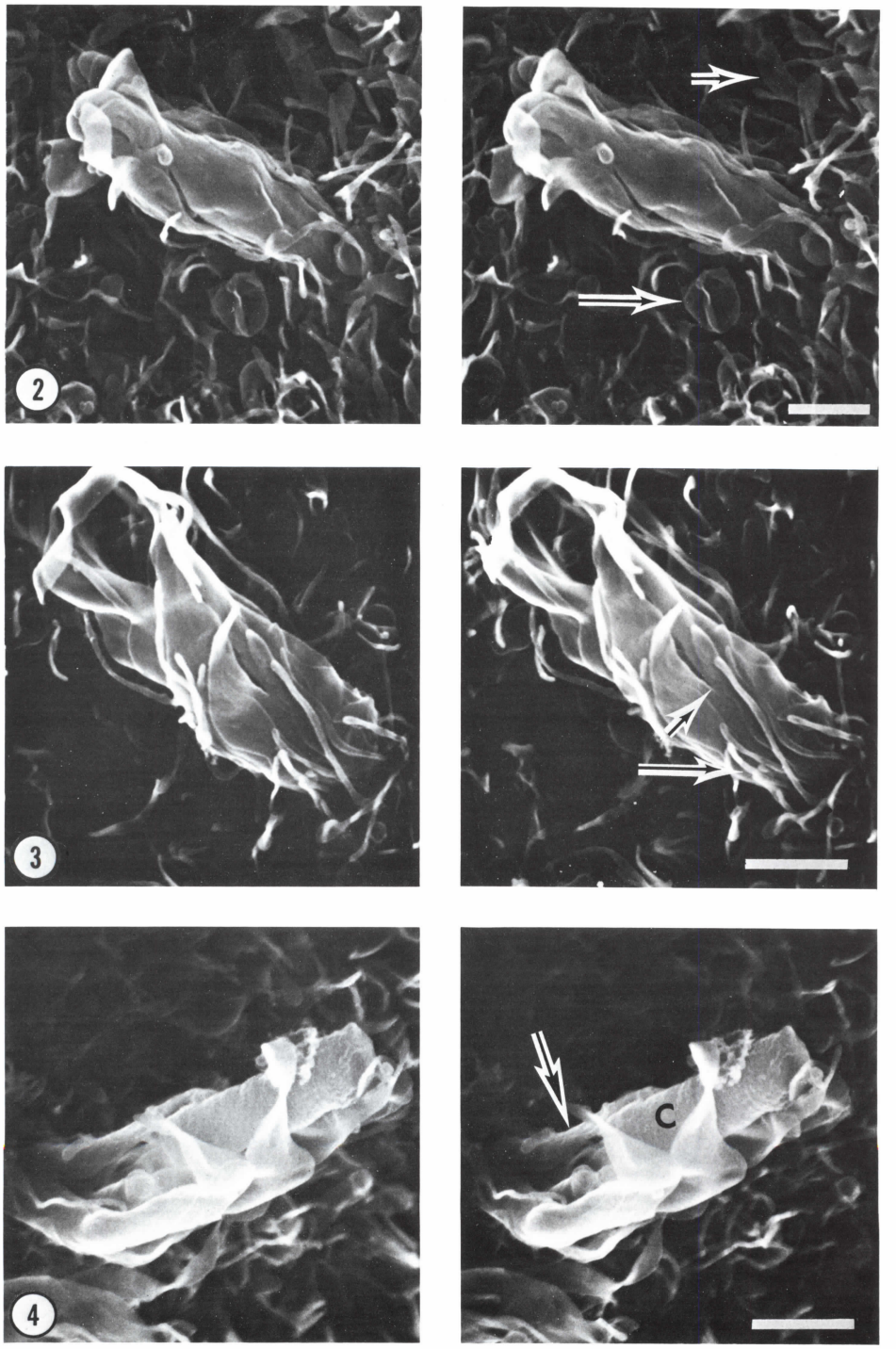

FIG. 3. Adult cat. SEM stereopair. This cone sheath has several long microvilli (long arrow) associated with it. Division of a cytoplasmic sheet into two leaflets is shown (short arrow). Bar = 2 μm.

FIG. 4. Adult cat. SEM stereopair. The COS (c) remains in this partially unravelled sheath. The supracone space (arrow) is visible between the tip of the COS and the RPE surface. Bar = 2 μm.

SEM (Figs. 1–4)[2] than was indicated by them. This variability has subsequently been observed in cone sheaths studied by serial section TEM (Fisher and Steinberg, submitted). The individual cytoplasmic sheets do not seem to be of either identical shape or dimension and usually the outer sheath lamellae are not broad enough to completely encircle the inner lamellae. Often a single sheet splits distally (Fig. 3) and each of the resulting leaflets continues to wrap around the more central sheets. A spiral pattern of organization is apparent within these sheaths. In a well-preserved sheath the individual sheets and their extensions taper at its open, distal end (Fig. 3). Often a sheath appears closed off due to the collapse of its distal end (Fig. 2). Other sheaths are partially unravelled, allowing observation of their internal, "cornhusk" (Steinberg and Wood, 1974) organization (Fig. 4). Occasionally all or part of a COS remains within the sheath (Fig. 4). The supracone space [i.e., the space between the tip of the COS and the apical surface of the RPE (Steinberg and Wood, 1974)] is seen as the slight narrowing of the entire sheath near its connection to the RPE cell perikaryon (Figs. 2, 4).

Along with the large cytoplasmic sheets, numerous slender microvilli extend from the RPE apical surface. Some are associated with the cone sheaths, extending as far as the apical tip of the sheath, while others extend only a fraction of this distance (Fig. 3).

In the adult cat the rod-associated apical projections are similar to, but smaller than, those comprising the cone sheath. Many of these processes branch into two or three "leaves" as do those of the cone sheath (Fig. 2). Some offshoots taper to a point and curl, while others widen into cup-like structures (Fig. 2). The "depressions" into which the ROS are inserted are usually obscured by the disarray of the processes resulting from tissue preparation.

Kitten Cone Sheath Development

A central-to-peripheral gradient of maturation exists within the eye for both retina and RPE (Johns *et al.*, 1979; Sidman, 1960; Vogel, 1978a,b). Thus, we preferentially examined the superior central region (within the tapetal area) of the RPE in each animal studied. Occasionally more peripheral regions were scanned in order to find well-preserved areas. Since it is possible to find developing cone sheaths in a variety of maturational stages, even within a relatively limited area of the same retina, the events in cone sheath morphogenesis described below are represented as spans of several days (rather than a single day) of postnatal age.

1–2 days. Prior to 12 days there is no hint of any structures resembling the cone sheath arising from the RPE apical surface. Just after birth, the apical surface is covered with a narrow zone (about 1.5 μm deep) of cytoplasmic processes. In tangential thin sections cut close to the apical border (Fig. 5) these appear as thin, branching ridges or sheets 0.02–0.04 μm wide. By SEM, the ridges are obscured by the long cylindrical microvilli, 0.1–0.2 μm in diameter, which dominate the interphotoreceptor space (Fig. 6). They appear more numerous and less organized than in the adult. Only rarely are broader, sheet-like processes seen by SEM. Irregularly shaped outer segments (Fig. 6) are inserted to varying depths in the microvillar zone. These observations correlate well with TEM pictures from the same stage of development, which show a random orientation of both outer and inner segments of photoreceptors (Vogel, 1978a,b). Within almost every outer segment, packets of discs lack orientation, contributing to the "boulder-like" ap-

[2] Figures presented here as stereoscopic pairs can be viewed best by placing the page on a flat surface in bright light and viewing with a "pocket" stereoscopic viewer (available from most electron microscopy supply firms). The viewer must be carefully centered and the interpupillary distance carefully adjusted for the proper stereoscopic effect.

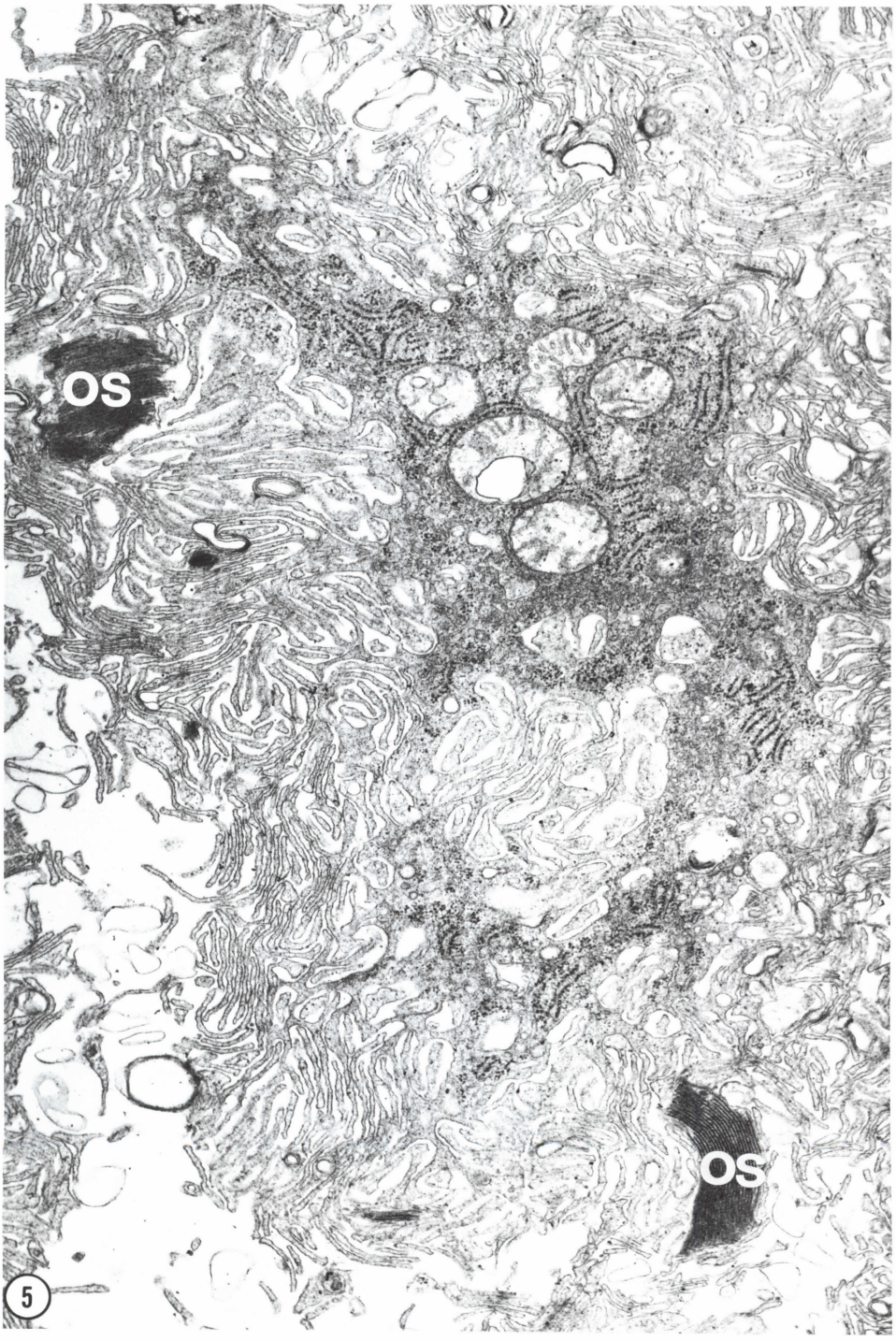

FIG. 5. Kitten RPE, 1 day postnatally. A tangential section cut very close to the RPE cell body shows that the surface is covered with numerous short, branching ridges. Because the cells have a mounded contour, a portion of the apical cytoplasm containing rough endoplasmic reticulum and mitochondria occurs in the center of the field. The tips of two outer segments (os) can also be seen amid the apical processes. × 20 000.

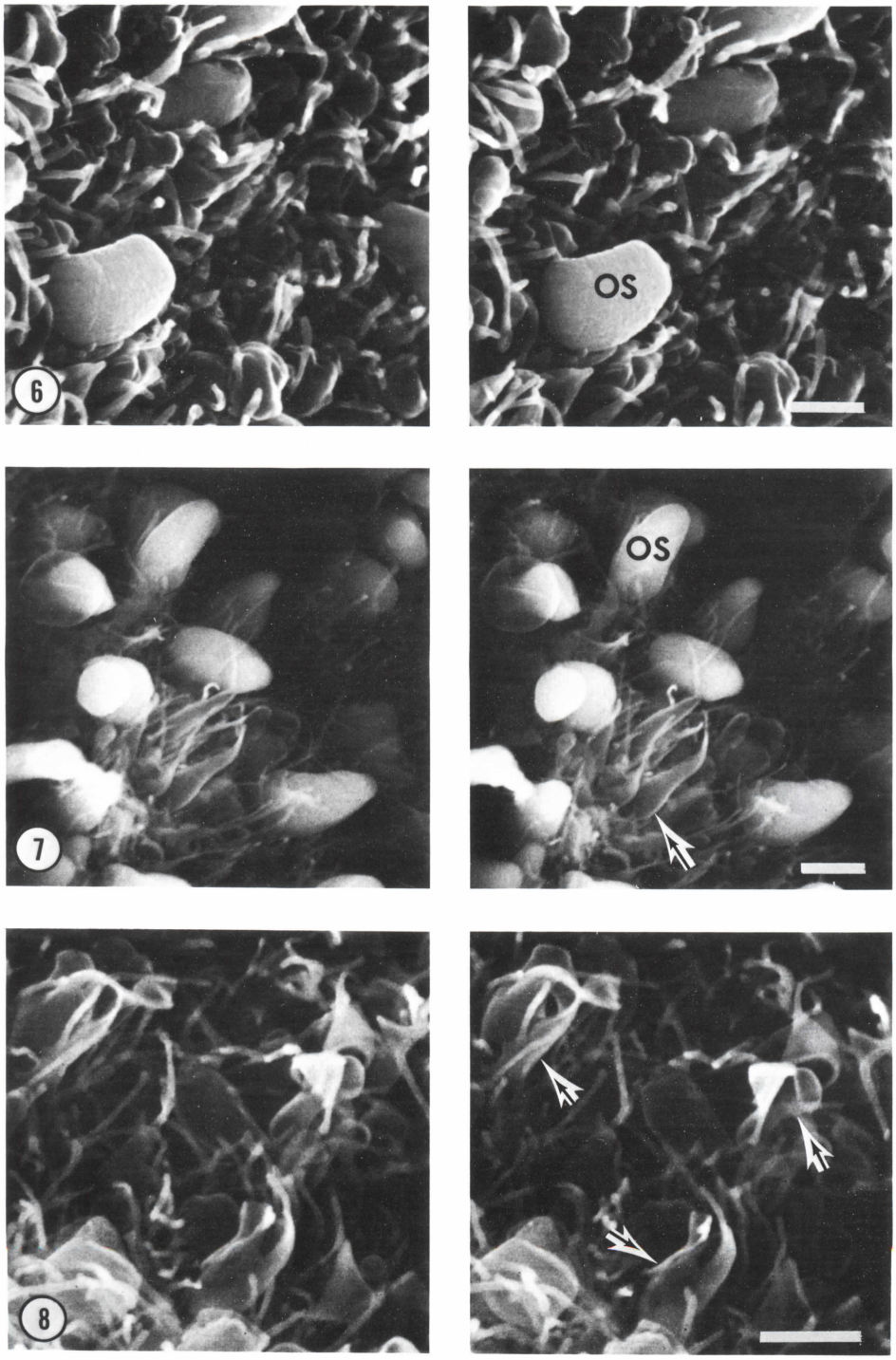

FIG. 6. Kitten RPE, 10 days postnatally. SEM stereopair. By SEM the apical surface of the RPE is dominated by cylindrical microvilli during the first 2 postnatal weeks. Much shorter lamellae can be seen close to the apical surface. The section in Fig. 5 is apparently through the latter. Several photoreceptor outer segments (os) adhere to the apical processes. Bar = 1 μm.

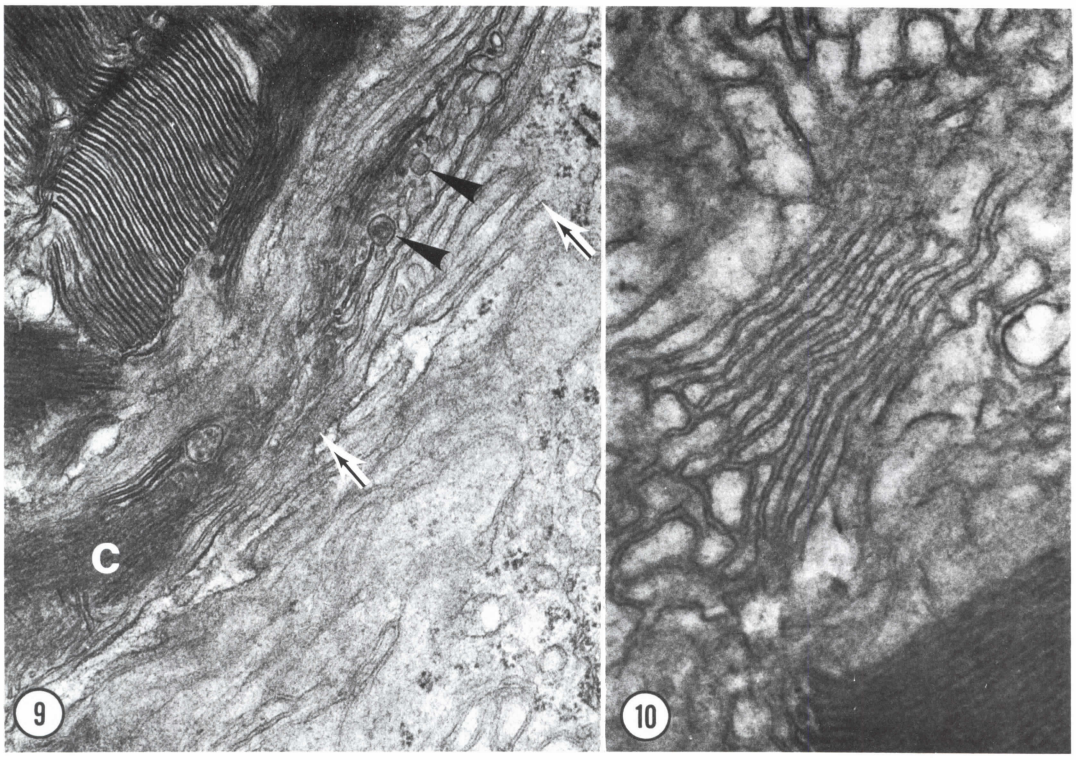

FIG. 9. Kitten apical RPE, 15 days postnatally. Section through the tip of a COS (c) and the supracone space (between arrows). Only two of the cone sheath processes extend past the tip of the cone. The portions of outer segments shown exhibit the disorientation of discs typical of the very young retinas. The inner processes within the supracone space contain several vesicles (arrowheads). × 50 000.

FIG. 10. Kitten apical RPE, 15 days postnatally. Tangentially oriented thin section very near the apical surface of the RPE. The collection of linear cytoplasmic sheets is the base of a cone sheath. × 48 000.

pearance of the outer segments seen by SEM (e.g., Figs. 6, 7, 9). Only later are the disc saccules oriented in parallel.

2 weeks. At 2 weeks the apical RPE surface remains dominated by microvilli, but some of these have lengthened, broadened laterally, and flattened (Fig. 7). Most of the morphogenetic events giving rise to the cone sheath occur near the RPE cell body and are inaccessible to SEM. In longitudinal sections (Fig. 9), both rod- and cone-associated RPE processes appear very similar. In Fig. 9, a cluster of these apical processes extends for about 7.0 μm alongside the outer segments. Neither the ROS nor the COS usually reach the apical surface of the RPE, so there is a supracone *or* -rod space above the distal tip of both types of outer segment. Numerous vesicles, 0.03–0.08 μm in diameter, some containing densely staining material, are associated with the innermost lamellae of the devel-

FIG. 7. Kitten RPE, 14 days postnatally. SEM stereopair. The apical surface consists mostly of microvilli. os = outer segment. Some tapering cytoplasmic sheets are grouped in a way suggestive of a developing cone sheath (arrow). Dehydration and critical-point drying via acetone. Bar = 2 μm.

FIG. 8. Kitten RPE, 16 days postnatally. SEM stereopair. Three groupings of leaflets which may be developing cone sheaths (arrows), barely reach beyond the apical microvilli. Dehydration and critical-point drying via acetone. Bar = 2 μm.

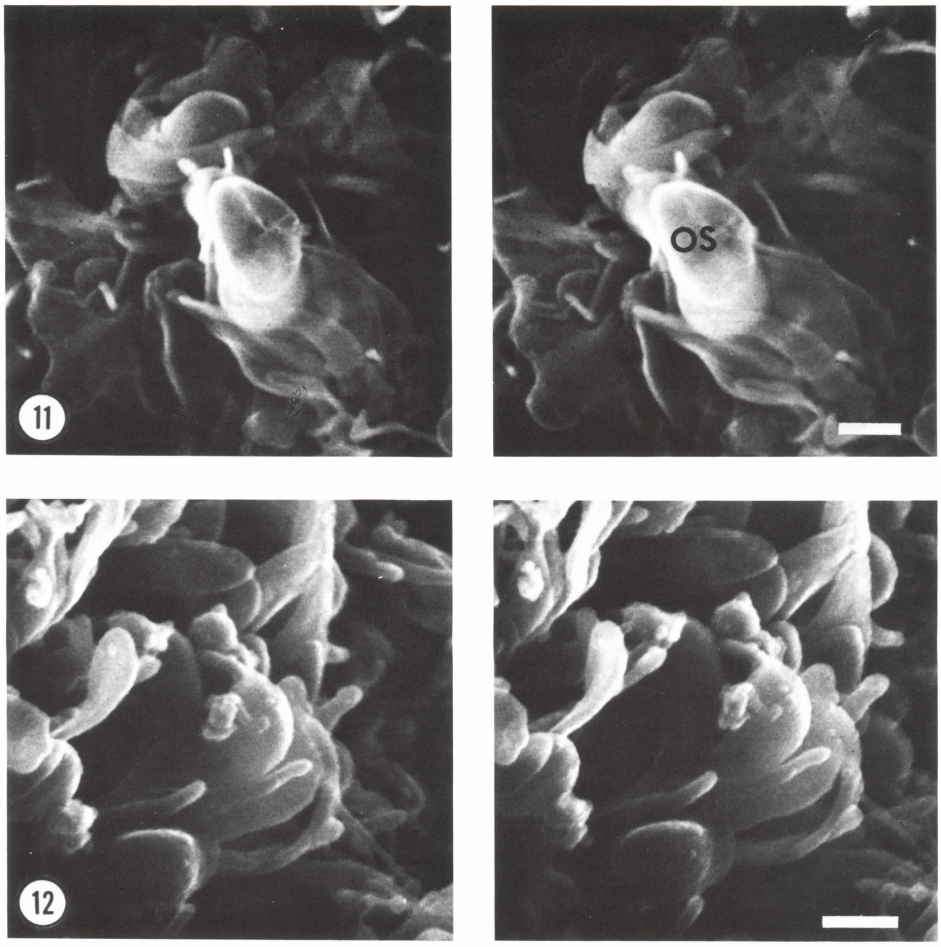

FIG. 11. Kitten apical RPE, 16 days postnatally. SEM stereopair. A developing cone sheath appears as an assemblage of lamellae surrounding a COS (os). Numerous rod-associated processes appear in the background. Dehydrated and critical-point dried via acetone. Bar = 1 μm.

FIG. 12. Kitten apical RPE, 21 days postnatally. SEM stereopair. An assembly of overlapping and branching cytoplasmic sheets, among individual rod-associated processes, forms a developing cone sheath. Bar = 1 μm.

oping cone sheath (Fig. 9). Tangential sections also illustrate the organization of the developing cone sheath. A grouping of about a dozen parallel, elongated, cytoplasmic sheets, each about 0.03 by 1.0 μm in dimension, is pictured in Fig. 10. This array is what would be expected from a plane of section oriented at right angles to the supracone space (see Fig. 9).

16–21 days. During the latter part of the third postnatal week, cone sheaths become recognizable by SEM as organized assemblages of broadened sheet-like processes. The sheaths barely extend beyond the numerous apical microvilli (Figs. 8, 11). The sheet-like processes tend to become dissociated during tissue processing (Fig. 8) because they are not yet broad enough to overlap substantially with each other. The branching of individual sheets at this stage is shown in Fig. 12. TEM (Figs. 13, 14) shows that the organization of the sheath has progressed considerably from the previous stage. Here the supracone space is laterally bordered by ROS, since most of them are now longer than the COS (Figs.

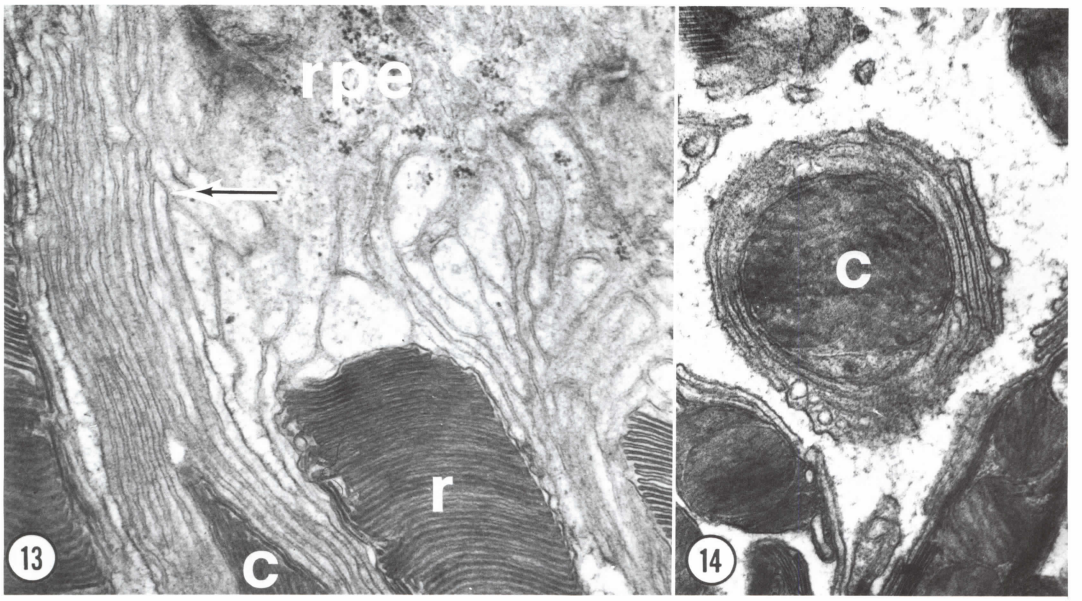

FIG. 13. Kitten apical RPE, 18 days postnatally. Thin section showing the tip of a cone (c) and a rod (r). Above the cone, the parallel lamellae of the cone sheath occupy the supracone space. One leaf of the cone sheath (arrow) does not connect to the apical surface in this plane of section. At this stage rods are longer than cones. Rod-associated processes show varied cross-sectional profiles. × 27 500.

FIG. 14. Kitten, 18 days postnatally. Thin section oriented tangentially to the long axis of outer segments shows the tip of a cone (c) surrounded by three to four layers of cytoplasm. × 27 000.

13, 15). In longitudinal thin sections the lamellae comprising the cone sheath are organized in closely packed arrays of parallel sheets, while those around the rods are more loosely packed and less parallel (Fig. 13). Tangential and longitudinal sections show that the apical portions of the COS are ensheathed in several concentric layers of cytoplasm and that the number of layers increases with age (Figs. 13–16). The basal portions of the COS are not yet ensheathed.

24–27 days. By the end of the fourth postnatal week the cone sheaths are obvious on the apical RPE surface by SEM. Their prominence is enhanced by the shortened rod-associated processes, the lengthened ROS, and the narrowed interphotoreceptor space.

A distinct supracone space is now visible by SEM as the tapering of the cone sheath near the RPE apical surface (Fig. 17). The processes forming the cone sheath overlap considerably, so that many sheaths appear intact, some even retaining an outer segment broken off during tissue preparation (Fig. 17).

30–40 days. Between 4 and 6 postnatal weeks the cone sheath has a morphology similar to the adult. At this time the supracone space is thicker, the cone sheath lamellae are more overlapped, and the ROS approach adult length (Fig. 18). Besides the cone sheaths, the apical surface of the RPE is covered with tapering sheets and microvilli which encircle the distal tips of ROS.

DISCUSSION

The adult cone sheath of the cat retina is a remarkable specialization of the apical membrane of the RPE cell. We have described the morphological changes in the RPE apical processes which form this structure during postnatal development. At the earliest stage, a fringe of numerous,

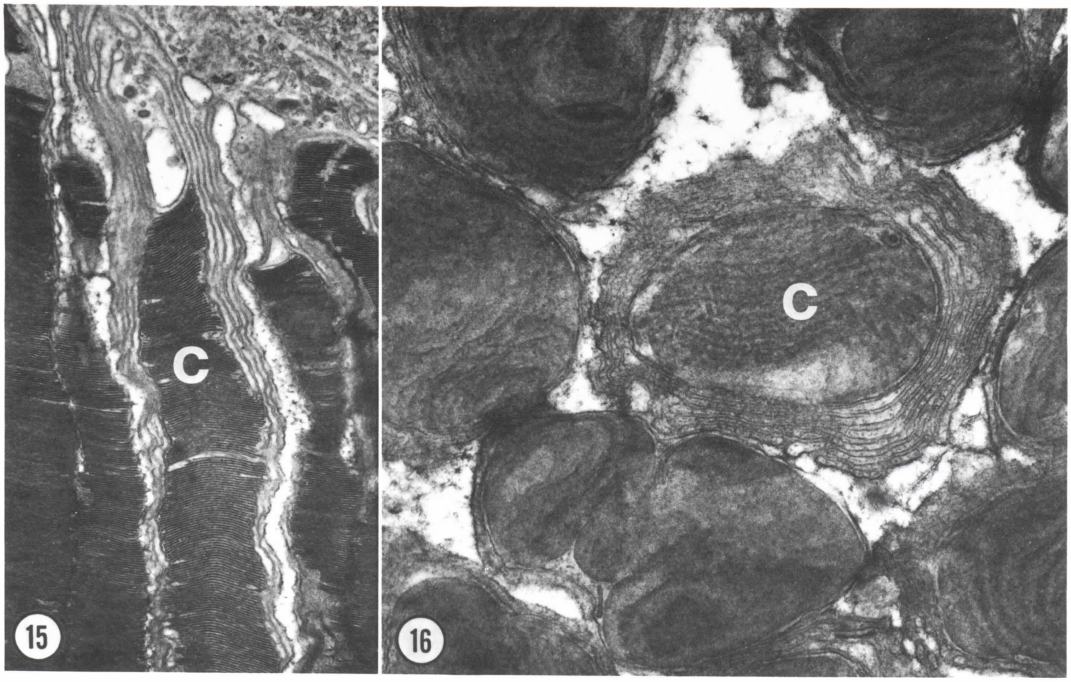

FIG. 15. Kitten, 21 days postnatally. A longitudinal thin section shows a COS (c) ensheathed for its entire length by RPE apical processes. × 12 000.

FIG. 16. Kitten, 21 days postnatally. A section 90° to that in Fig. 15 shows that the COS (c) are ensheathed by up to six layers of cytoplasm. The COS is surrounded by ROS. × 27 000.

small cytoplasmic processes covers the RPE apical surface and extends into the interphotoreceptor space. By using both TEM and SEM, we determined that these are thin, branching ridges close to the apical surface. By SEM, these ridges are largely obscured by the numerous apical microvilli, which are structurally identical to those described in other adult retinas (Hansson, 1970; Hebel, 1971; Steinberg *et al.*, 1977). The presence of microfilaments is believed to be a general property of RPE apical processes and microvilli (Burnside and Laties, 1976).

Significant events in sheath morphogenesis occur around 2 weeks after birth. This is supported by the nearly adult structure of cone sheaths seen at the next stage (16–21 days). In well-preserved tissue for TEM the flattened sheets which envelope both ROS and COS at 2 weeks are ultrastructurally similar to the microvilli, inasmuch as they contain an internal microfilamentous cytoskeleton but few other organelles (Steinberg and Wood, 1974; Fisher and Steinberg, submitted). In tangential sections the aggregation of several parallel lamellae (as in Fig. 10) represents the specialization of RPE processes associated with the adult cone sheath (Fisher and Steinberg, submitted). Numerous vesicles found in the developing cone sheath may reflect rapid growth and proliferation of cytoplasmic processes occurring at this stage. The growth of cell membranes by the fusion of vesicles to plasma membrane has been postulated to occur at the growth cone during neurite elongation (Bunge, 1973; Skoff and Hamburger, 1974) and at the photoreceptor inner segment during outer segment elongation and renewal (Kinney and Fisher, 1978; Besharse and Pfenninger, 1980).

During the third postnatal week, both the

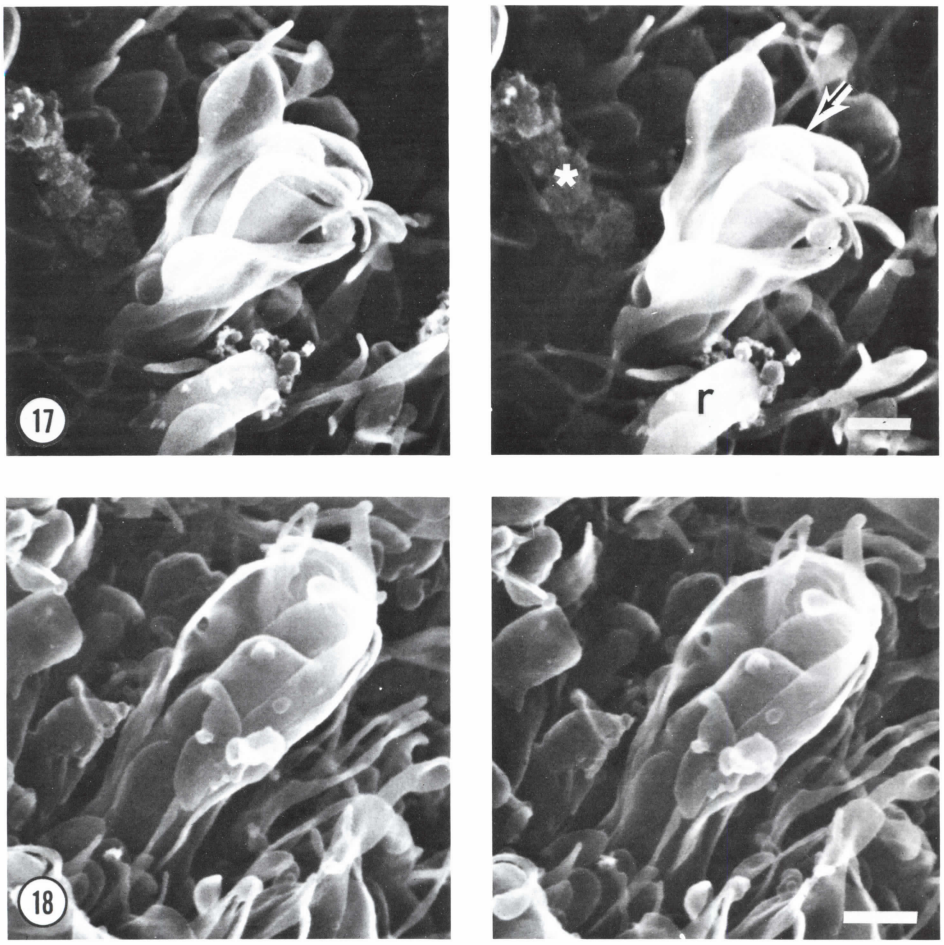

FIG. 17. Kitten apical RPE, 27 days postnatally. SEM stereopair. By this stage the base of the sheath is visible and its outer lamellae are very broad. The COS (arrow) can be seen within this sheath. A ROS (r) appears below the sheath. Asterisk indicates interphotoreceptor matrix adhering to the surface of the tissue. Bar = 1 μm.

FIG. 18. Kitten apical RPE, 30 days postnatally. SEM stereopair. An adult-like cone sheath extends well beyond the shorter rod-associated processes. Bar = 1 μm.

sheath and the supracone space are first recognizable by SEM. Both are manifestations of the elongation of the ROS, and the shortening and winnowing of rod-associated RPE processes. During this time the interphotoreceptor space decreases and the diameter of ROS increases (Tucker *et al.*, 1979). The extent of ROS ensheathment is still greater than in the adult RPE since there is extensive wrapping of ROS as far vitread as the COS. In adult cats, a transverse section just distal to the COS tip rarely shows rod-associated processes. The cone sheath at this stage has nearly the same number of lamellae as in the adult. At this and later stages, many cone sheaths have outer sheets that bifurcate in the supracone space, explaining why some of the lamellae bordering the COS in longitudinal sections appear to lie free in the interphotoreceptor space.

While in the third week the individual cytoplasmic leaflets of the sheath can often be seen by SEM, at 4 weeks the sheaths

are more developed and their lamellae more tightly wrapped. The apical processes generally appear adult-like at about 30 postnatal days, the same time the ROS and COS attain their adult length and form (Vogel, 1978a,b; Tucker *et al.*, 1979). There is, however, continued addition of lamellae to the cone sheath between 30 and 40 postnatal days.

As the kitten retina matures, the manner by which RPE processes ensheath the distal tips of the rods changes. During earlier stages, the rod "sheath" may be several lamellae thick. But the lamellae become narrower and more tapered until several thin, narrow, sheets form a single layer around the ROS tip as described in the adult retina by Steinberg and Wood (1974).

Many functions have been speculated for the cone sheath in the cat (see Steinberg and Wood, 1974), but the only proven function is that of phagocytosis (Anderson *et al.*, 1978). If Steinberg *et al.* (1977) are correct in their model for the mechanics of phagocytosis in cone sheaths (using the human cone sheath as a model, their p. 469), the innermost layers of the sheath are directly involved in the phagocytic process. Thus, it is clear that the central sheath process utilizes its membrane in the engulfment process. This membrane is then subsequently lost during degradation of the phagosome. How this membrane is replaced is also unknown. The fine structure and ontogeny of the sheath suggests two possible mechanisms. Either the phagocytosing sheet is constantly rebuilt by the addition of new membrane [as in Fig. 50 of Steinberg *et al.* (1977)], or is laterally replaced by elaboration of new lamellae at the sheath's perimeter. This dynamic loss and elaboration of membrane by the RPE may be analogous to the phenomenon of "ruffling" described for various motile cells *in vitro* (Abercrombie *et al.*, 1970, 1971).

It has been observed here and in other retinas that apical microvilli tend to occupy most of the interphotoreceptor space in the earliest developmental stages (Weidman and Kuwabara, 1968; Braekevelt and Hollenberg, 1970; Caley *et al.*, 1972; Feeney, 1973). These apical microvilli could function in several different ways. *In vitro* studies have demonstrated that reaggregation of retinal cells is reduced under experimental conditions which decrease the number of filopodia on the cell surface (Ben-Shaul and Moscona, 1975); the RPE microvilli are structurally similar to the filopodia seen on cells *in vitro* and thus may play a role in cell recognition and adhesion during development of the photoreceptor–RPE interface. They could also be a reserve source of membrane (Trinkhaus and Erickson, 1974) to be utilized indirectly in the expansion of the sheet-like processes, or directly by transformation into cytoplasmic sheets. Various cell-surface specializations (filopodia, lamellipodia, lobopodia, and surface blebs) are thought to be capable of transforming interchangeably into each other (Trinkhaus and Erickson, 1974; Albrecht-Buehler, 1976; Dipasquale, 1975). Indeed, we observed that some of the microvilli of 2- to 3-week-old kittens display flattening as if they were in the process of transforming into sheets.

Surface structures similar to RPE microvilli (filopodia) have also been proposed to be sensory specializations mediating the extension or retraction of other adjacent cytoplasmic processes (Albrecht-Buehler, 1976). Perhaps a microvillus in contact with a COS mediates the expansion of adjacent (or its own) surface membrane into a cytoplasmic sheet. Whether or not microvilli play this role, some kind of interaction—effecting development of the sheath—transpires between that portion of the RPE cell apposing the cone and the COS itself. Moreover, it has been demonstrated here that Steinberg and Wood (1974) were correct in concluding that rod- and cone-associated processes are equivalent structurally up to a certain age, after which they differentiate in distinct ways depending on the type of photoreceptor contacted. It is not simply a matter of COS being shorter

than ROS, since we have found that differentiation of the cone sheath commences at a stage when the COS and ROS are not significantly different in length.

Interactions similar to those postulated above have been proposed for the ensheathment of CNS axons in myelin (Brady and Quarles, 1973; also see Steinberg and Wood, 1974). Similar mechanisms may operate during development of the individual cone sheath lamellae and the myelin sheath as each "fans out" from its cytoplasmic connection with the cell body (Bunge *et al.*, 1962). Moreover, the scheme proposed for CNS myelination, in which an inner tongue of cytoplasm wraps around the axon continually insinuating itself beneath the previously formed layers (Raine, 1977), would also be a plausible scheme for the genesis of the individual whorls of cone sheath lamellae. The sheath, in striking contrast to myelin, is formed from several lamellar processes which arise independently from the RPE surface and encircle the COS.

From about 15–30 postnatal days, when the cone sheath undergoes its period of significant growth, other anatomical, physiological, and behavioral parameters of the kitten's visual system are also experiencing dramatic maturation. For example, most kittens yield a positive visual orienting response at 21 days, while visual pursuit is detectable slightly later, at 24 days (Norton, 1974). ROS reach about 95% of their adult length at 30 days and full length at 45 days (Tucker *et al.*, 1979). On the other hand, ROS diameter reaches that of the adult at 20 days, with dramatic growth during the 3 preceding days. This corresponds to the stage exhibiting diminution of both rod-association RPE processes and interphotoreceptor space.

Although there are still no specific physiological roles (other than phagocytosis) known for the elaborate cone sheaths found in a few mammalian retinas, it seems clear that whatever its role, the anatomical relationship between COS and the RPE processes is probably vital for normal visual functioning. At least we have established here that several maturational events, including maturation of the cone sheath, occur at about the same time in development of the kitten eye.

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