Retinal Detachment in the Cat: The Pigment Epithelial-Photoreceptor Interface

Don H. Anderson,* Walter H. Stern,† Steven K. Fisher,* Page A. Erickson,* and Gerard A. Borgula‡

Twenty-six cat retinae were surgically detached by injecting fluid into the subretinal space (SRS). The retinae were then studied by light and electron microscopy at detachment intervals ranging from 1/2 hr to 14 months. Degenerative and proliferative changes occur at the retinal pigment epithelial (RPE)-photoreceptor interface very soon after detachment, and the severity of these changes depends upon both the duration and height of the detachment. The specialized apical RPE processes that ensheath the outer segments are replaced by a uniform fringe of short, undifferentiated processes. The apical RPE surface becomes mounded, and this mounding becomes more pronounced at longer detachment durations. Labeling experiments with ^{3}H-thymidine showed that some cat RPE cells enter a phase of stimulated DNA synthesis 12–24 hrs after detachment; RPE mitotic figures are first apparent 48 hrs after detachment. In the cat, discrete regions of proliferated RPE cells usually appear in one of several configurations. A number of different cell types, including polymorphonuclear neutrophils, monocytes at various maturational stages, photoreceptor cells, Müller cells, and RPE cells, appear in the expanded SRS of detached retinae. Rod and cone outer segments degenerate rapidly and become membrane bound sacs by 3 days postdetachment; the assembly of new outer segment membrane apparently does not stop completely even at moderately long detachment intervals (ie, 2 months). Degenerative changes in the inner segments do not take place with the same rapidity as those in the outer segments. The changes that occur at the RPE-photoreceptor interface are rapid, progressive, and sometimes irreversible events that have significant implications for photoreceptor recovery following retinal reattachment surgery. Invest Ophthalmol Vis Sci 24:906–926, 1983

The retinal pigment epithelium (RPE) mediates the transfer of ions and metabolites between the choroidal capillaries and the neural retina.[1] When the retina is detached from the RPE and this intercellular relationship is altered, morphologic changes occur in the RPE cells, the photoreceptors, and in other retinal cells as well. Some of these changes are proliferative in that certain cells that, under normal conditions, are mitotically inactive begin to divide[2]; and some changes are degenerative, particularly within the neural retina, because cell death ensues if the two tissues are not reapposed.[3]

There have been numerous attempts over the years to develop experimental animal models that have the characteristics of human retinal detachments. Of these, the investigations by Kroll and Machemer in the owl monkey (*Aotes trivirgatus*) retina remain the most comprehensive[4–7] despite the fact that they were completed over a decade ago. The normal interactions between the RPE and retina, and their significance for retinal function, are more clearly defined now. New information has been added with respect to the metabolism of the RPE and photoreceptors,[1] the process of outer segment renewal in rods and cones,[8–11] and the morphology of the outer segment-RPE interface.[12–16]

These findings, and a consideration of their implications, provided the impetus for our re-examination of experimental detachment and reattachment. We selected the cat retina because it has several desirable features that distinguish it from other species used in previous studies. In contrast to the nearly all-rod owl monkey retina, the cat retina has a moderate number of easily identified cones and an area centralis similar in several anatomical respects to the primate fovea.[13,15] Unlike the rabbit and owl monkey retinae, the vasculature of the cat retina is very similar to the human retina in that it extends to the inner border of the outer plexiform layer.[17] Furthermore, the physiology and morphology of the RPE, photo-

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receptors, and retinal neurons in the cat have been, and continue to be, studied intensively.

In this paper we describe the degenerative and proliferative changes that occur at the RPE-photoreceptor interface of the cat retina at detachment intervals ranging from 1/2 hour to 14 months.

Materials and Methods

Animals

The eyes from 26 cats with unilateral rhegmatogenous retinal detachments were fixed at: 0.5, 1.0, 12, 24, 48, and 72 hrs; 6, 13, 22, 30, 50, 70, and 83 days; and at 3, 6, and 14 months after detachment. All of the animals were maintained on a (12L:12D) lighting cycle during the course of the study.

Surgery

Cats were anesthetized first with an intramuscular injection of ketamine hydrochloride (Bristol, 50 mg/ml), and maintained under deep anesthesia with intravenous sodium pentobarbitol (Nembutal, 50 mg/ml). In addition, a retrobulbar injection of 0.5 cc of 2% Xylocaine was given. Extracapsular cataract extraction was performed through a 180° corneal incision, and was followed by excision of the posterior capsule and a partial open-sky vitrectomy. The cornea was then sutured closed and the eye was allowed to heal for several weeks. In the second stage of the surgery, the posterior vitreous cortex was removed using a vitreous suction cutter, and the cutter was used to place a small retinal hole in the superior nasal retina. The suction cutter was replaced with a curved needle through which lactated mammalian Ringer's solution or, in several cases, a 0.5% aqueous solution of Healon® (sodium hyaluronate) was slowly injected into the subretinal space (SRS) using a mechanical drive syringe. This procedure resulted in a bullous retinal detachment that radiated outward from the retinal hole toward the optic disc.

Transmission Electron Microscopy

Animals were fixed by intracardiac perfusion of 1% glutaraldehyde and 1% paraformaldehyde in phosphate buffer (pH 7.1). After perfusion, the eyes were enucleated, the anterior one-third of the globes were excised, and the eye cups were immersed overnight in the aldehyde mixture. The following morning the specimens were washed in phosphate buffer (plus sucrose at 45 mg/ml), postfixed in veronal acetate buffered osmium tetroxide (2%), dehydrated in a graded ethanol and H_2O series, and embedded in Araldite (6005).

Autoradiography

Two hundred microcuries of ^{3}H-thymidine (50 mCi/mmol specific activity) in 0.2 cc of phosphate buffer was injected intraocularly into control eyes, and into eyes that had been detached for specific intervals. All of the eyes were injected in the middle part of the light cycle in order to control for diurnal fluctuations in the cell cycle. After an incubation interval of 3 hrs the eyes were enucleated, the anterior one-third of the globe was dissected away, and the eye cup was fixed by immersion as described above. Light microscopic autoradiograms were prepared from 1 μm tissue sections that were dipped in a 1:1 solution of Kodak NTB-2 and distilled H_2O maintained at 41 C. The slides were exposed from 5–10 days at 4 C, developed for 2 min in full strength D-19 (at 20 C), washed, fixed, and stained with methylene blue-azure II or with 1% basic fuchsin in a 1:1 solution of ETOH and H_2O.

Scanning Electron Microscopy

Specimens consisting of sclera, choroid, RPE, and retina were pinned to the surface of a dissecting dish and immersed in phosphate-buffered solution. The retina overlying the detached area was carefully peeled away thereby exposing the apical RPE surface. Then the RPE-choroid-sclera complex was fixed by replacing the phosphate buffer with the glutaraldehyde- formaldehyde mixture such that the meniscus never fell below the level of the tissue. Specimens were postfixed in three to five changes of phosphate-buffered 2% osmium tetroxide alternating with phosphate-buffered tannic acid (1–5%). The samples were then dehydrated in tertiary-butyl alcohol and ethanol, transferred to amyl acetate, and critical point dried in a CO_2 chamber. Finally, the tissue was coated with a 10 nm layer of gold-palladium alloy in a vacuum evaporator before examination in a JEOL JSM-2 scanner.

Light Microscopic Staining Techniques

Thick (1 μM) sections were stained for light microscopic examination using a saturated aqueous solution of paraphenylenediamine (PPDA) or 1% basic fuchsin in a 1:1 solution of ETOH and H_2O. Araldite-embedded sections (1 μm) were stained for the presence of collagen using Humphrey and Pitman's trichromatic stain.[18] Using this technique, collagenous material stains pink to red, elastin appears violet, and fibrin is stained blue.

After removing the araldite from 1 μM sections by incubating in a solution of saturated NaOH for 2–5 min, sections (taken from animals at different de-

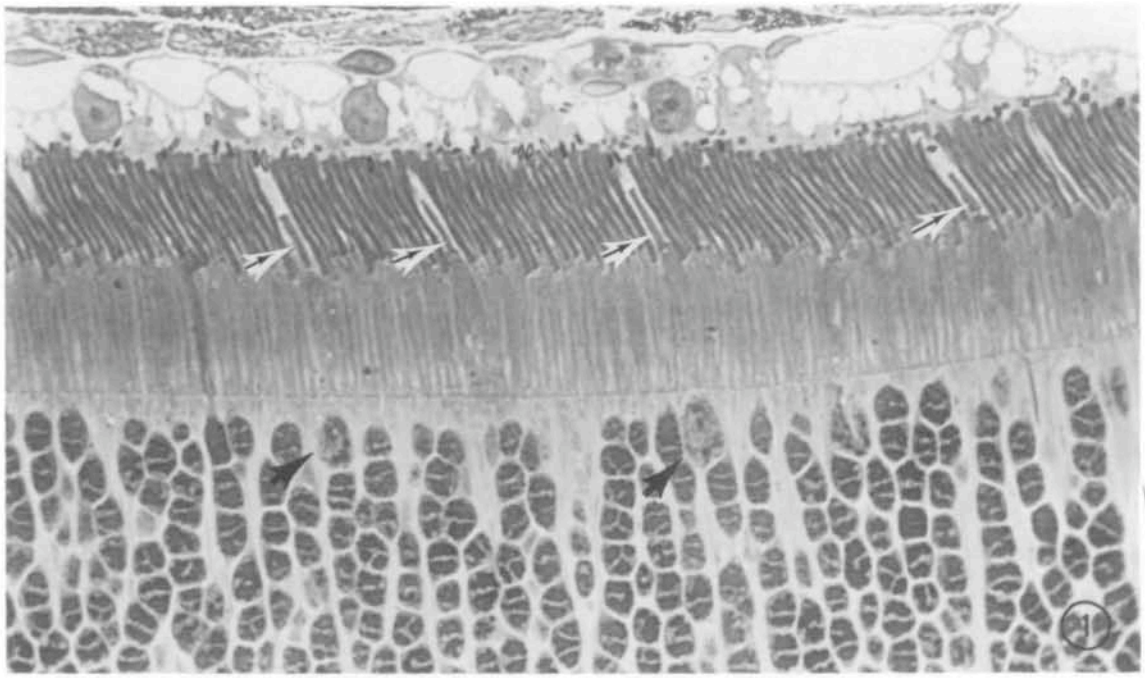


Fig. 1. A pigmented area from the posterior retina in a lensectomized and vitrectomized cat eye. The RPE and photoreceptors are normal. The cone outer segments (arrows) are somewhat shorter than rod outer segments, with the cone sheaths occupying the space between the outer segment tips and the apical RPE surface. Cone nuclei (black arrows) always lie just vitreal to the outer limiting membrane (×720 phase contrast).

tachment intervals) were stained with PAS using procedures modified from DiBella and Hashimoto[19] and Litwin and Kaspryzyk.[20]

Retinal Tissue Examined

Tissue specimens were examined by light and electron microscopy from several different retinal regions: (1) normal areas from the opposite nonoperated eyes (Fig. 1); (2) normal-appearing, nondetached areas from the detached retinae; (3) transition zones between detached and attached regions; (4) shallow detachments arbitrarily defined as a separation between retina and RPE of less than three retinal thicknesses; and (5) high detachments greater than three retinal thicknesses.

Results

The Cat Retina

The morphologic features of the photoreceptors and RPE of the cat retina have been described in a number of previous studies.[13,15-16,21] Here, we only need to note that the cat has a tapetum cellulosum located in the superior retina that terminates just above the midline; below the midline the RPE-choroid is usually pigmented. It has a duplex retina with a maximum cone density (26,000–27,000/sq mm) in the area centralis,[21] which is located just nasal and superior to the optic disc. The cone outer segments are recognized easily in the light microscope because they are shorter than rod outer segments, and they are ensheathed by a highly organized array of microvillous and sheet-like processes, termed the cone sheath,[13] which emerge from the apical RPE surface (Fig. 1).

The Ciliary Epithelium

Neither the pigmented nor nonpigmented ciliary epithelia showed any morphologic changes in response to detachment in the adjacent retina. A few cells of unknown origin bordered the ora serrata and ciliary epithelium in the vitreous cavity. In autoradiograms adjacent to detached regions of 12, 24, or 48 hrs duration, labeled nonpigmented epithelial nuclei were very rare; no labeled pigmented nuclei were found at the times sampled.

The Pigment Epithelial Cells

One day postdetachment: One of the earliest detectable histologic changes following detachment takes place at the apical RPE surface. The distal tips of rod outer segments and all of the cone outer segments are normally ensheathed by apical processes 5–15 μM in length. As early as 1–2 hrs following detachment surgery, these complex surface special-

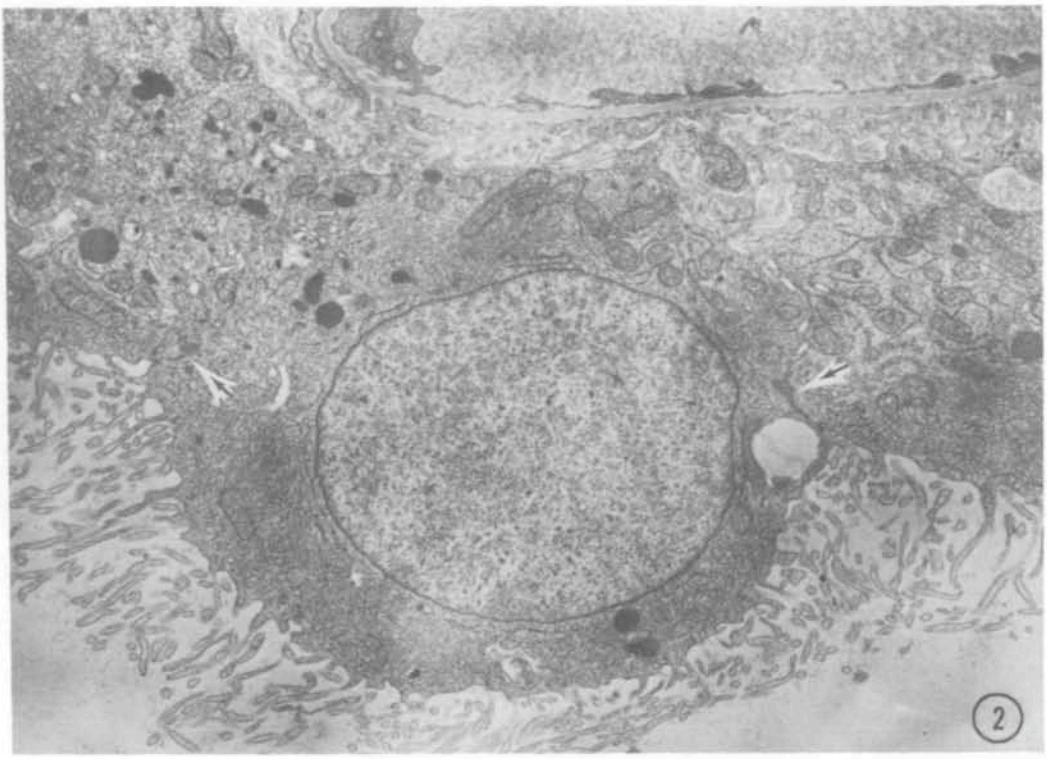


Fig. 2. The RPE 1 day after detachment. In comparison to normal RPE cells, the apical surface is slightly mounded. The sheet-like apical projections that normally ensheath the outer segments have been replaced by a homogeneous fringe of short, microvillous processes. In this particular cell, the nucleus is displaced into the mounded region. The cell's lateral junctions are indicated by arrows (×6200).

izations, including the cone sheaths, are replaced by a "fringe" of microplicae about 5 μM in length (Fig. 2). RPE cell organelles and the junctional complexes between adjacent RPE cells remain unaffected at this stage. There is no evidence of RPE proliferation up to about 12 hrs after detachment.

One to three days postdetachment: The shape of the RPE cells' apical surface changes dramatically about 24 hrs after detachment. The formerly flat surface appears mounded (Fig. 2), and this mounding becomes more pronounced as detachment duration lengthens (Figs. 3, 4). This is sometimes accompanied by a decrease in the amount of basal surface apposed to Bruch's membrane, and by migration of the nucleus toward the apex of the mound.

Pulse labeling experiments with ^{3}H-thymidine showed that some RPE cells enter a phase of stimulated DNA synthesis between 12 and 24 hrs after detachment. By 48 hrs the number of labeled nuclei per mm of RPE increases substantially, and RPE mitotic figures are present at both 48 and 72 hrs postdetachment (Fig. 5). Studies in rabbits with traction retinal detachments induced by intraocular injection of cultured RPE cells[22] confirmed that ^{3}H-thymidine labeled RPE nuclei are first apparent about 24 hrs after detachment.

By 72 hrs, the monolayer of RPE cells is occasionally interspersed with small areas of hyperplasia (Fig. 6). Such areas are particularly common at transition zones between detached and attached retina (Fig. 7). The daughter cells are joined by adhering junctions to each other and to cells in the original monolayer. They lack the apical-basal polarization that is characteristic of normal RPE cells. Instead, their entire surface is lined with many short processes that interdigitate with similar processes from adjacent cells. There is no evidence of any extracellular matrix or basement membrane associated with the proliferating cells at this time. Their organelle content is not different from normal RPE cells (except for phagosomes) or from those cells occupying the original monolayer. In contrast to the spherical shape of normal RPE nuclei those of the daughter cells, as well as some in the monolayer of detached regions, are lobulated (Fig. 6).

In pigmented regions of the inferior retina, the melanin distribution within the daughter RPE cells is irregular. Some cells have few or no pigment granules in their cytoplasm, while others are hyperpigmented. In areas of hyperplasia both unpigmented and pigmented cells intermingle. Autophagic vacuoles containing melanin and other material are pres-

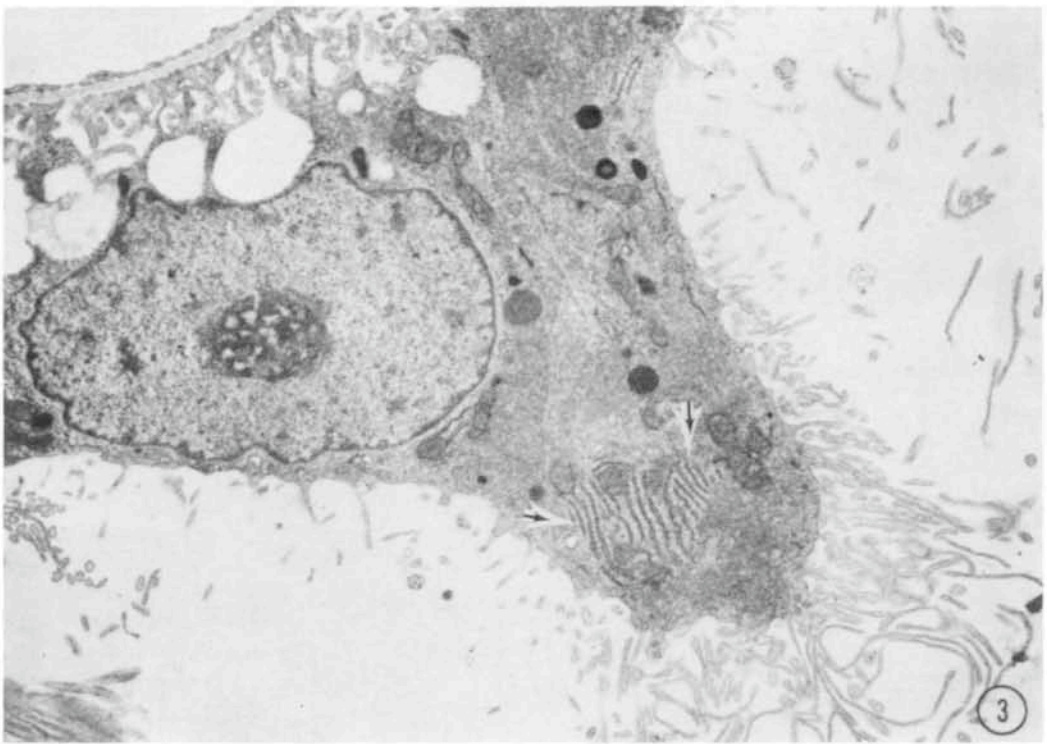


Fig. 3. The RPE 3 weeks after detachment. At 3 weeks, the mounding is more pronounced than it is after 1 day. The microvillous processes are longer near the apex of the mound. The nuclear border is indented and stacks of rough-surfaced endoplasmic reticulum (arrows), not present in normal RPE cells, appear in the apical cytoplasm (×6000).

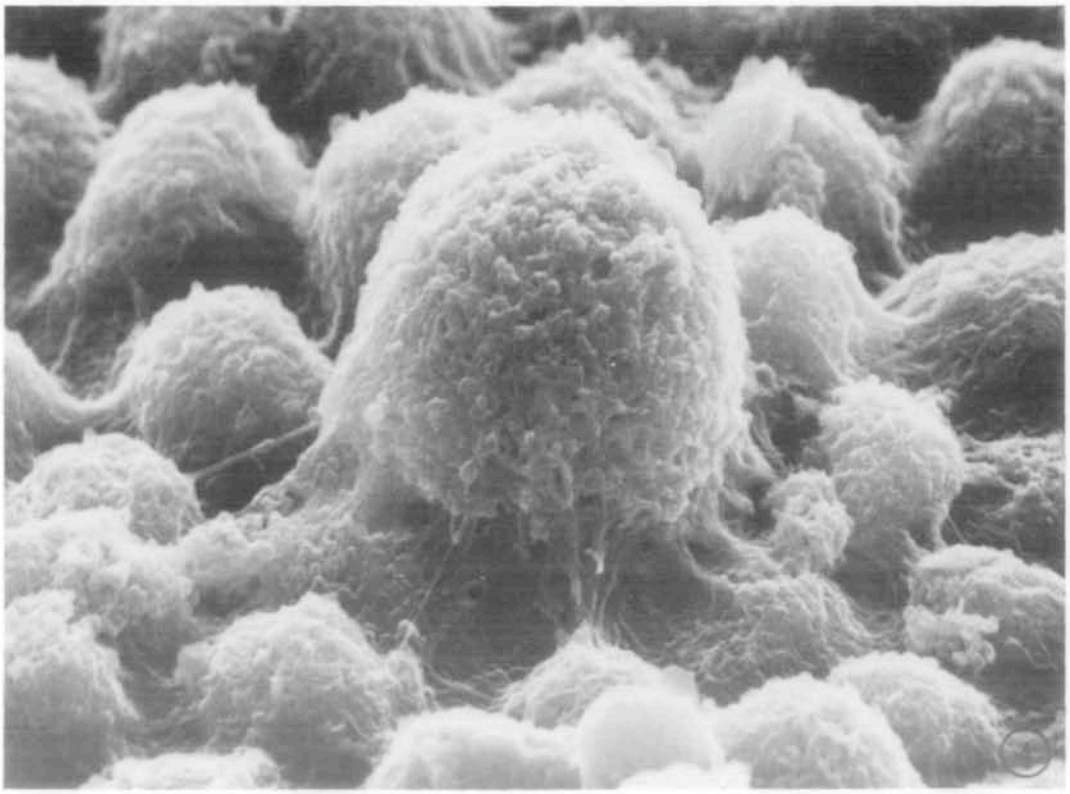


Fig. 4. The RPE apical surface 6 weeks after detachment. This scanning electron micrograph shows the pronounced mounding of the RPE cells from a different perspective (×4800).

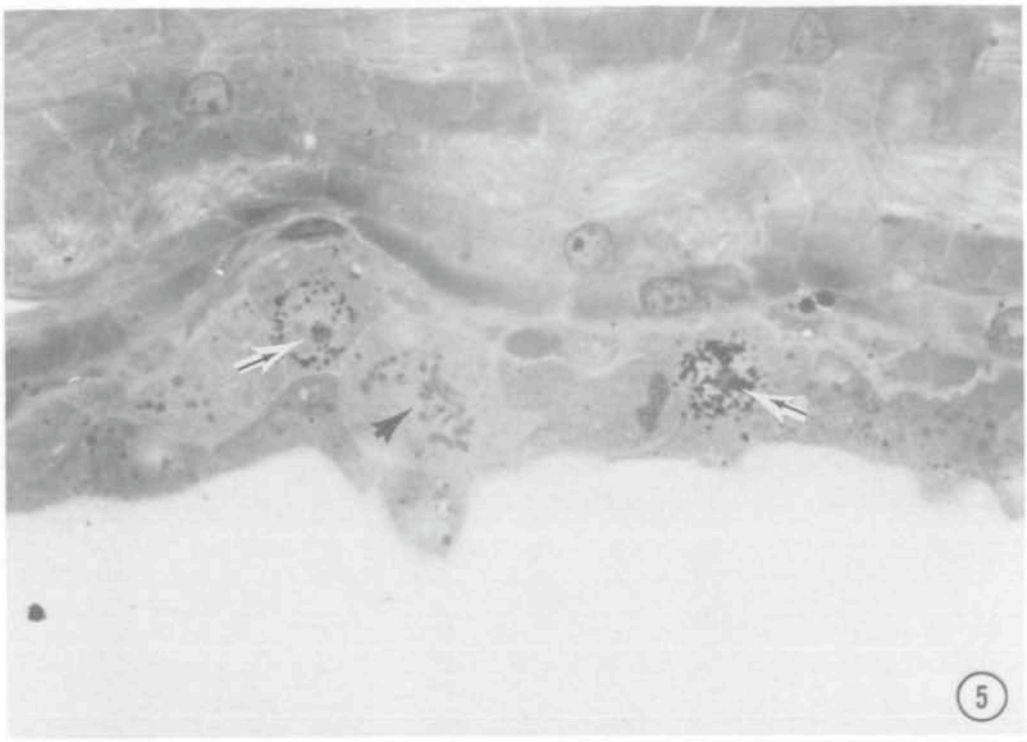


Fig. 5. Light microscopic autoradiogram of the cat RPE 2 days after detachment. ^{3}H-thymidine was injected intravitreally 3 hrs prior to fixation. Two ^{3}H-Thymidine labeled RPE nuclei (arrows) and an adjacent mitotic figure (black arrow) indicate that some RPE cells are proliferating at this stage (×850).

ent in these cells. Most of the pigment granules, however, are not part of a phagolysosomal complex.

One to 4 weeks postdetachment: There are few changes in the RPE cells occurring at this stage that have not been identified in earlier detachments. The mounding of the apical surface is much more pronounced in areas where the RPE still remains a monolayer. Prominent stacks of rough-surfaced endoplasmic reticulum, unlike that found in mature RPE cells but similar to that found in human fetal retina at 12 weeks,§ are seen within the apical cytoplasm of the mounded cells (Fig. 3). In many mounded cells, the processes along the apical border have increased in length. Areas of hyperplasia are more numerous than in earlier detachments, although the extent of proliferation is variable from region to region. In some areas, the RPE is several layers thick.

One to 14 months postdetachment: In long-term detachments, proliferated RPE cells appear in one of several different configurations: as discrete mounds of cells (Fig. 7) joined by adhering junctions to each other and to the original monolayer; as multiple layers of cells that parallel the original RPE monolayer; as papillary-type protrusions into the subretinal space (Fig. 8); or, infrequently, as placques of spindle-shaped cells embedded in an extracellular matrix that stains positively for collagen. Generally, the mounding of cells still in the monolayer configuration remains pronounced. There is no indication that the normal shape of the cells returns even at the longest, ie, 14 months detachment time. In the 14-month detachment, numerous clusters of RPE cells appear next to the original RPE apical surface, free in the SRS, or adjacent to the photoreceptor remnants where they are surrounded by Müller cell processes (Fig. 9). However, ^{3}H-thymidine-labeled RPE nuclei are absent.

§ Linberg KA, and Fisher SK: Unpublished observations.

The Subretinal Space

Zero to 72 hrs postdetachment: Up to 12 hrs after detachment, the SRS contains outer segment debris and some erythrocytes but few other cell types. An amorphous ground substance usually surrounds the outer segments that remain attached to inner segments (Fig. 10). The few cells that are present tend to be located next to the outer segments or adjacent to the apical RPE surface.

At about 24 hrs after detachment cells with PAS-positive cytoplasm appear in the subretinal space, in the outer nuclear layer (Fig. 11), and within the inner retina. Such cells are also present in the choroidal

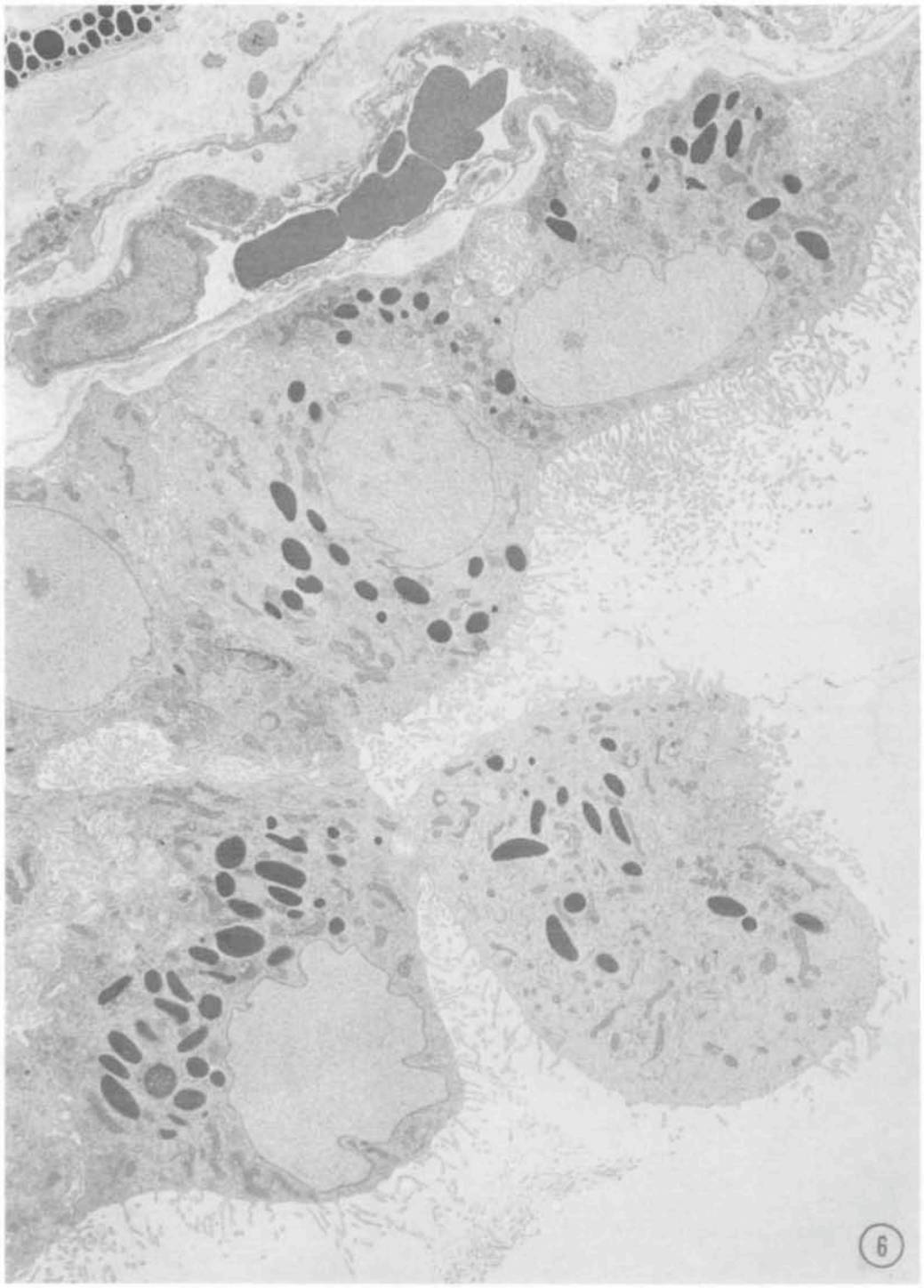


Fig. 6. The cat RPE 3 days after detachment. The RPE monolayer is interspersed with small clusters of RPE cells as a result of proliferation. The apical surface is covered with a fringe of shortened microvilli, the nuclei are indented, and pigment granules are scattered throughout the cytoplasm (×9600).

capillaries, and in the capillaries of the inner retina. These cells appear to be of two types: polymorphonuclear neutrophils (PMNs) and monocytes at different maturational stages. The PMNs were identified in the light microscope by their characteristic multilobed nuclei and dense heterochromatin pattern. Monocytes were recognized by their kidney or horseshoe-shaped nucleus, pseudopodia, and clusters of

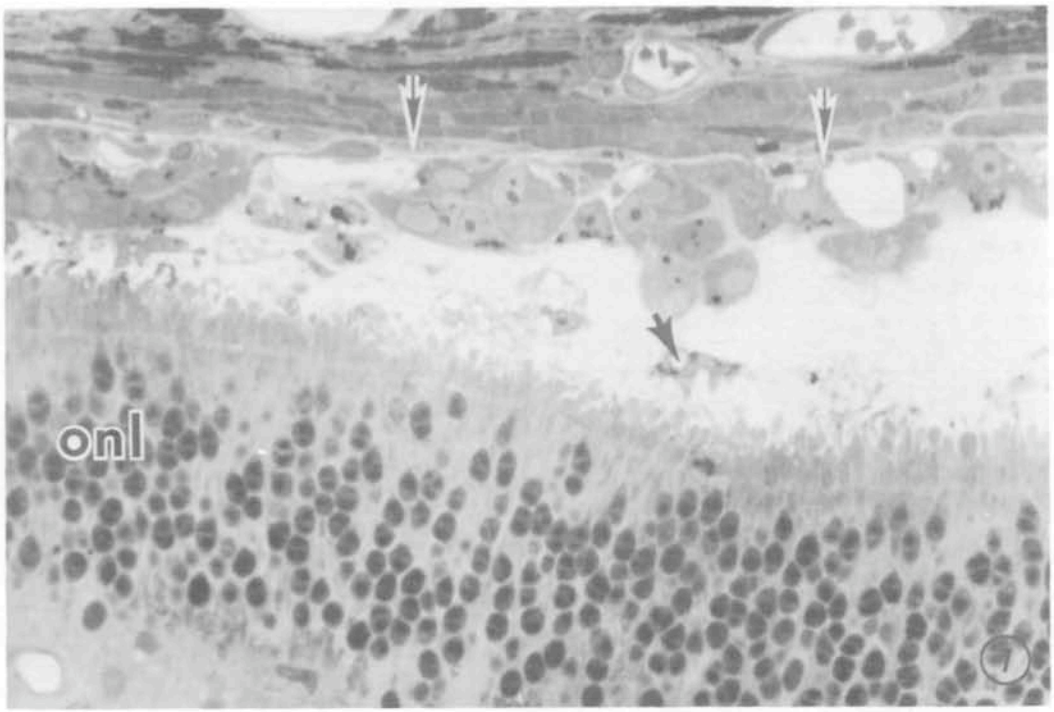


Fig. 7. A transition zone between attached and detached cat retina 13 days after detachment. Clusters of proliferated RPE cells are common in such areas (arrows). At 13 days, the outer segments are nearly gone. Phagocytic cells (black arrow) are found adjacent to the inner segments. Some thinning of the outer nuclear layer (ONL) is apparent (×525).

azurophilic granules. In a few instances, monocytes are positioned between adjacent RPE cells after having apparently migrated between the capillary endothelial cells and through Bruch's membrane (Fig. 12).

By 72 hrs after detachment, the number of PAS-positive cells in the SRS appears lower. Maturing monocytes, ie, tissue macrophages, are more numerous at this time. These cells are characterized by long and numerous pseudopodal processes, multilobed

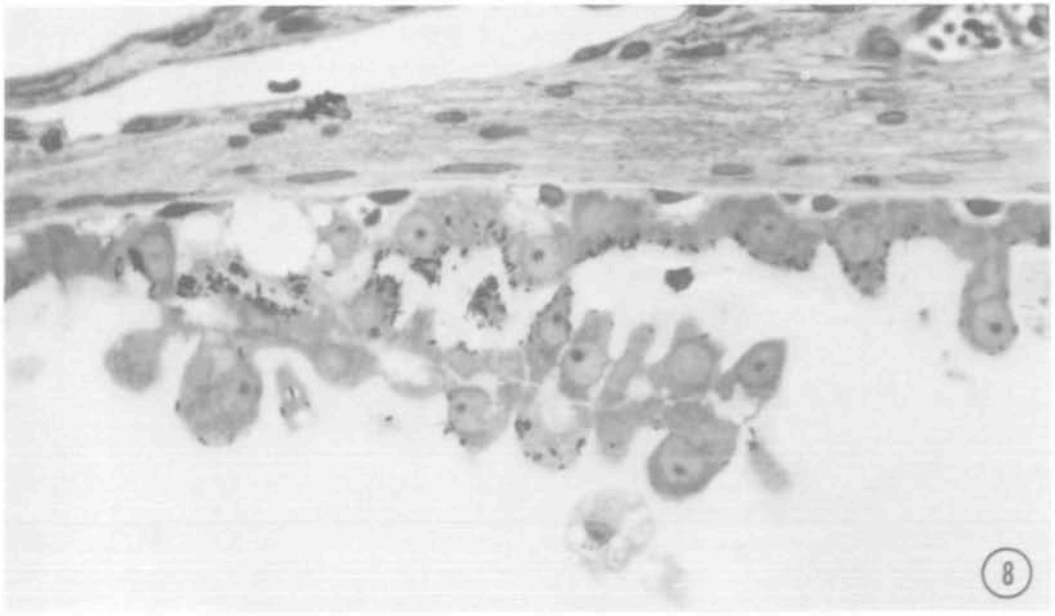


Fig. 8. The cat RPE 50 days after detachment. In longer term detachments tongue-like extensions of proliferating RPE cells, sometimes called papillary proliferations, project into the subretinal space. The number of pigment granules, as well as their intracellular distribution, in proliferating cells tends to be irregular (×600).

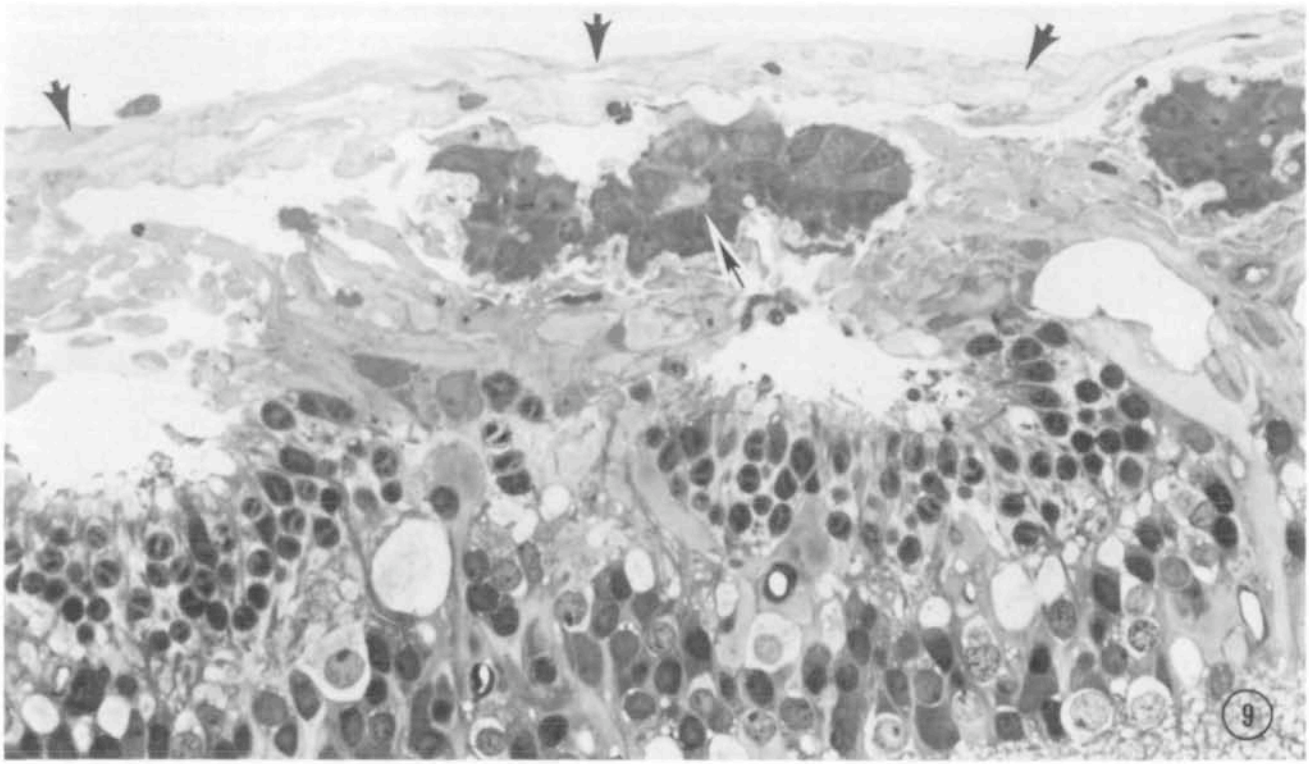


Fig. 9. The cat retina 14 months after detachment. At the longest detachment interval examined, dark-staining clusters of RPE cells (arrow) and Müller cell processes (black arrow) line the outer margin of the retina. The outer segments have disappeared, as have most of the inner segments by this time. The outer limiting membrane has numerous discontinuities through which Müller cells project into the subretinal space. The outer nuclear and outer plexiform layers are thinned considerably and absent in some locations. The inner nuclear layer is disrupted as well (×450).

nuclei with prominent nucleoli, and large numbers of phagocytic vacuoles. The cytoplasm contains both primary and secondary lysosomes and residual bodies. They tend to congregate near the apical RPE surface and also close to the distal tips of the degenerating outer segments where they phagocytize large amounts of outer segment debris (Fig. 13). Processes of these cells envelop the truncated outer segments (Fig. 14) much like the RPE apical processes in control retinae.

At least two additional cell types that are derived from the retina-RPE are found in the subretinal space at this time: RPE and photoreceptor cells. The first definite signs of RPE cells separating from Bruch's membrane are found at about 72 hrs postdetachment (Fig. 6). Electron micrographs suggest that cells in the initial stages of migration progressively reduce their basal surface area that apposes Bruch's membrane (Fig. 15) until only a small segment of lateral surface remains attached by adhering junctions with adjacent RPE cells. The cells leaving the monolayer are characterized by a narrow protruding tail, a trailing edge of cytoplasm resembling the uropods found in motile lymphocytes (Fig. 16).[23]

After their migration away from the RPE layer the fate of these cells is uncertain. However, in pigmented retina, a number of cells have elliptically shaped granules that are indistinguishable from normal melanin granules. These cells are located above the tips of the degenerating outer segments and next to the apical RPE surface. Some of these cells and other cells with no pigment granules contain homogeneous particles 0.75 μM in diameter that also appear frequently in normal cat RPE cells. Many cells also contain large packets of phagocytized outer segment debris.

As early as 24 hrs after detachment a few photoreceptor cells appear in the SRS. They appear with increasing frequency in longer term detachments. Many of these cells were identified as rods by their two distinct clumps of heterochromatin. Their presence in the SRS is strongly correlated with "gaps" in the outer limiting membrane where the zonulae adherentes between adjacent Müller cell processes, and between Müller cell processes and photoreceptor inner segments, are absent.

Long-term detachment: At detachment durations longer than 8 weeks, the numbers of cells in the SRS appears to decline. PMNs are no longer present in

Fig. 10. Cat photoreceptors 12 hrs after detachment. Large and small packets of outer segment debris appear above the outer segments in the subretinal space. The outer segment tips are distended (asterisks), although the rest of the disc stack is not nearly as disrupted (×6200).

significant numbers, and the number of monocytes is also lower. Mature macrophages, RPE phagocytes, Müller cells and some photoreceptor cells are the significant cell types remaining in the SRS. In long-term detachments Müller cell processes, characterized by their sparse cytoplasm and numerous 10 nm diameter filaments, emerge into the SRS from discontinuities in the outer limiting membrane. These processes can

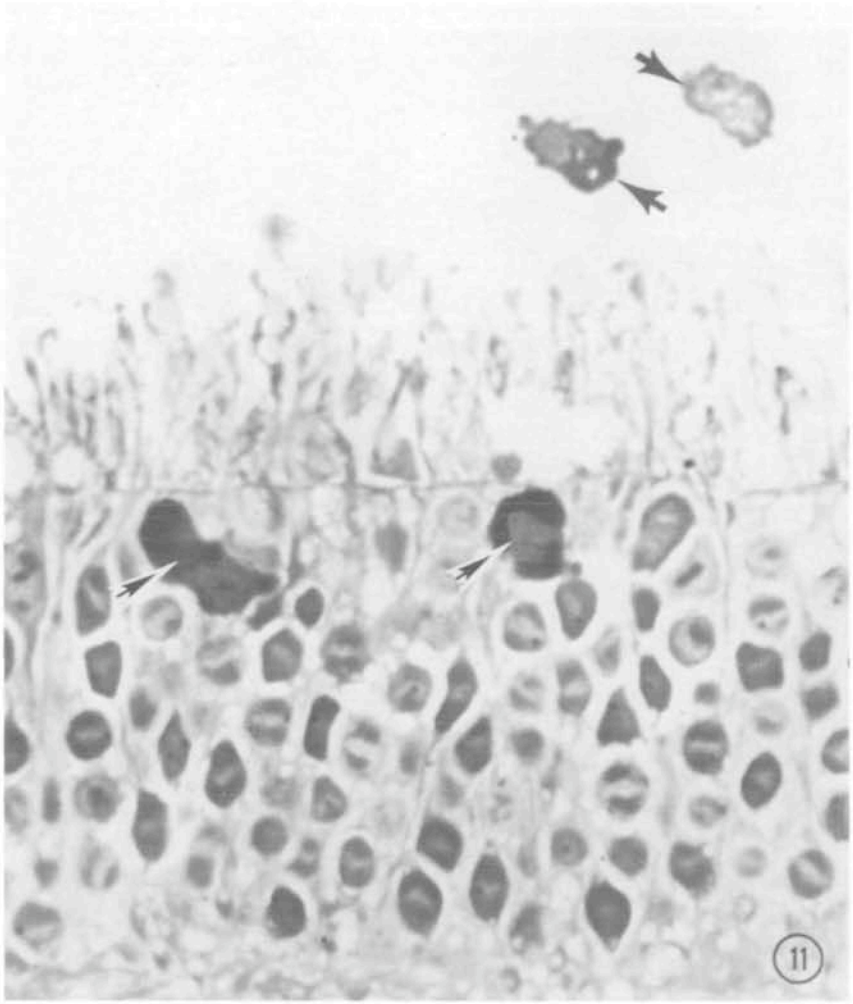


Fig. 11. PAS stained cells; 1 day after detachment. At the early detachment times a number of PAS positive cells, presumably polymorphonuclear neutrophils and monocytes, are found in the subretinal space (black arrows) and in the retina (arrows) (×1125 phase contrast).

extend laterally several hundred microns from their point of origin within the retina (Fig. 9).

The Photoreceptor Outer Segments

The structure of some outer segments is almost normal up to about 12 hrs following detachment. However, many outer segments show evidence of immediate damage. Those that remain nearly intact retain their inner segment connection and their cylindrical shape; however, the distal tips appear distorted or vacuolated (Fig. 10). Some damaged outer segments are broken, some pieces remain adjacent to the apical RPE surface, and others appear as large membrane-bound packets in the subretinal space.‖

‖ It is possible that some outer segment damage could have been produced by the injection of fluid into the SRS, damage that might not be expected from detachments induced by traction on the inner retinal surface. However, our results indicate that most retinal areas where such damage occurred were located close to the injection site.

Both rod and cone outer segments show evidence of disruption between 24 and 72 hrs after detachment. Toward the end of this period they consist of membrane bound sacs, partially filled with disc membrane, still connected to their ciliary stalks. In some cases, evaginations of the plasma membrane can be identified at the outer segment bases adjacent to the connecting cilia (Figs. 17A–C), although the evaginating membrane is usually not organized into an orderly disc stack. Membrane evaginations are still present in detachments 2 weeks after surgery (Fig. 17A), and even in longer term detachments some disorganized disc material is contained within the outer segment plasma membrane (Figs. 17B, C).

Outer segment structure progressively deteriorates as detachment duration lengthens. There is no apparent difference between rod and cone outer segments in the rate of degeneration. In general, outer segment morphology is most affected in highly detached areas or in retinae detached for a long time.

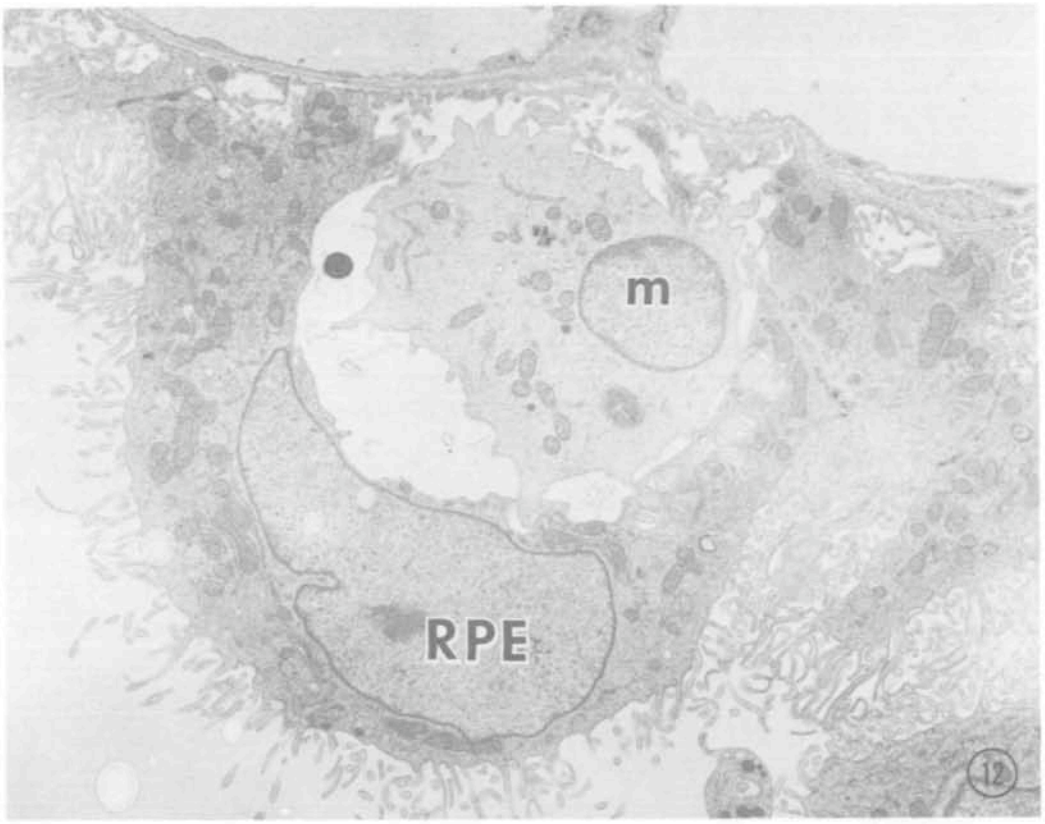


Fig. 12. Cat RPE 13 days following detachment. A presumptive monocyte (m) after having apparently passed through Bruch's membrane from the choroidal capillaries. The RPE cell (RPE) surrounding the monocyte appears to have lost most of its lateral surface contact with neighboring cells and its basal contact with Bruch's membrane (×5000).

The Photoreceptor Inner Segments

After the first day of detachment, the inner segments show little evidence of disruption, although their regular parallel alignment is sometimes altered. The mitochondria are normally aligned, not vacuolated, and the connecting cilia are intact.

Degenerative changes in inner segment morphology do not take place with the same rapidity as they do at the level of the outer segments. However, between one and three days after detachment the inner segments at most locations are affected, albeit to varying degrees. Inner segment morphology appears to depend upon the detachment height. Figure 18A is a low power electron micrograph from a shallow 13-day detachment; Figure 18B is a micrograph from an adjacent high detachment area. The inner segment organelles from the area of shallow detachment appear near normal, while those from the highly detached region are severely disrupted and fewer in number.

In retinae detached for 2 weeks or longer, the inner segments' parallel alignment is usually disrupted. Vacuolization at the distal tips of the inner segments ranges from mild to severe. The area occupied by mitochondria is greatly reduced from normal; they are frequently swollen, with their cristae appearing distended and fragmented (Fig. 18B). However, even in severely affected areas the connecting cilia, their basal bodies, and striated rootlets can still be identified.

Discussion

Several different types of controls were used in these experiments to assure that the changes observed were related to retinal detachment and not to some other aspect of the experimental procedure. Three retinae were examined for ultrastructural changes that could be attributed solely to the vitrectomy surgery. With the exception of a mild inflammatory response in the first week after the vitrectomy, no abnormalities in any of the retinal layers or at the outer segment-pigment epithelial interface were apparent by either light (Fig. 1) or electron microscopy. Retinas detached by subretinal injection of mammalian Ringer's solution or by Healon were compared at identical detachment intervals to determine whether

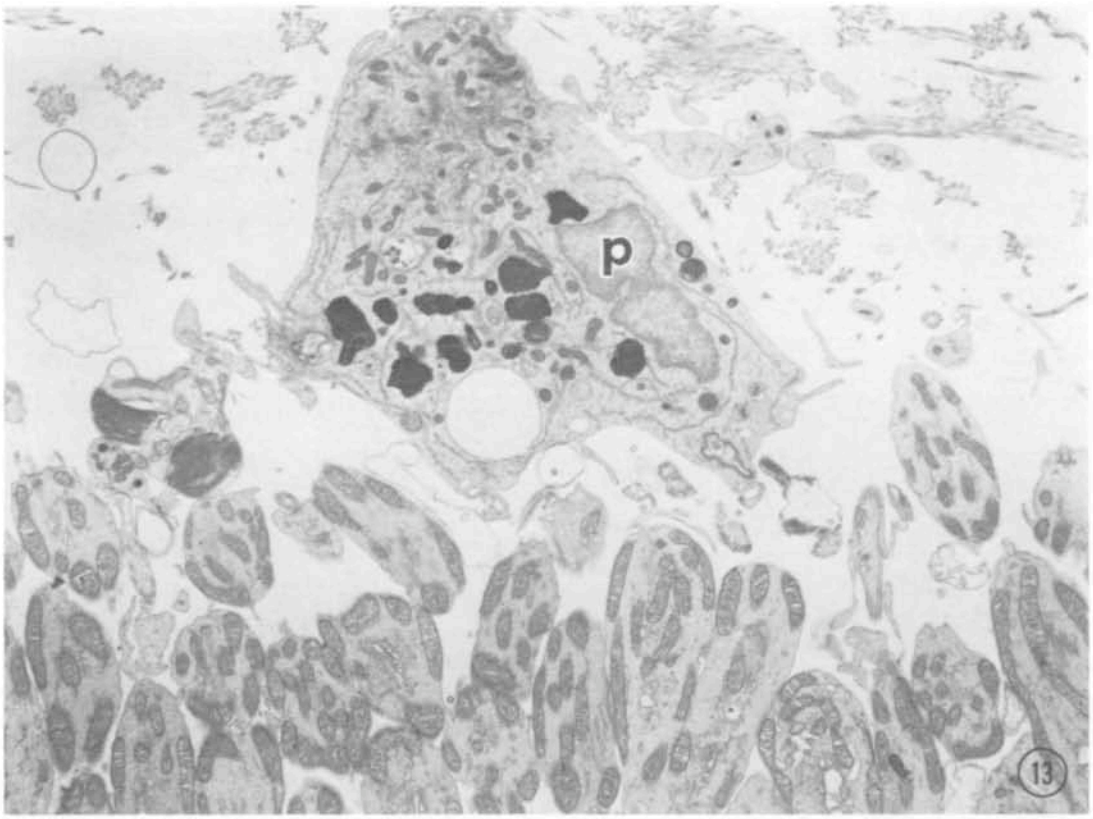


Fig. 13. Cat photoreceptors 13 days after detachment. RPE and mononuclear phagocytic cells, similar to the cell shown here (P), scavenge the outer segment debris found above the inner segment tips (×5000).

any morphologic changes depended upon the type of fluid injected. No differences were noted. In addition, cat retinae detached by subretinal fluid injection were compared to rabbit retinae detached by intraocularly injected RPE cells.[22] All of the major morphological changes identified in the fluid injection detachments were also found in the traction detachments.

The RPE in Experimental Retinal Detachment

Ultrastructural studies of experimental retinal detachment have been reported in several mammalian species: in the owl monkey,[6-7] the rhesus monkey,[24] the rabbit,[25-29] and now in the cat. These studies are fairly consistent in identifying the general changes that take place at the photoreceptor-RPE interface after retinal detachment. However, there are discrepancies concerning the specific nature of these changes and the timing at which they occur.

In all species examined, the RPE cells undergo dramatic morphologic changes very soon after detachment. Within several hours after detachment in the cat and rabbit,[28] and by 24 hrs in the owl monkey,[6] the specialized microvillous and sheet-like processes that normally interdigitate with the outer segments disappear. An array of homogeneous microplicae, that normally underlies these specialized processes,[15] is all that remains on the apical surface of the cat RPE. Such changes may occur even more rapidly after detachments produced by hyperosmotic intravitreal injections; the configuration of the apical processes changes after 2-3 min in the rabbit and within 3 hrs in the monkey.[29]

By 1 day after detachment in the cat and rabbit,[26] and by 3 days in the owl monkey,[5] the apical RPE surface becomes mounded and protrudes into the subretinal space. This change in cell shape becomes more pronounced with time, and, unlike the situation in the owl monkey,[6] the apical surface in the cat does not flatten again at detachment intervals as long as 14 months. Phagosomes rapidly disappear from the RPE cytoplasm,[6] and melanin granules and mitochondria may be misplaced and/or irregularly oriented. These morphologic changes imply that rapid metabolic changes also occur in these cells in response to their loss of contact with the photoreceptor outer segments.

We have recently shown that the onset of RPE proliferation in the cat retina occurs about 24 hrs

after experimental rhegmatogenous retinal detachment.[2] We found similar results in rabbit retinae with experimental traction detachments thereby eliminating the possibility that proliferation is merely a result of damage induced by subretinal fluid injection or is attributable to some other aspect of the detachment procedure. In other species, the onset of the RPE proliferative response after detachment has not been studied specifically. However, examples of stimulus dependent DNA synthesis and cell division in other mitotically inactive tissues indicate that an appropriate stimulus is almost always preceded by a "prereplicative" interval of 12–72 hrs.[30,31] Thus, it is highly likely that the results in cat retinae are good predictors of the onset of the RPE response in the human retina. In other tissues, such as liver,[31] the proliferative response can involve a sizable fraction of the total cell population. Although the magnitude of the RPE proliferative response is not known, it may have important implications for the RPE's apparent involvement in massive periretintal proliferation,[32,33] and for other pigment epithelial disorders as well.

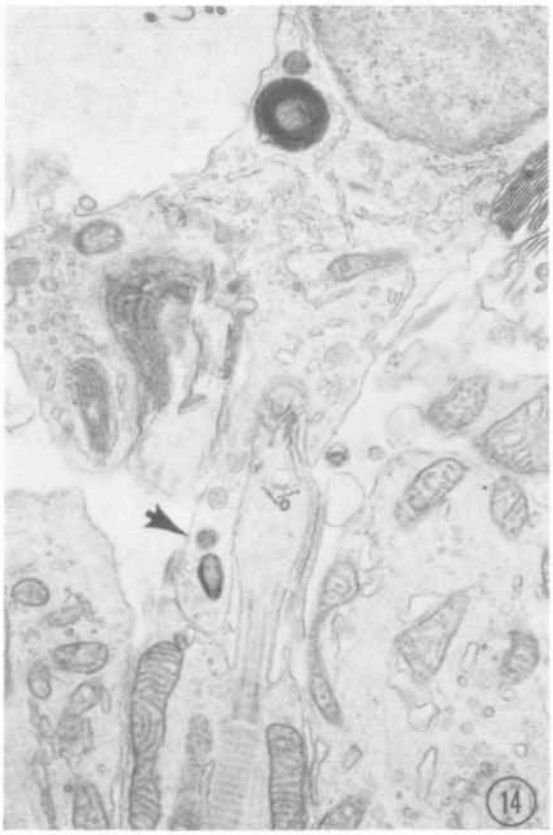


Fig. 14. Thirteen days after detachment. Pseudopodial extensions (arrow) of the phagocytes drape the ciliary stalks of the photoreceptors (×16200).

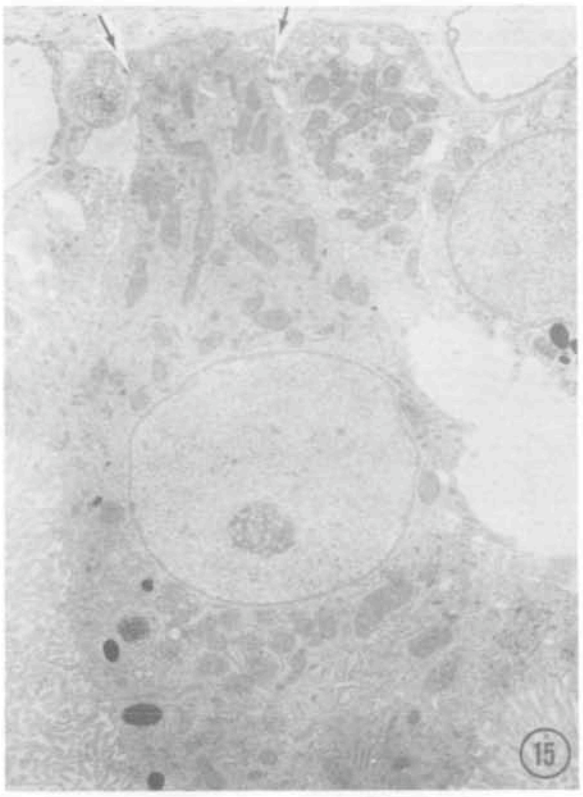


Fig. 15. The cat RPE 50 days after detachment. RPE cells in the process of migrating into the subretinal space progressively reduce the amount of basal surface (arrows) apposed to Bruch's membrane (×3500).

The fact that RPE proliferation begins so quickly after detachment suggests that close apposition of the RPE and retina is a prerequisite for keeping the RPE in a mitotically inactive state. If this is correct, the reapposition of the neural retina and RPE by reattachment surgery could be expected to stop proliferation. In the reattached cat retina, outer segment regeneration tends to be poorest in areas of RPE proliferation or Müller cell hypertrophy.[34] Thus, RPE and Müller cell proliferation, as well as their adverse effects upon subsequent outer segment recovery, could conceivably be averted if reattachment took place prior to the onset of proliferation, or if proliferation was inhibited prior to reattachment.

Evidence of ^{3}H-thymidine labeled RPE nuclei and RPE mitosis was absent in the 14 month detachment. Machemer and Laqua[32] found similar results in longstanding detachments in the owl monkey retina. These findings suggest that the proliferative response could be a self-limiting process that does not continue indefinitely, and there is some experimental support for this conclusion.[35]

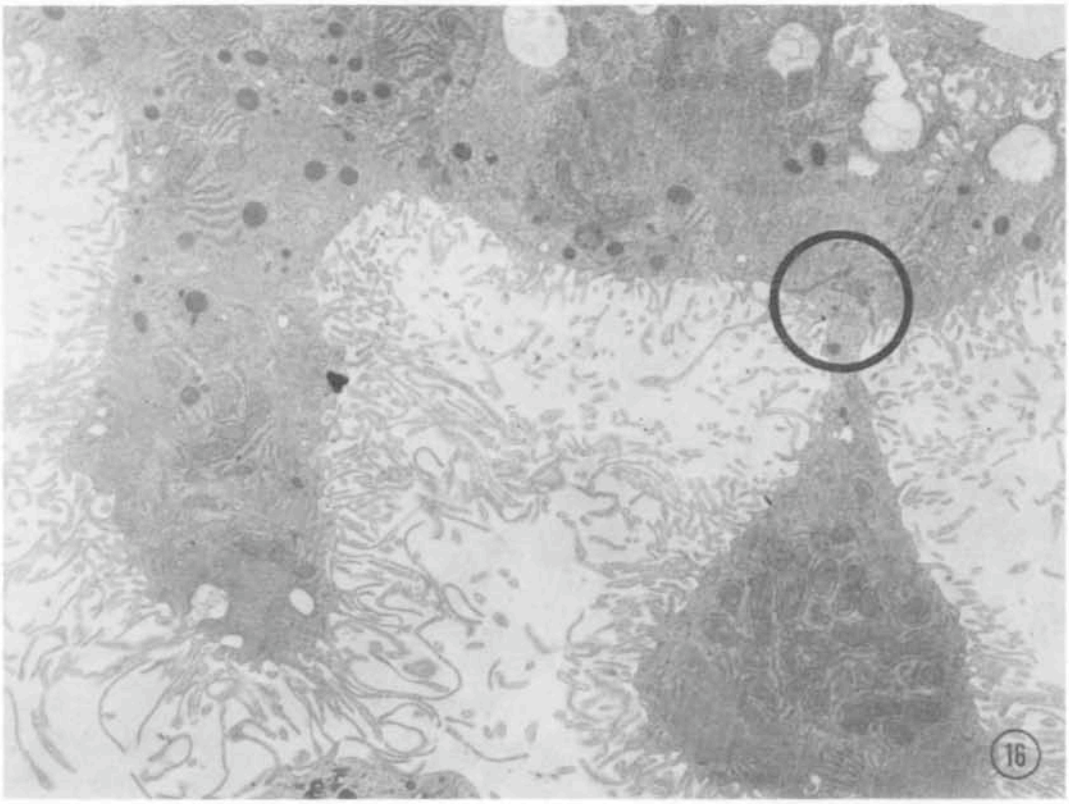
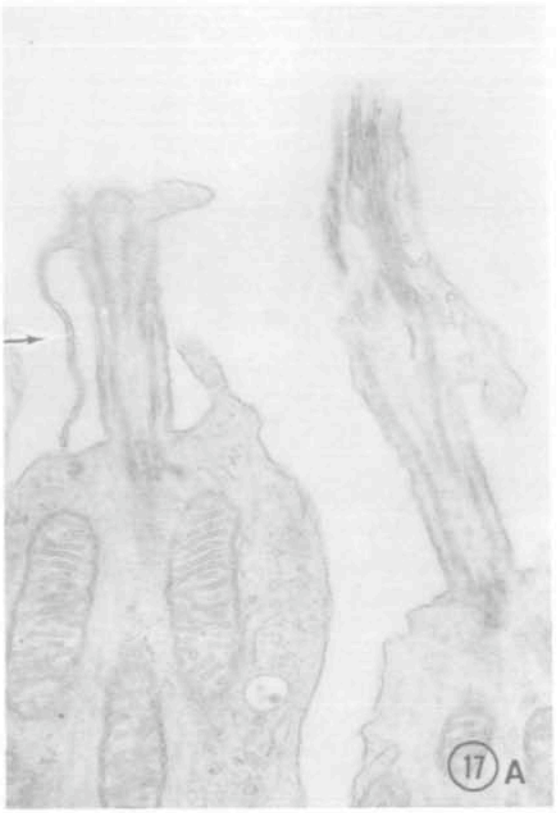


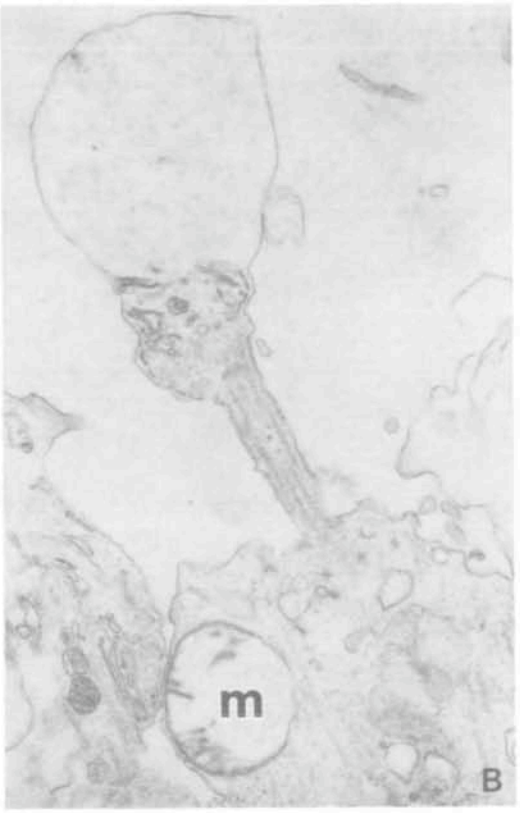
Fig. 16. 22 days after detachment. The RPE cell on the right has nearly separated from the monolayer. A narrow protruding tail of cytoplasm retains junctions to neighboring cells (circle). The apical processes of these highly mounded cells are longer than those found at earlier detachment intervals (×5300).

The Origin of Subretinal Phagocytes

There is some controversy concerning the origin and prevalence of subretinal phagocytes after retinal detachment. By several days postdetachment, RPE cells begin migrating away from the RPE monolayer in the detached owl monkey[32] and rabbit[27,28] retinae. They congregate close to the distal tips of the degenerating outer segments where they engulf packets of outer segment material. In the rabbit, the migration of RPE cells into the subretinal space is thought to represent the major source of subretinal phagocytes.[25] In the owl monkey, RPE derived cells are also believed to be one of the major subretinal cell types.[32] Feeney et al[36] concluded that macrophages, rather than RPE-derived cells, are the predominant cell type found in human subretinal fluid samples. However, Feman and Lam,[37] who differentiated between RPE derived cells and blood-borne tissue macrophages by their napthyl acetate staining properties, found a highly variable distribution of the two cell types in subretinal fluid samples from 19 human patients. Recent in vitro studies have shown that isolated RPE cells have immunophagocytic properties similar to tissue macrophages.[38] Thus, migrating RPE cells apparently do have the capacity to function like macrophages in the SRS.

In the detached cat retina, both macrophages and RPE-derived cells can be identified in the SRS. Immature macrophages presumably enter the SRS via the choroidal capillaries, and perhaps through the retina as well, traversing Bruch's membrane and migrating between adjacent RPE cells (see Fig. 12). Transepithelial migration of white blood cells is also known to occur in other tissues.[39] In the cat, RPE cells in the process of migrating into the SRS can be identified positively (Fig. 16). Also, in detached retinae many subretinal cells possess elliptically shaped pigment granules and other organelles that identify them as RPE cells. However, the pigmentation of proliferating RPE cells is quite variable in that some cells are filled with granules while others have none at all. Thus, in the cat SRS it is impossible, without a specific cellular marker, to distinguish quantitatively between blood-borne tissue macrophages and RPE-derived cells that contain no melanin granules.





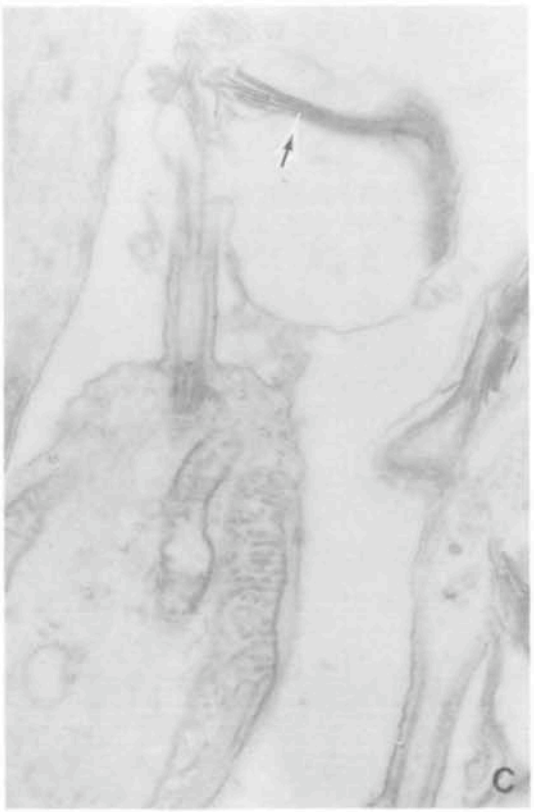
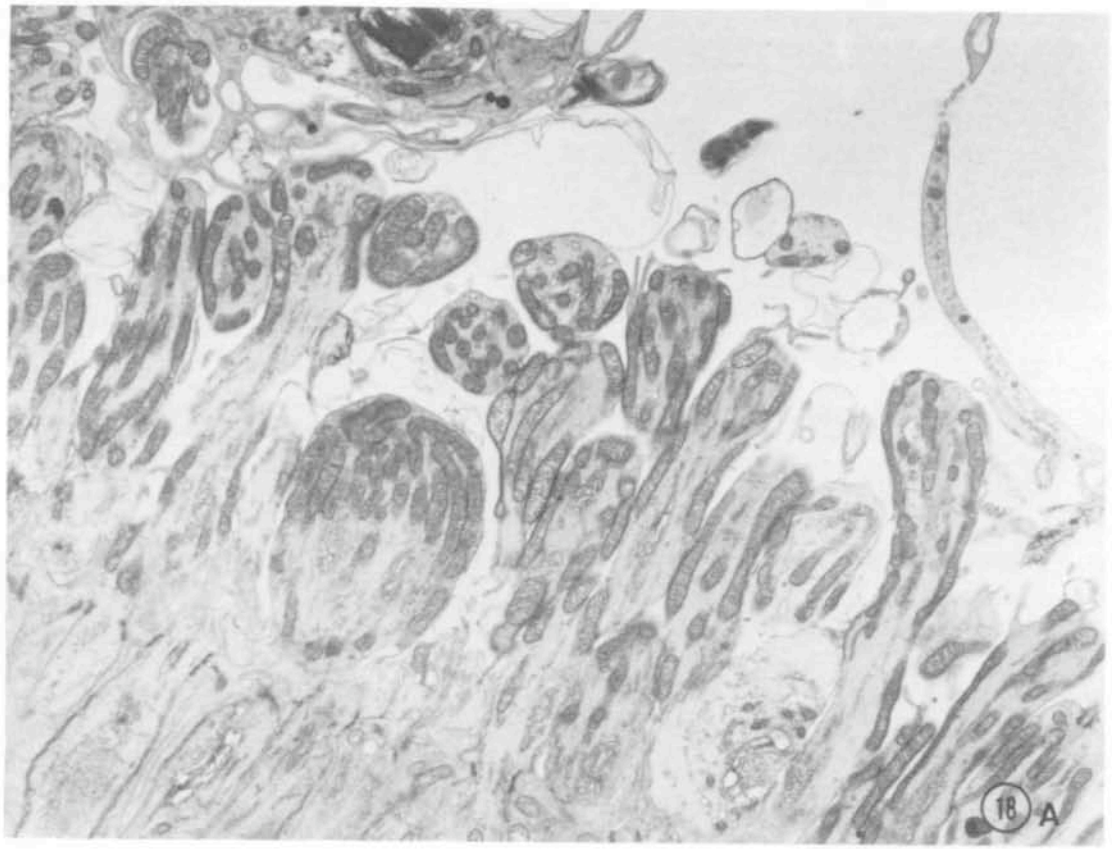


Fig. 17. Cat photoreceptors. **A**, 13 days after detachment. Evaginations of outer segment membrane are occasionally seen at the tips of the ciliary stalks (arrow). Inner segment mitochondria still appear intact (×22500). **B**, 50 days after detachment. At longer intervals, most remaining outer segments appear as empty sacks at the tips of the ciliary stalks. Mitochondria (m) are swollen (×20000). **C**, 70 days after detachment. In a few areas, small stacks of disc membranes (arrow) can even be found at lengthy detachment intervals (×20000).


18 A

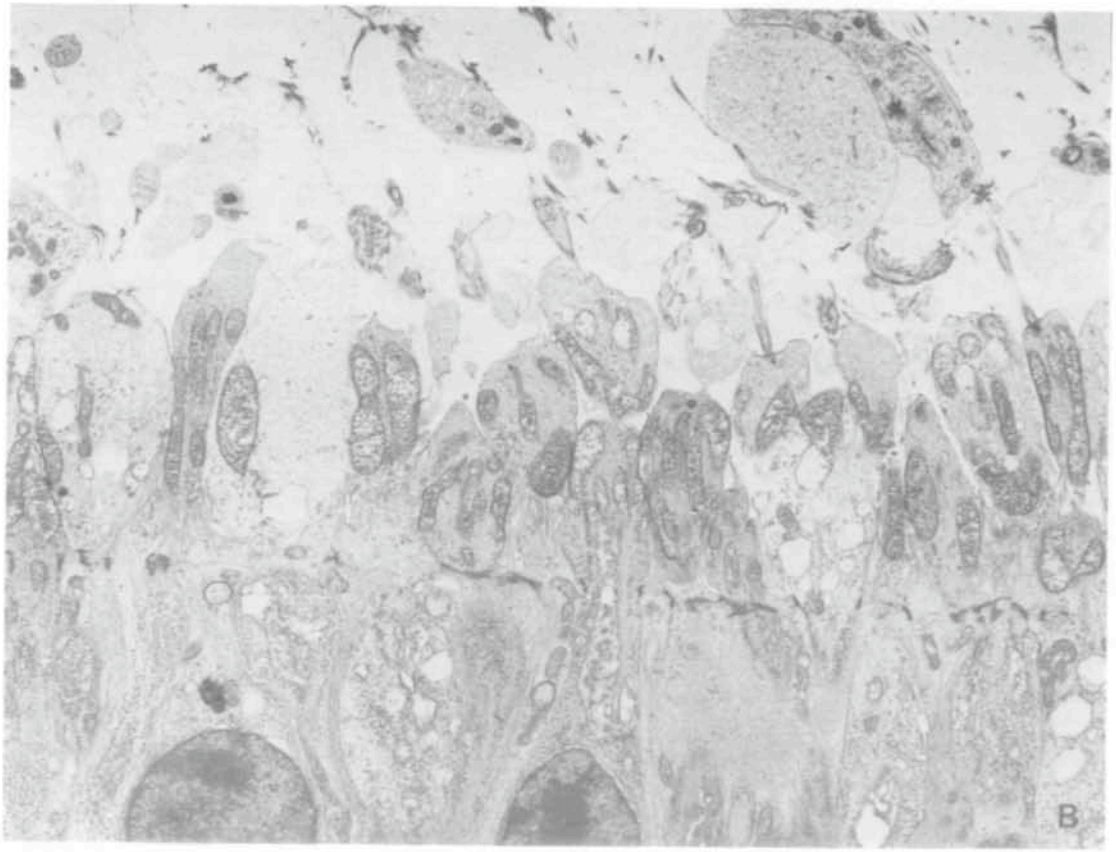

B

Fig. 18. Thirteen days after detachment. **A**, Shallow detachment. Although the outer segments have degenerated and the number of mitochondria is reduced, inner segment morphology is close to normal (×5000). **B**, High detachment from an adjacent region. In high detachments, some ellipsoids appear fragmented; others are vacuolated. Mitochondria are swollen and fewer in number. The myoid region also appears vesiculated (×5000).

Johnson and Foulds[25] concluded that migrating rabbit RPE cells leave a membrane-bound portion of their cytoplasm remaining with the monolayer. In the cat, we found no evidence for such a process. On the contrary, we concluded that migrating RPE cells progressively reduce their basal surface area, sever their lateral junctions, and then appear in the SRS as free phagocytes.

New Disc Assembly in Relation to Detachment

In all species examined, the normal structure of the photoreceptor outer segments deteriorates rapidly after detachment. One week after retinal detachment in the owl monkey, the outer segments appear vacuolated, and the discs are irregularly oriented and swollen.[6] Photoreceptors in the detached rhesus monkey retina apparently follow a similar time course.[24] In the rabbit, the outer segments appear vacuolated and disorganized 1 day following detachment.[27] In the cat, the structure of most outer segments is nearly normal up to about 12 hrs postdetachment; but by 1–3 days they are severely disorganized.

Despite the rapid loss of normal outer segment morphology in response to detachment, the assembly of new outer segment membrane apparently does not stop completely. In a detachment of 4 weeks duration, Machemer and Kroll[7] found evidence of labeled outer segment material 2 days after injection of tritiated amino acids. In the detached cat retina, electron micrographs taken at the level of the connecting cilium suggest that newly synthesized membrane appears as disorganized discs and membranous whorls that are not assembled into uniform stacks. Evaginations of disc material are occasionally identified at the tips of the ciliary stalks up to several weeks after detachment (Figs. 17A, C). In the reattached retina, electron microscope autoradiograms of photoreceptors similar to those shown in Figures 17A–C indicate that new protein continues to be incorporated into the disorganized outer segment membranes.# Immature rat photoreceptors maintained in tissue culture without an overlying RPE can synthesize outer segment material although, as in detached retinae, it is not arranged into discrete stacks.[41] Photoreceptor degeneration in retinal detachment is quite similar to that observed in the degenerate retina of the cave salamander where newly synthesized protein also continues to be incorporated into the outer segment remnants.[42] It is possible that degenerating photoreceptors may synthesize new disc membrane at an abnormally low rate, or that the new membrane may be biochemically defective. Nevertheless, the evidence indicates that the apposition of the RPE is not required for new membrane synthesis, but it is necessary for the production of a normally oriented disc stack.

Anderson DH, Fisher SK, and Ward RE. Unpublished observations.

Factors Limiting Visual Recovery

The degenerative and proliferative changes that occur at the RPE-photoreceptor interface and elsewhere in the detached cat retina[3] are rapid, progressive, and sometimes irreversible events that undoubtedly play a major role in defining the limits of recovery after reattachment. Figure 19 summarizes the rapid onset of these morphologic changes in the experimentally detached rabbit, owl monkey, and cat retinae.

In the reattached cat retina our results strongly suggest that two of the most prominent changes after detachment, RPE proliferation and subretinal Müller cell migration and proliferation, adversely effect outer segment regeneration.[34] The involvement of RPE cells and retinal glia in massive periretinal proliferation is now widely recognized.[32,33,43] However, their roles in relation to retinal recovery following reattachment have not been assessed. Outer segment regeneration underlying such regions in the reattached cat retina is usually poor.[34] The presence of intervening cellular material in the subretinal space, in conjunction with poor outer segment regeneration particularly within the macula, is likely to be an important factor that contributes to persistently reduced vision after reattachment.

There are other clinical signs of subretinal pathology that may be attributable wholly or in part to RPE and/or Müller cell proliferation. Local areas of proliferating RPE cells are likely to be the basis of the various pigmentary changes often seen in detached human retinae. For example, it is likely that demar-

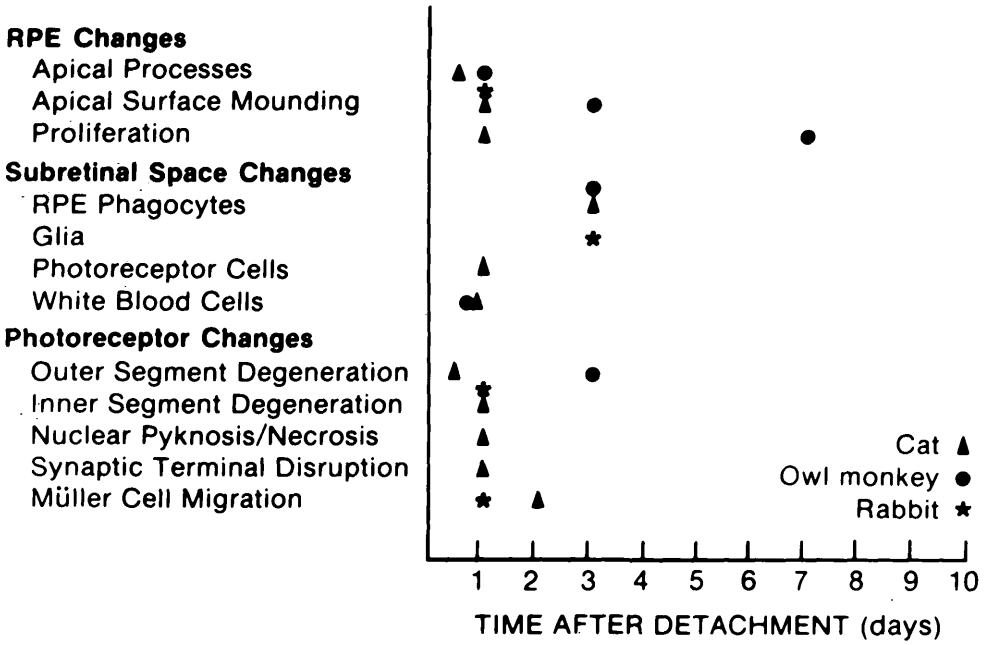


Fig. 19. Summary diagram of the onset of degenerative and proliferative changes in the experimentally detached cat, rabbit, and owl monkey retinae as a function of detachment duration. Most ultrastructural changes in experimentally detached retinae appear very soon after detachment—from a few hours to three days after detachment.

cation lines are actually zones of pigmented or unpigmented RPE cell proliferation that mark the boundary between attached and detached retina.[44] In the detached cat retina, the frequency of RPE cell clusters at "transition zones" between attached, and detached regions support that contention. Subretinal glial proliferation involving Müller cells and/or retinal astrocytes has been described in two experimental models of retinal detachment,[43] including the cat.[34] Subretinal fibrosis, clinically characterized as multiple opaque strands in the subretinal space,[45] may be an advanced form of Müller cell proliferation in the human retina.

In the cat, the extent of morphologic change in the detached retina is strongly correlated with the duration of the detachment. In general, the morphologic effects tend to be more pronounced as the time since detachment lengthens. Thus, our results strongly suggest that detachment duration should be related inversely to the capacity for subsequent retinal recovery and, therefore, to eventual visual recovery after reattachment.

Acuity measurements in patients having macular detachments show that postoperative improvement is best in those cases where detachment duration is less than 8 weeks; however, no statistically significant difference in improvement is found at durations between 1 and 8 weeks.[46,47] Similarly, Cleary and Leaver[48] noted that patients with macular detachments of 8 weeks or longer are likely to experience reduced vision after surgery. Gundry and Davies[49] found that the best final acuity is obtained in patients whose macula had been detached for less than 1 week. But they also found no significant difference in final acuity at detachment intervals ranging between 1 week and 6 months, although acuity at all intervals continued to improve up to 2 years after reattachment. On the other hand, hue discrimination, photopic acuity, and static field testing in a group of 35 selected patients indicated that visual recovery in all three parameters is inversely related to macular detachment duration.[50] In light of the dramatic morphologic changes that occur between 1 and 8 weeks after detachment in the cat retina and in other animal models of detachment, it is difficult to explain why no differences in postoperative acuity are detected in human patients at short detachment intervals. However, most of the above investigations found final acuity to be highly variable even in patient groups with similar detachment durations. Various macular abnormalities,[48] the type of surgical procedure, the etiology of the detachment, and other as yet unidentified variables may obscure the differences related to detachment duration. More sensitive psychophysical tests, such as those employed by Chisolm et al,[50] and by Fitzgerald et al,[51] may be required to reveal subtle differences in visual capacities at short detachment intervals.

In addition to duration, it is clear that many of the detachment changes in the cat are accentuated in regions where the separation between retina and RPE is great (see Figs. 18A, B). Machemer[5] noted a similar effect in the owl monkey retina. The implication is that the molecular interactions that contribute to the

maintenance of normal photoreceptor structure and metabolism, require close apposition between the retina and RPE. A thorough understanding of these interactions in normal, detached, and reattached retinae could lead to successful efforts to retard or even prevent the degenerative and proliferative changes that take place with such rapidity in the detached retina.

Key words: retinal detachment, pigment epithelium, proliferation, photoreceptors, degeneration

Acknowledgments

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