Morphological Recovery in the Reattached Retina

Don H. Anderson, Christopher J. Guérin, Page A. Erickson, Walter H. Stern, and Steven K. Fisher

After experimental retinal detachment in the cat, a number of morphological changes take place in retinal and RPE cells. Following reattachment, the ultrastructural relationship between the photoreceptors and the RPE is re-established, but it does not return to the predetachment state even after short detachment episodes coupled with prolonged recovery periods. All of the reattached retinae show some degree of abnormality, ranging from subtle changes in photoreceptor ultrastructure to dramatic degenerative effects in the outer retina. Abrupt transitions in morphology from one reattached area to an adjacent area are not unusual. Photoreceptor recovery varies widely between animals, and between adjacent regions within the same retina. Ensheathment of outer segments by RPE apical processes is abnormal. In some reattached areas rod outer segment dimensions and disc structure are near normal as is the displacement rate of rod outer segment discs. In others, especially in areas of RPE or Müller cell proliferation and hypertrophy, the outer segments are shortened or absent completely, and there is a reduction of cell bodies in the outer nuclear layer. In some retinae, recovery in cones is inferior to that in rods. At short detachment durations (<1 wk) morphological recovery in the reattached retina is optimal while at long intervals (>1 month) recovery is poor. The changes at the photoreceptor-RPE interface identified in the reattached cat retina probably have adverse effects on visual recovery when they occur within the human macula.
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When the retina is detached from the retinal pigment epithelium (RPE)* normal vision is impaired, and a number of morphological changes occur in retinal and RPE cells.[1-5] After the retina is reattached to the RPE, a complex process of recovery begins that can lead to partial, and sometimes nearly complete, restoration of vision.[6] The fact that at least partial vision is restored in most human patients implies that the adverse anatomical and physiological effects of retinal detachment can be halted or even reversed after successful reattachment. The cellular events in this recovery process are not clearly understood, however, and those variables which significantly influence the outcome have not been rigorously defined. Histopathological observations on human reattached retinas have not been reported. Therefore, our current understanding of visual recovery after reattachment is derived solely from animal models of experimental reattachment,[7-11] and from clinical and psychophysical studies of visual recovery in human patients.[12-19]

Recently we introduced a new experimental model of retinal detachment by documenting the degenerative and proliferative changes that occur in the cat retina as a function of detachment duration.[3-5] In this study we report our findings in the reattached cat retina, emphasizing the cellular changes at the photoreceptor-RPE interface. After reattachment, morphology does not return to normal even after prolonged recovery periods (ie, 6 months); instead, a modified version of the normal photoreceptor-RPE relationship is re-established. Overall morphological recovery is optimal at the shortest detachment durations (less than 1 week), and is less successful at longer intervals (greater than 1 month). These morphological abnormalities in the reattached cat retina probably underlie the lack of complete visual recovery after detachment in humans, especially if such changes occur within the macula.

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* Abbreviations used in the text: OLM = Outer Limiting Membrane; SRS = Subretinal Space; ONL = Outer Nuclear Layer; ROS = Rod Outer Segment; DNA = Deoxyribonucleic Acid; RPE = Retinal Pigment Epithelium; SF6 = Sulfur Hexafloride gas; ARGs = Autoradiograms; COS = Cone Outer Segment.

Materials and Methods

Animals

The eyes from 16 cats with unilateral retinal detachments were reattached and subsequently fixed for electron microscopy at selected intervals after reattachment. The detachment (d) and reattachment (r) intervals (in days) were as follows: 1d:10r, 1d:14r, 3d:

7r, 3d:30r, 3d:210r, 5d:1r, 5d:7r(2), 7d:210r(2), 8d:7r, 10d:7r, 11d:3r, 14d:30r, 42d:30r, 42d:210r. The boundaries and other characteristics of each detachment were recorded on a standard ophthalmic detachment chart immediately after surgery. After detachment, and periodically after reattachment, the experimental eyes were examined by indirect ophthalmoscopy. After perfusion fixation, eyecups were examined and the fundi were photographed using a 35-mm camera fitted with a 50-mm macro lens. The animals were killed several hours after the onset of light (8L:16D schedule) by deep anesthetization followed by intracardiac perfusion of fixative. All animals were cared for and treated in accordance with the ARVO Resolution on the Use of Animals in Research.

Surgery

Unilateral retinal detachments were produced in cats using a refined version of the procedure recently published elsewhere.[4] Animals were anesthetized with an intramuscular injection of ketamine HCl (100 mg/ml) and acepromazine maleate (10 mg/ml), and maintained under deep anesthesia with periodic injections of ketamine. For local anesthesia a retrobulbar injection of 0.5 cc of lidocaine (2%) was given. Extracapsular lens extraction was performed leaving the posterior capsule intact. The corneal incision was closed and the eye allowed to heal for a minimum of 2 wk. For the detachment surgery an infusion cannula was sewn in place in the inferotemporal quadrant of the clear cornea using a 5.0 nylon mattress suture. A 20-gauge incision was made in the superotemporal quadrant of the cornea and an Ocutome (CooperVision; Irvine, CA) was used to remove the posterior capsule and vitreous. After vitrectomy a fluid–gas exchange was performed. A glass micropipette with a flat 80–100 μm tip diameter was mounted on a micromanipulator and then inserted into the incision. Using a syringe fitted to a Harvard infusion pump, sodium hyaluronate (Healon, Pharmacia; Uppsala, Sweden 0.5 mg/ml) was injected slowly as the pipette was advanced into the retina. When the pipette tip reached the subretinal space (SRS) a small bleb formed creating a retinal detachment. The detachment was enlarged to the desired size by regulating the amount of Healon injected into the SRS. Retinae were reattached using the following procedure: Animals were anesthetized as before. A 5.0 dacron mattress suture was placed in the inferotemporal quadrant of the cornea approximately 1.5 mm from the limbus. A 20-gauge incision was made and an infusion cannula was secured. A second 20-gauge incision was made in the superotemporal quadrant. Air was infused through the cannula and fluid was drained using a 20-gauge blunt beveled tip on a Charles fluted needle. A mixture of 50 or 75% sulfur hexafluoride (SF6) and air was flushed through the eye until a complete exchange was achieved. Finally, the incisions were sealed, and the eye was allowed to heal. In several animals intraocular pressure was monitored by pneumotonometry at 6-hr intervals over a 24-hr period. No abnormal pressure elevations were detected. No cryotherapy or photocoagulation was used around the retinal hole. In several animals, the retina flattened spontaneously without requiring surgery.

Light and Electron Microscopic Autoradiography

The fixation protocol and staining techniques employed in this study have been previously described.[4] Light microscopic autoradiograms were prepared as follows: 1–2 mCi of ^{3}H-Leucine in 0.2 ml of phosphate buffer was injected intraocularly 24 or 48 hr prior to fixation; or, 200 mCi of ^{3}H-thymidine in buffer was injected 2 hr prior to fixation. After fixation and embeddment in Araldite (6005), 1-μm sections were processed for light microscopic autoradiography by the dipping method. Sections were placed on cleaned glass microscope slides. The slides were dipped into a 1:1 aqueous solution of NTB–2 nuclear track emulsion (Eastman Kodak; Rochester, NY) under sodium vapor illumination. After drying, they were transferred to light-tight slide boxes and exposed in the dark at 4°C for 3–7 days. The slides were developed in full-strength D–19 at 20°C for 2 min, stopped in distilled H_2O, fixed, for 10 min, and washed in running deionized H_2O. Autoradiograms were stained sequentially with a mixture of Azure II and methylene blue, and then with basic fuchsin as described previously. Electron microscope autoradiograms were prepared according to a method modified from Young and Droz.[20] Thin sections (60–80 nm) were transferred to parlodian-coated (1% in isoamyl acetate) glass microscope slides. The slides were stained with aqueous uranyl acetate and lead citrate, coated with a thin layer of carbon by vacuum evaporation, and dipped (under sodium vapor illumination) into a 1:4 aqueous solution of Ilford L–4 nuclear track emulsion. After drying, the dipped slides were placed in light-tight boxes along with packets of dessicant, sealed, and exposed at 6°C for 2–4 months. The slides were then developed in freshly prepared phenidon developer for 1 min at 15°C, fixed in 2 changes of 30% thiosulfate, and washed in running distilled H_2O for 15 min. Next, the parlodian film was floated off the glass slides onto a surface of distilled H_2O, and 200-mesh copper grids were placed directly on top of the sections. The film and grids were then transferred to filter paper and allowed to dry. Finally, the grids were cut away from the film and thinned by immersion in isoamyl acetate for 2 min.

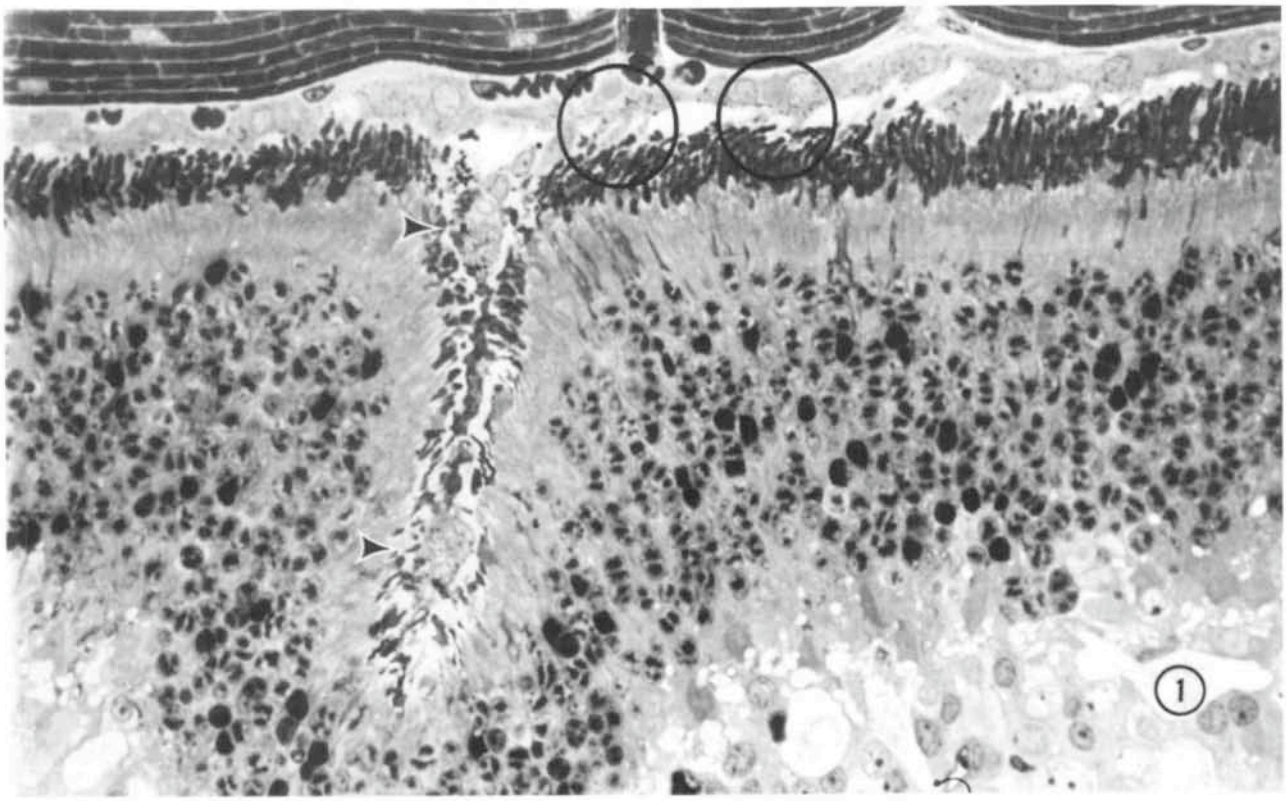

Fig. 1. An area of folding in a reattached retina. There is outer segment debris and a few cell bodies within the SRS (arrows). The encircled areas show locations where abnormal extensions of the apical RPE processes contact the outer segments. Note the pyknotic ONL nuclei adjacent to the fold. (42d:30r) (×1,125).

Light Microscopic Measurements

Measurements of outer segment length were obtained using a Zeiss Universal Research Microscope (Carl Zeiss, Inc.; Oberkochen, West Germany) and 100× oil immersion objective (final magnification = ×1250). The outer segments were carefully aligned along their longitudinal axes by systematically varying the tissue cutting angle. One-micron sections were cut and stained using the protocol described above. An ocular micrometer was used to measure the width of the outer segment layer. Repeated measurements were made at approximately 500-μm intervals in multiple tissue blocks in order to provide a rough estimate of the range of outer segment lengths for a given area of reattachment.

Results

At low magnification in the light microscope, most of the reattached retinae show few obvious signs of disruption. The normal stratification of the retina is usually preserved, although in retinae detached for lengthy intervals, some reduction in the thickness of the outer nuclear layer is usually apparent. At higher magnification all of the reattached retinae show some degree of abnormality, ranging from subtle changes at the outer segment–RPE interface to dramatic degenerative effects in the outer retina. In addition, we noted that within the area of reattachment adjacent retinal regions vary considerably in their degree of abnormality. Abrupt transitions in morphology from one region to an adjacent region in the same eye are not unusual. As such, it is impossible to establish a precise timetable of morphological recovery or to identify critical periods in the recovery process with certainty. But we were able to identify a number of general trends, and we did find reattached regions in different retinae that shared many characteristics. Therefore we grouped these recurring morphological patterns into several different categories, and these are described below.

Retinal Folds

About 60% of the reattached retinae have occasional pleats or tucks where the outer nuclear layer (ONL) is folded over upon itself. As shown in Figure 1, the photoreceptor outer segments face each other rather than apposing the apical surface of the RPE which is not folded. Some of the larger folds form small bulges that project out into the vitreous. Within the folds, the outer segments are reduced in number, with individual outer segments appearing abnormally short and disorganized.

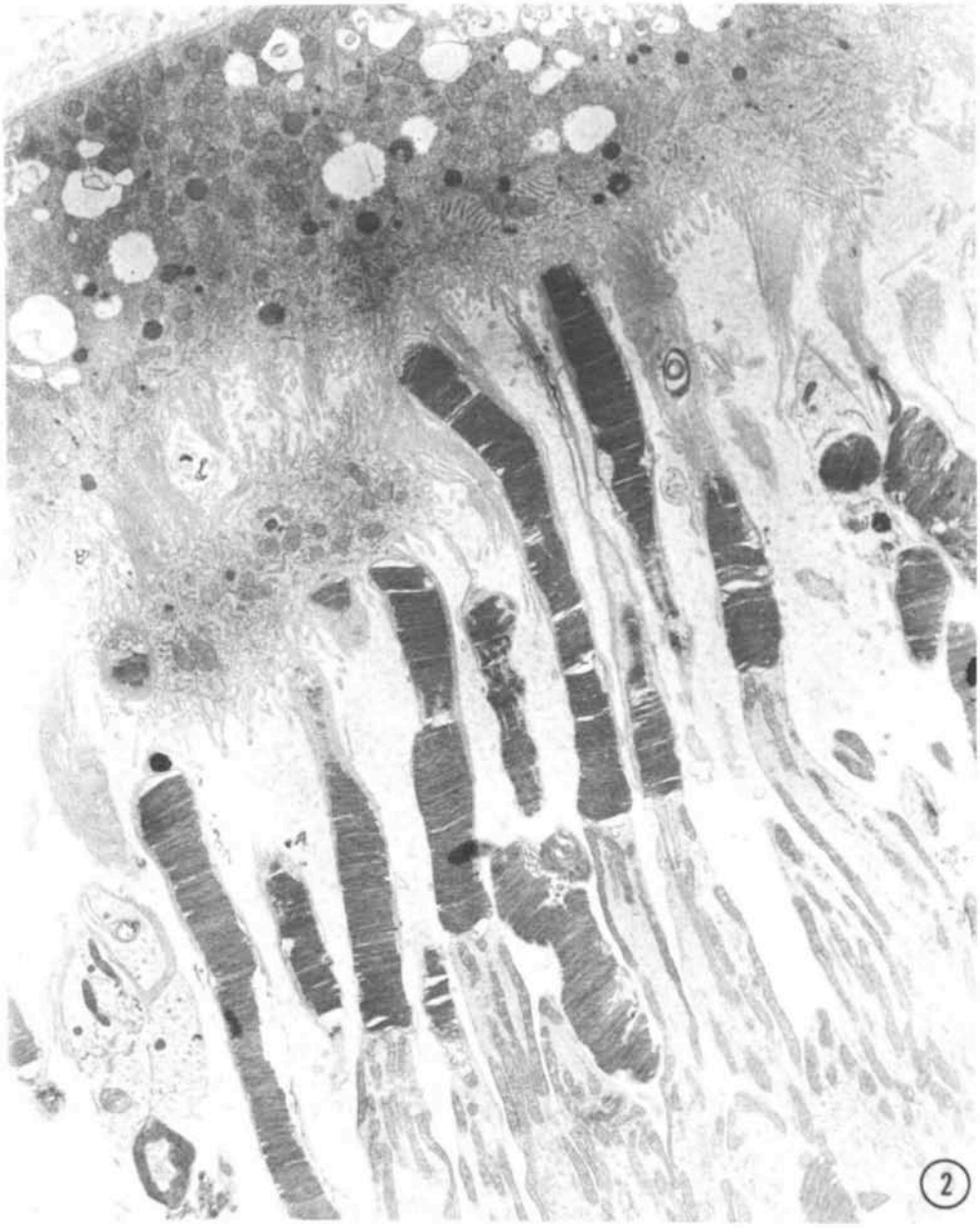

Fig. 2. Electron micrograph from an area of reattached retina (3d:210r). In many reattached regions, such as this one, the configuration of the apical RPE processes and their interdigitation with the outer segments is highly unusual. The dimensions and organization of some regenerated ROSs are normal. However, they do not appear in a densely packed, parallel row. (×18,500).

The space between the outer segments contains lamellar debris and phagocytic cells that have many of the characteristics of RPE cells. We have called these cells RPE phagocytes to distinguish them from blood-borne phagocytic cells.[4] Proliferating RPE cells as well as Müller cell processes are often found at the base of the folds next to the apical RPE surface. Pyknotic cell bodies, primarily in the outer nuclear layer (Fig. 1), are also evident within and adjacent to the folds.

The Apical RPE Surface

The apical RPE surface acquires a mounded or scalloped profile shortly after the cat retina is detached. This mounding persists in the reattached retina, and is evident to varying degrees in almost all reattached regions from both short and long-term reattachments (see Figs. 2–3). Apical processes project from the mounded surface and contact the photoreceptor outer segments or, in the absence of outer segments, the distal inner segment tips. In some of the long-term reattachments, closely packed, parallel arrays of sheet-like processes, similar to the sheaths that normally envelop cat cone outer segments, emerge from the mounded apical surface (Fig. 2). These processes interdigitate with both rod and cone outer segments and appear more numerous than in normal cat retina (Fig. 4). Some arrays follow long and tortuous routes before they contact the

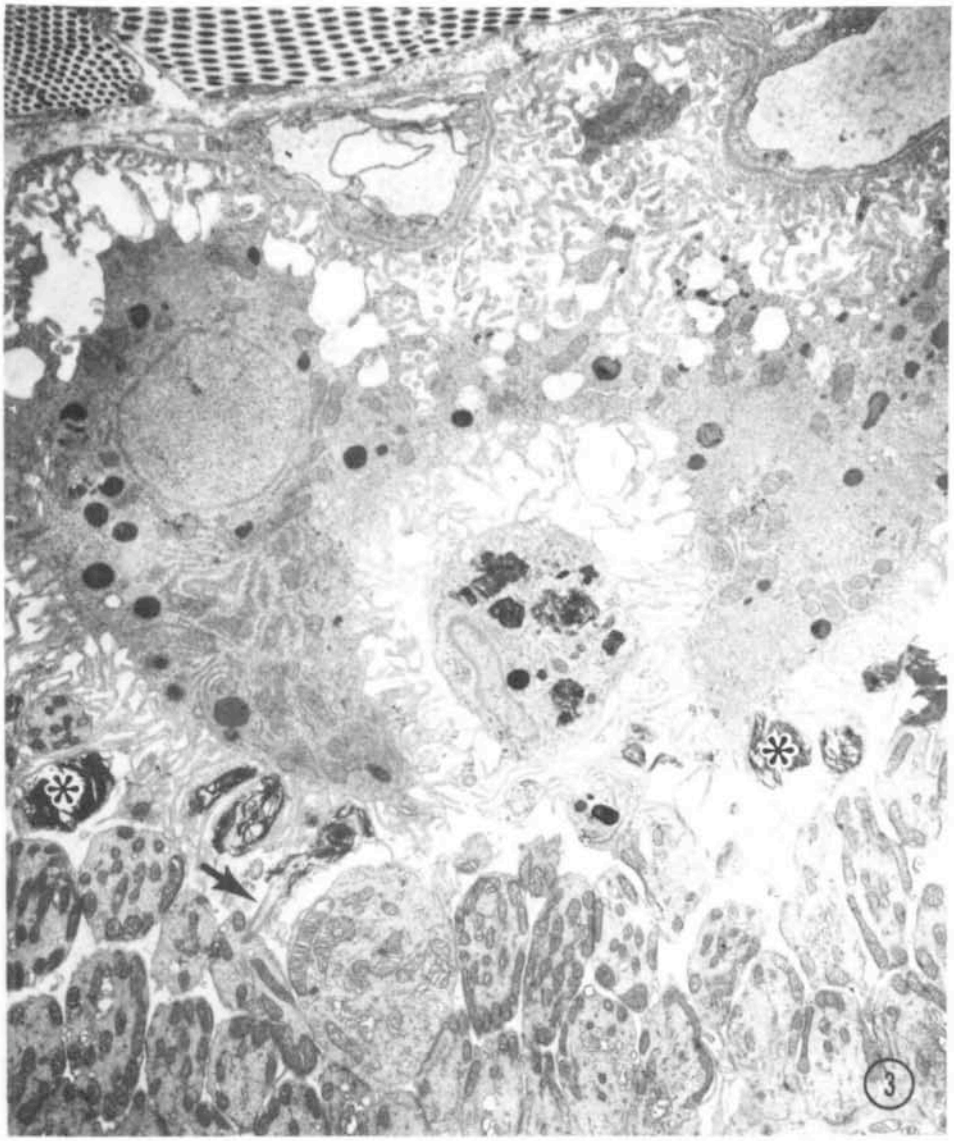

Fig. 3. Photoreceptor–RPE interface 1 wk after reattachment (5d:7r). In this low power view, the scalloped apical surface of the RPE is clearly evident. The shortened outer segments in this region consist of disorganized disc membranes that appear along the perimeter of the apical surface (asterisks). Microvilli envelop the shortened outer segments. One outer segment (arrow) shown is connected to its cilium. (×4,000).

outer or inner segments. Large packets of phagocytized outer segment discs occur within these processes. In contrast to the uniform alignment of normal cat outer segments (Fig. 4), those in the reattached retina often appear misaligned in longitudinal section because they are inserted along the perimeter of the scalloped apical surface (Fig. 3).

RPE Proliferation

We observed some degree of RPE proliferation in all reattached retinae that had been detached for longer than 1–2 days. There was significant variability in the extent of proliferation between animals. The most extensive proliferation occurred in retinae that had been detached longer than 1 wk. Proliferation in each of the long-term reattached retinae (210 days) was not substantially different from what could have occurred during the detachment interval alone. Despite the presence of a large number of proliferated RPE cells, light microscopic autoradiograms (ARGs) from the 42d:210r retina showed no evidence of ^{3}H-thymidine incorporation into RPE nuclei during the 2 hr prior to fixation.

In short-term retinal detachments, we identified no collagenous matrix or basal lamina in association with proliferated RPE cells. However, in the reattached retinae, a collagenous-type matrix and an associated basal lamina are often present. Segments of basal lamina occur along the cell surfaces next to this extracellular matrix. Within the subretinal space (SRS), the proliferated RPE cells occur in two general configurations. In one, the daughter RPE cells are arranged in small clusters (Fig. 5) that do not display any apical-basal surface polarity. These cells are connected by adhering junctions, but apparently lack the occluding junctions

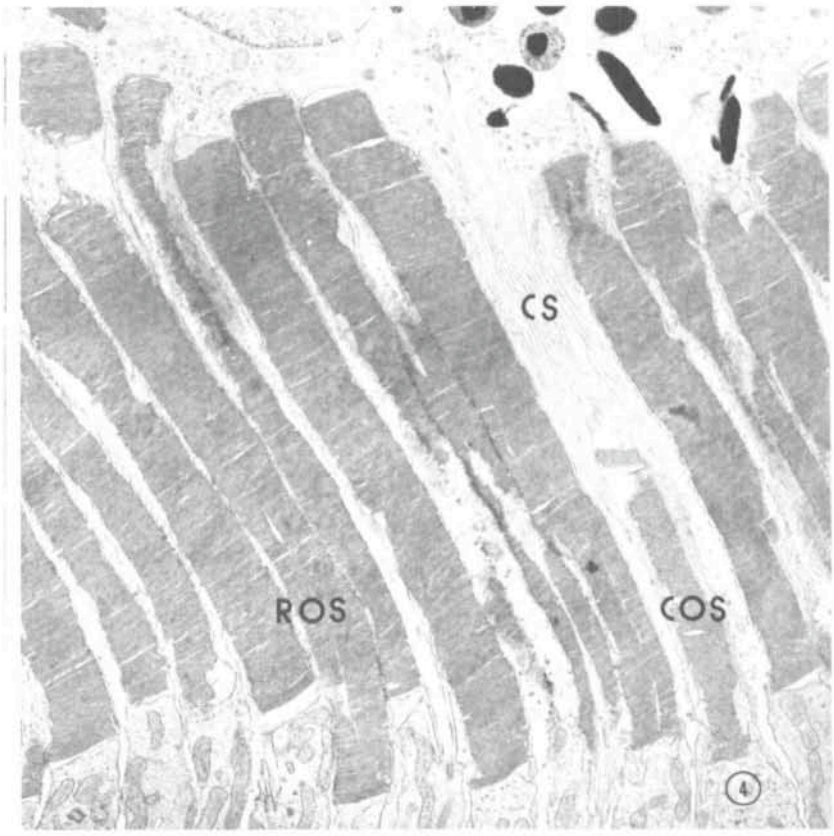

Fig. 4. Electron micrograph of the photoreceptor and RPE in the normal cat retina. The outer segments are oriented along their longitudinal axes in a uniform, densely packed row. Cone outer segments (COS) are somewhat shorter than rods (ROS and are ensheathed by a complex array of apical processes known as the cone sheath (CS). The apical surface of the RPE is not scalloped or mounded as it is after detachment and reattachment. (×11,500)

and gap junctions found normally in RPE cells. In the other configuration, the new cells form additional monolayers of polarized cells that parallel the original monolayer (Figs. 6, 7). The surface polarities of the new monolayers need not be the same as the original one. For example, the polarities of the three monolayers shown in Figure 6 (L_1–L_3) are reversed with respect to each other. The polarity of L_2 is the opposite of the original monolayer (L_1) and, similarly, the polarity of L_3 is the reverse of L_2. The net result is that the apical surfaces of L_1 and L_2 and the basal surfaces of L_2 and L_3 appose each other.

When the RPE cells assume a multi-layered configuration and when the additional layer adjacent to the

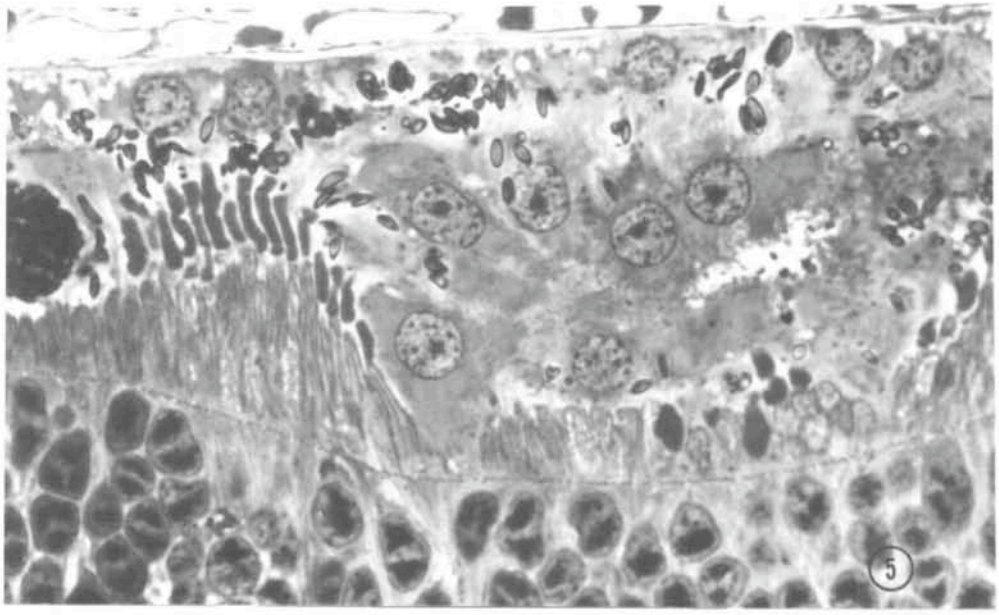

Fig. 5. An area of RPE proliferation in a reattached retina (7d:210r). Only a few outer segment remnants remain under the cluster of proliferated RPE cells. The dimensions of the outer segments adjacent to the zone of proliferation are normal. (×1,800).

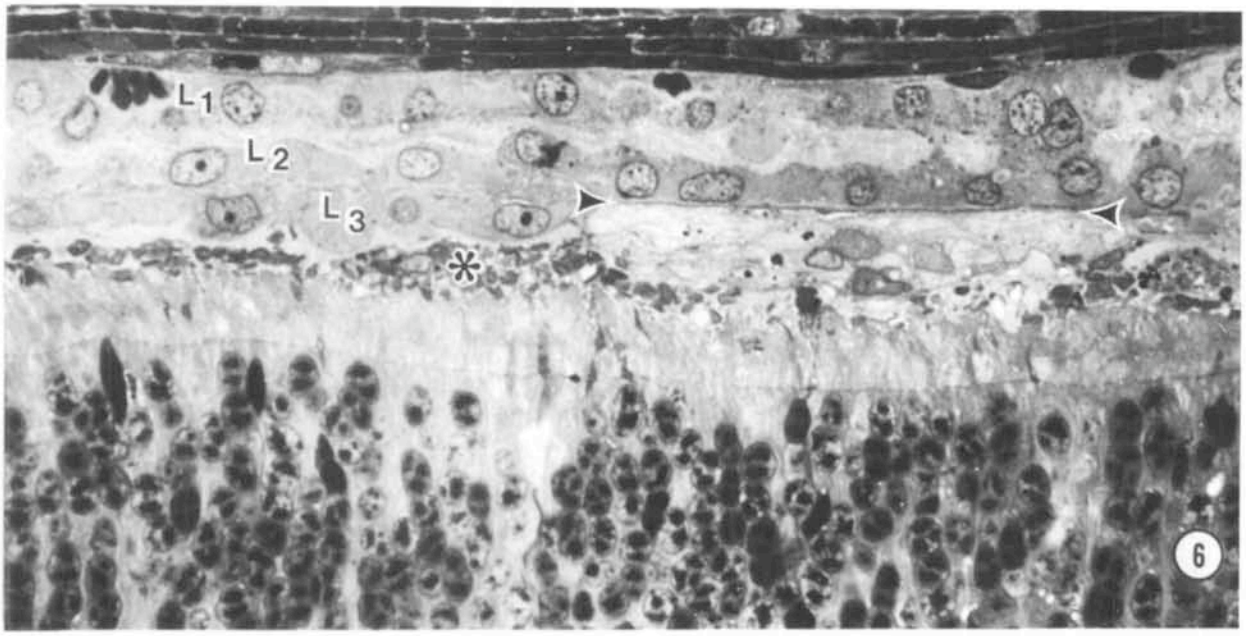

Fig. 6. An area of multi-layered RPE in a reattached retina (14d:30r). Three monolayers of RPE cells are present (L_1, L_2, L_3), each displaying different surface polarity. The apical surfaces of L_1 and L_2 face each other, as do the basal surfaces of L_2 and L_3. The basal lamina of L_2 is clearly evident (arrows). Only outer segment fragments (asterisk) appear near the inner segment tips. A number of pyknotic ONL cell bodies are also apparent. (×800).

outer segments expresses the correct surface polarity, apical processes from the new monolayer interdigitate with the outer segments and phagocytize shed packets of discs. The cells that comprise the new monolayer contain a number of inclusions, including phagosomes, while inclusions are notably absent in the cells that make up the original monolayer (Fig. 7).

Outer segment recovery directly underlying a cluster of proliferated RPE cells is usually poor. Rod outer segments (ROSs) underlying a region of proliferated cells are usually less than one half the length of ROSs in the immediately adjacent region (Fig. 5). Inner segments remain intact, but the number of mitochondria and other organelles is reduced when compared to controls. The same pattern occurs on a smaller scale near so-called hyperpigmented RPE cells. These cells, which usually occur singly or in association with normally pigmented RPE cells, are interspersed through-

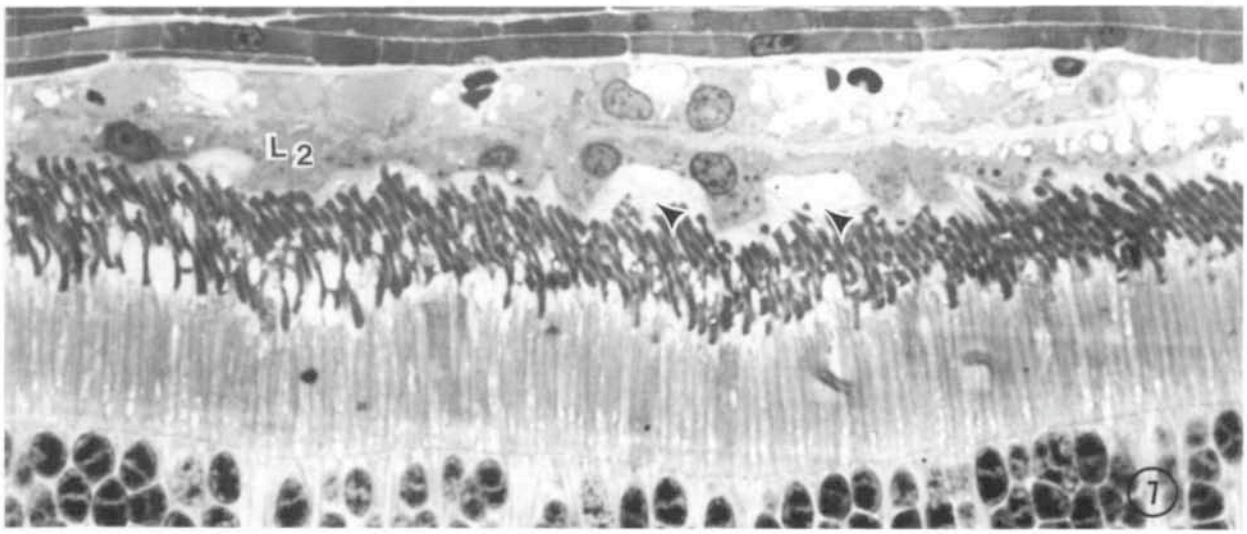

Fig. 7. In this reattached region, the second monolayer of RPE cells (L_2) displays normal surface polarity. The cells have an even basal border and long microvillous processes (arrows) extend from the highly mounded apical surface to contact the outer segments. (42d:30r) (×800).

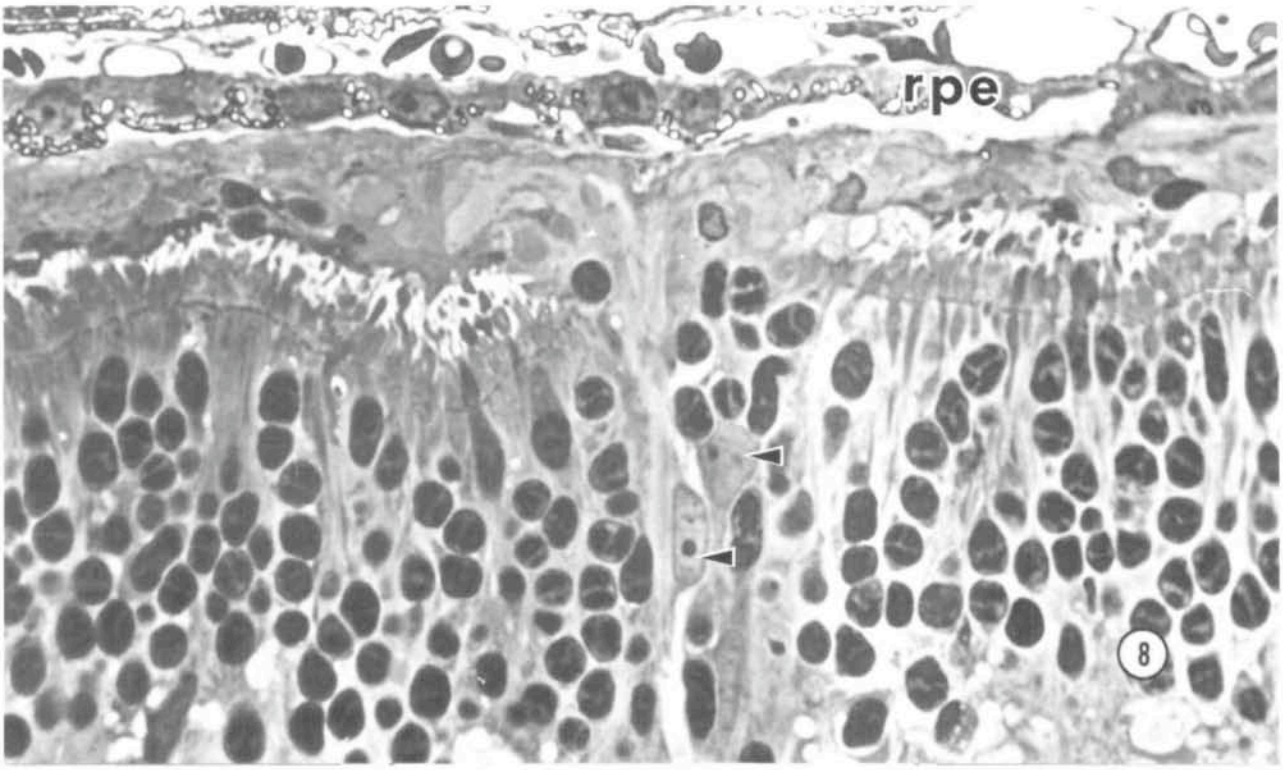

Fig. 8. An example of subretinal Müller cell hypertrophy in a reattached retina (42d:30r). Müller cell processes and photoreceptor cell bodies project into the SRS through a discontinuity in the outer limiting membrane creating a barrier between the photoreceptors and the apical RPE surface. No outer segment regeneration is apparent in this region. The arrows show two Müller cell bodies that have migrated from their normal location in the inner plexiform layer to the ONL. (×1,125).

out the outer segment layer (Fig. 5). They do not appear to form junctions with neighboring cells, and they too apparently retard the regeneration of underlying outer segments.

Subretinal Gliosis

In long-term retinal detachments, Müller cell processes and photoreceptor cell bodies appear in the SRS forming multiple layers between the retina and RPE.[2-5] We also identified such areas in the reattached retinae that had been detached for 42 days. In Figure 8, two Müller cell nuclei are located immediately adjacent to the disrupted outer limiting membrane (OLM). Müller cell processes and displaced photoreceptor nuclei project into the SRS through a discontinuity in the outer limiting membrane (OLM). Processes project laterally from the "gap" creating a barrier between the photoreceptors and the apical RPE surface. Photoreceptor recovery in such regions is always poor. Almost all photoreceptors underlying this subretinal barrier have no outer segments. There is also an obvious reduction in the number of photoreceptor nuclei beneath gliotic regions. The photoreceptors that remain have smaller ellipsoids with a reduced number of inner segment organelles.

Photoreceptor Morphology and Renewal

Photoreceptor recovery after reattachment varied considerably from retina to retina and from one reattached region to adjacent regions. In general, however, those retinae that were detached for the shortest intervals (ie, 1 wk or less) showed the best recovery, while those detached for long intervals (6 wk) showed poor recovery. For example, in a comparison of several animals with similar reattachment times but different detachment times, measurements of ROS length differed sharply. ROSs in the central portion of the superior nasal quadrant ranged from 8.8–17.2 μm in the short-term detachments (3d:210r, 7d:210r) to 0.0–7.3 μm in the long-term detachment (42d:210r) (see Table 1). Almost all reattached regions in the 42d:210r retina show a considerable reduction in ONL thickness, many pyknotic photoreceptor nuclei, atrophied inner segments, and shortened outer segments (Figs. 9, 10); in some areas outer and inner segments are completely absent (Fig. 11). This was a consistent pattern in those animals whose retinae were detached for the longest times. In comparison, most reattached areas in the animals with detachments of 3 and 7 days fared much better (Fig. 2). A reduction in photoreceptor cell bodies and the concomitant thinning of the ONL is limited to a few

Table 1. Range of outer segment lengths after retinal reattachment

Detachment period	*Reattachment period*	*Outer segment length (range)*	
		Rods	*Cones*
0 (normal)	0	11.0–18.2 (μm)	8.8–13.1 (μm)
5 (days)	7 (days)	5.5–7.3	2.2–3.6
5	7	1.8–9.1	*
8	7	7.3–14.6	7.0–8.0
10	7	3.5–7.0	*
3	30	9.1–14.6	9.0–10.0
14	30	5.1–13.9	2.8–3.5
42	30	3.6–14.6	*
3	210	8.8–17.2	8.8–13.1
7	210	12.8–16.4	3.6–7.3
42	210	0.0–7.3	*

* Measurements unavailable because of disruption of the outer segment layer.

locations only. In some areas, ROS length as well as outer segment structure appears to be normal. Electron microscope autoradiograms in the 3d:210r animal show an advancing front of radiolabeled protein positioned 4–5 μm from the outer segment bases 48 hr after an intravitreal injection of ^{3}H-Leucine (Fig. 12). A similar displacement occurred in ROSs from the opposite control eye. In an adjacent area from the same animal (3d:210r) (Fig. 13), the outer segments are truncated and disorganized. No discrete band of labeled protein can be identified, but the silver grains over the outer segment fragments indicate that newly synthesized protein was incorporated into the outer segments during the preceding 48 hr. A comparison between the animals whose retinae were reattached for 30 days shows similar results. ROS length in the retina detached for 3 days is normal in most regions (Fig. 14A), but many ROSs in the retina detached for 42 days are shortened, and there is evidence of pyknosis in the ONL (Fig. 14B).

ROSs are also abnormally short in those animals reattached for brief periods (1 wk). Measurements from the central area of the superior nasal quadrant in 4 such animals (10d:7r, 8d:7r, 5d:7r(2)) yielded ROS ranges of 3.5–7.0 μm, 7.3–14.6 μm, 1.8–9.1 μm, and 5.5–7.3 μm respectively (Table 1) (see Figure 14C–E). This compares with values of 11.0–18.2 μm for ROSs at similar locations in normal control eyes. Each of the four animals was injected with ^{3}H-Leucine intraocularly 24 hr prior to fixation. Light microscopic ARGs from the reattached areas show a concentration of labeled protein at the expected location close to the outer segment bases.

Although ROS length is normal in several of the reattached retinae, photoreceptor inner and outer segments often retain subtle ultrastructural anomalies. In some cases, an extension of the rod inner segment appears alongside the outer segment plasma membrane creating an asymmetrical expansion of the ellipsoid. In other cases, there is a slight reduction in inner segment mitochondrial density, or an apparent elongation of the ellipsoid.

In many of the reattached retinae, cone outer segments (COS) were virtually impossible to identify because of the misalignment and disorganization of the outer segment layer. Therefore, we were able to obtain COS length data only from reattached retinae where the outer segment layer was not disrupted. In several of these retinae, we noted that the COSs appeared shorter than normal, with wide variability in their lengths. In the superior retina outside of the area centralis, COSs in normal cat retinae are about 60% of the ROS length, approximately 6–7 μm in the periphery and 10–13 μm nearer the posterior pole (in Araldite-

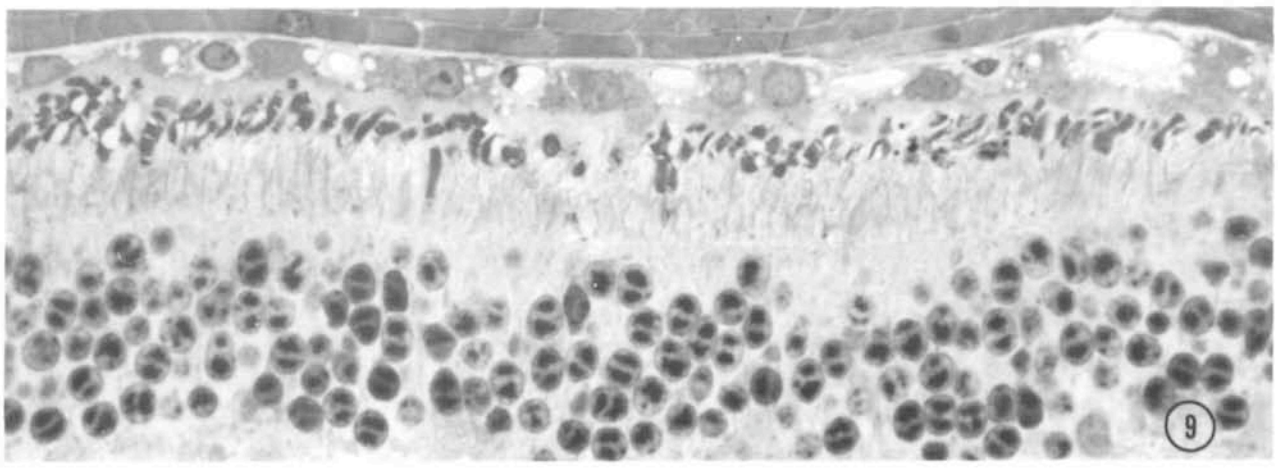

Fig. 9. After lengthy detachment episodes followed by long-term reattachment, the outer segments are usually shortened, and the number of nuclei in the ONL is reduced. The apical RPE surface retains the mounded profile acquired after detachment. (42:210 (×800).

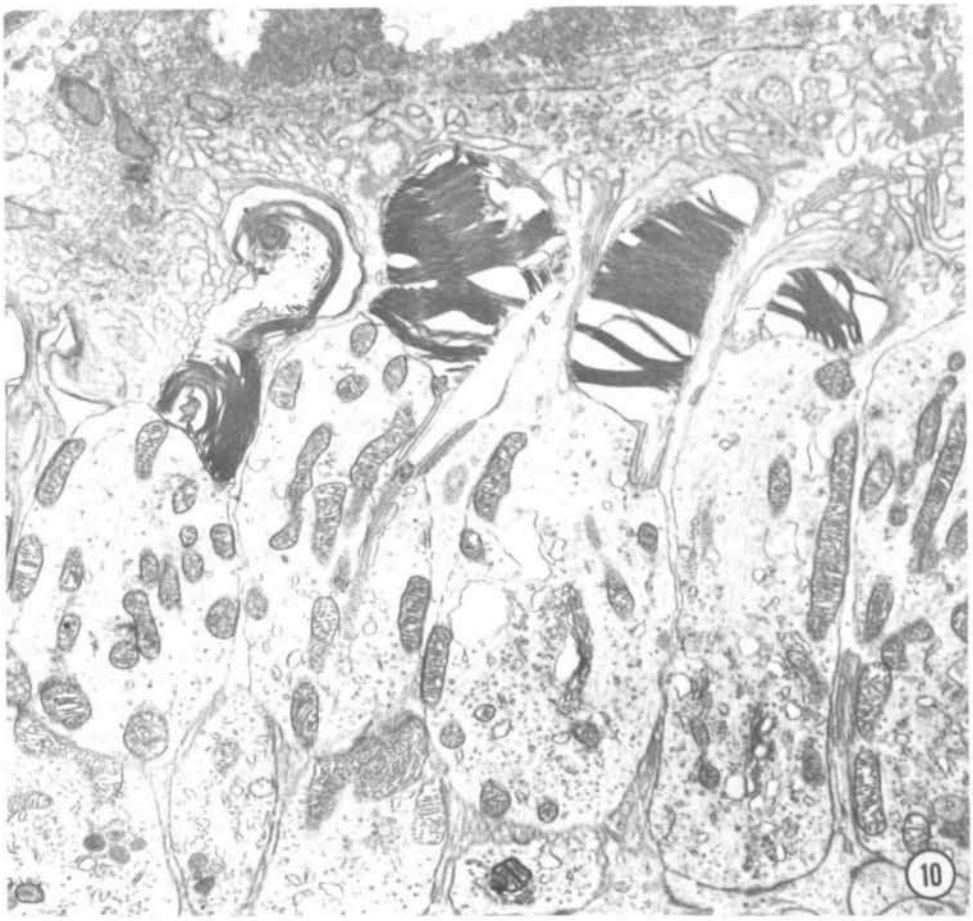

Fig. 10. In an electron micrograph from the area shown in Figure 9 (42d:210r) the regenerated outer segments are shortened substantially and inner segment mitochondria appear less numerous. However the basic organization of the disc stack, its ensheathment by the apical processes and its relationship to the connecting cilium are still intact (×6,600).

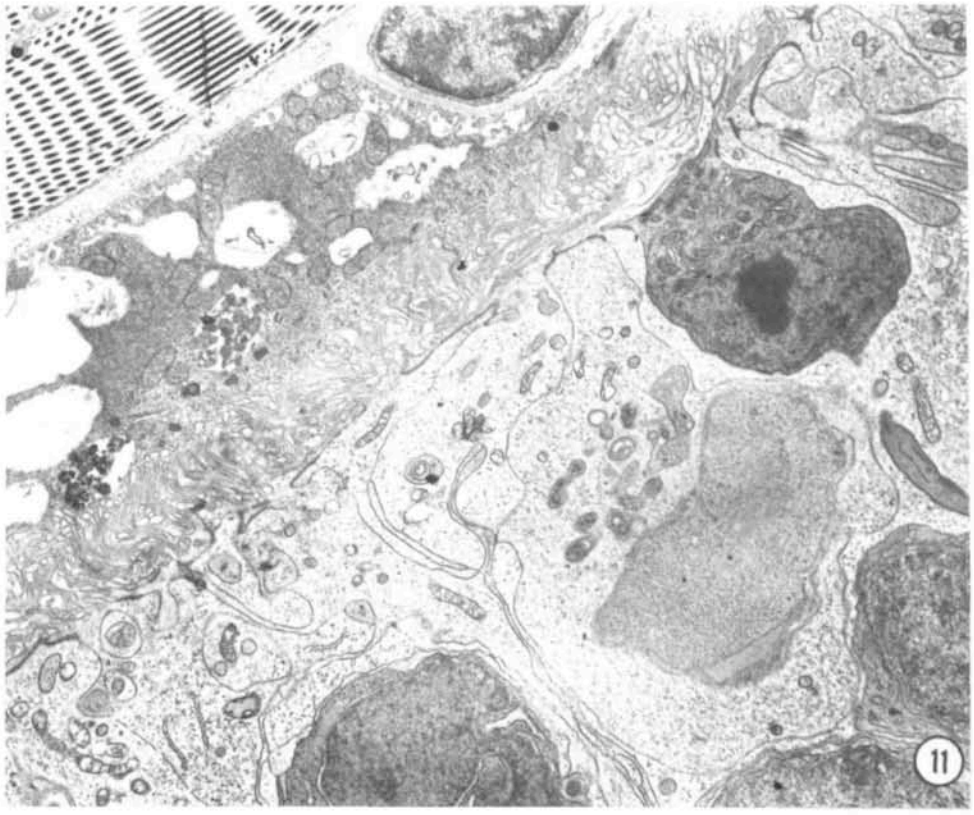

Fig. 11. An electron micrograph from the retina described in Figures 9, 10 (42d: 210r). There is no evidence of any outer or inner segments in this region. Only the photoreceptor cell bodies, including the nucleus and a small amount of cytoplasm, remain. The junctions which comprise the outer limiting membrane are apposed to the apical RPE surface (×3,200).

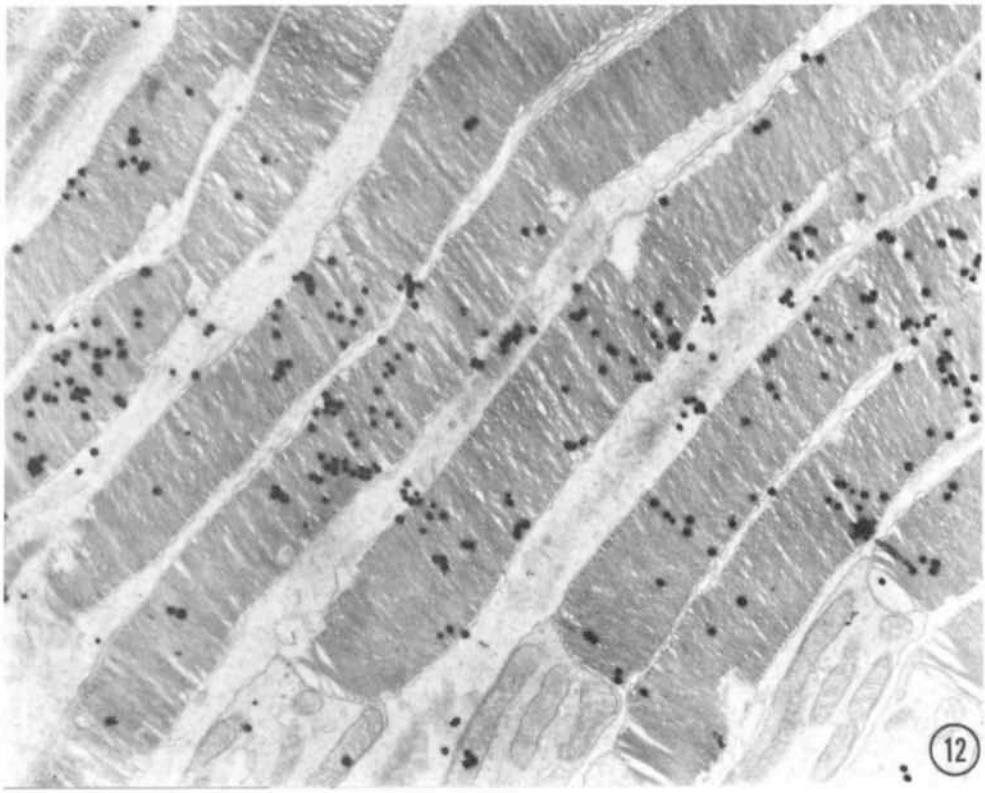

Fig. 12. Electron microscope autoradiogram of ROSs in a reattached retina (3d:210r). Forty-eight hours prior to fixation, ^{3}H-Leucine was injected intraocularly. An advancing front of labeled protein is positioned 4–5 μm from the ROS bases (×7,500).

embedded tissue). The values are somewhat broader in the reattached retinae (Table 1). In two of the animals (5d:7r, 14d:30r) COSs are quite short (2.2–3.6 μm) (Fig. 14D), whereas in two others (3d:180r, 3d:30r) their length appears near normal (8.8–13.1 μm). Short COSs may retain a normal cylindrical organization, but contain many fewer discs than normal outer segments. The disc membrane at the outer segment base, the site of disc morphogenesis, is structurally normal. The most basal discs are continuous with the

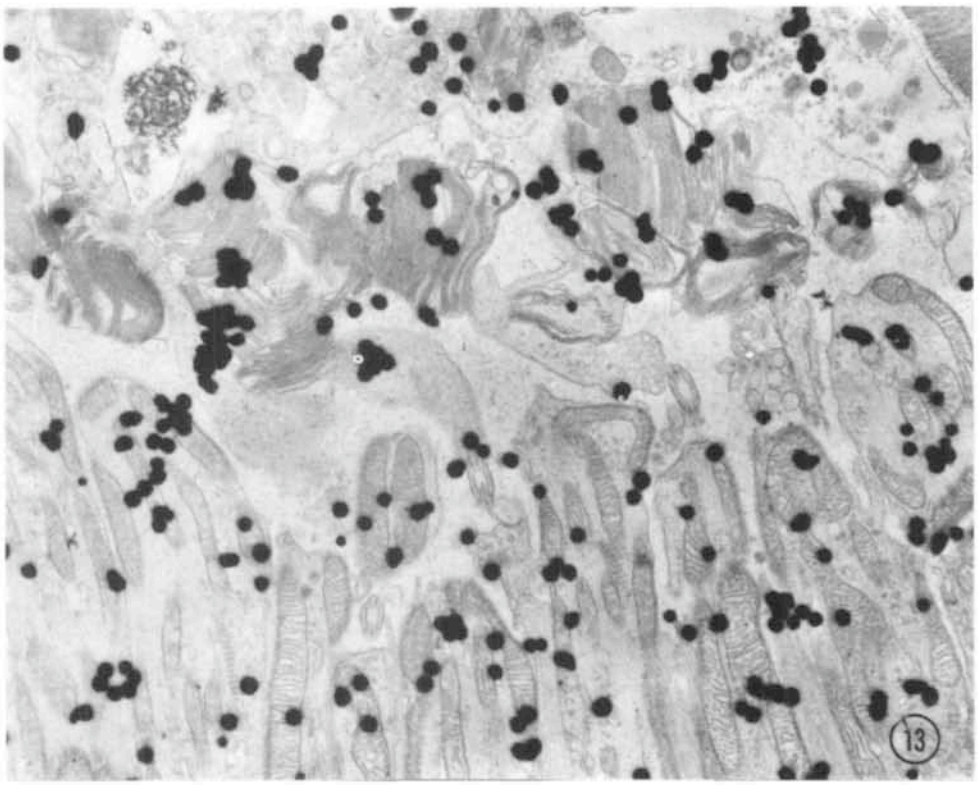

Fig. 13. Electron microscope autoradiogram of ROSs in an area adjacent to that in Figure 12. No pattern is observed, but the labeling of the disorganized disc membranes indicates that newly synthesized protein was incorporated into the outer segments during the preceding 48 hr (×6,000).

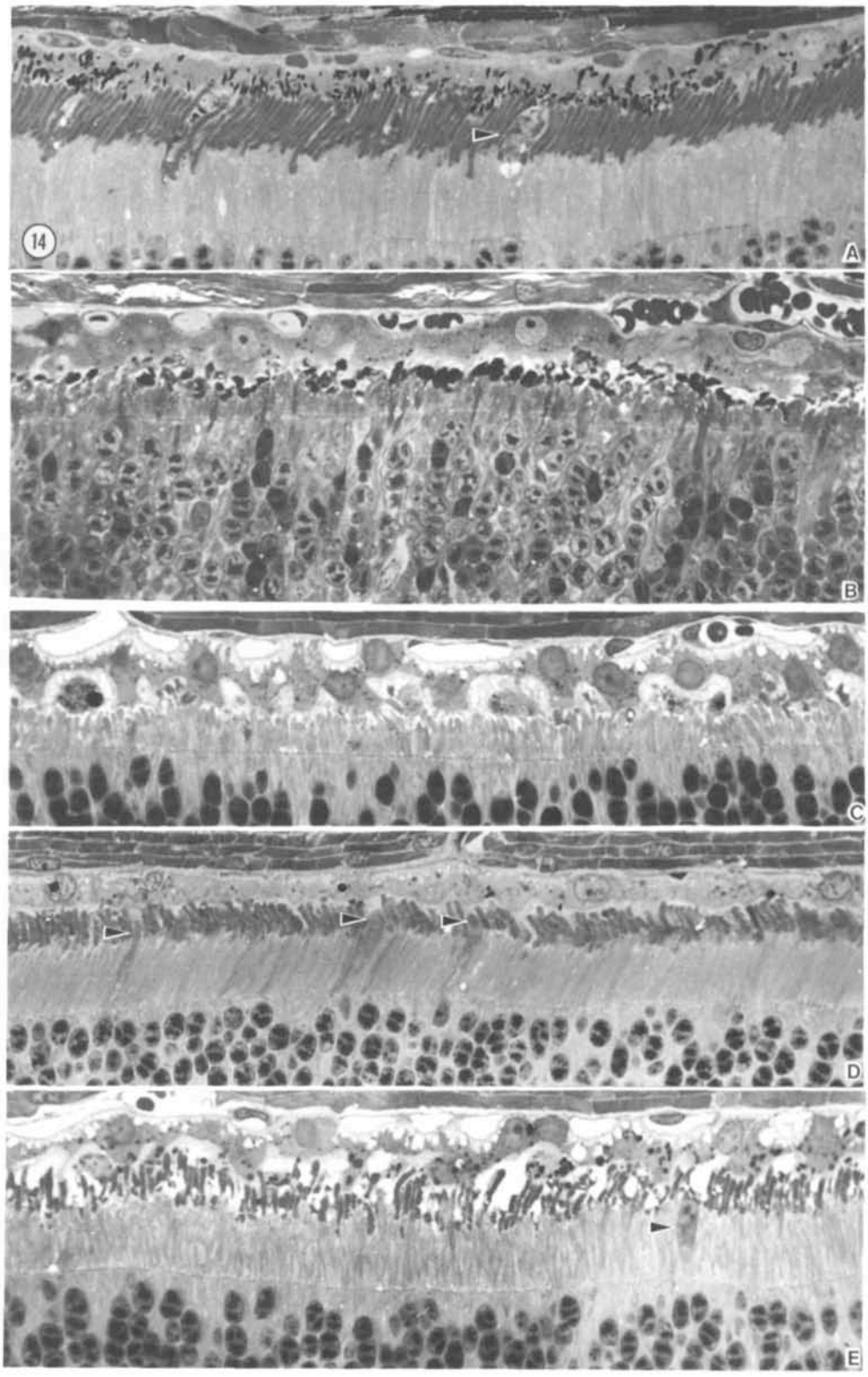

Fig. 14. **(A)** The photoreceptor–RPE interface after short-term detachment and 1-month reattachment. After short detachment intervals the outer segments can reattain normal length in a relatively short time frame. Discrete areas of disruption, however, can still be identified. Note the migrating cone nucleus in the center of the field (arrow) (3d:30r) (×800). **(B)** In contrast to 14A, lengthy detachment intervals (42 days) coupled with a 30-day reattachment period (42d:30r) result in distinctly inferior outer segment regeneration. Note the pyknotic photoreceptor nuclei in the ONL (×800). **(C)** Light micrograph from a retina detached for 5 days and reattached for 7 days (5d:7r). There is virtually no evidence of outer segment regrowth in this region. The apical RPE processes extend down to the inner segment tips. This probably represents one of the earliest stages in the recovery process (×800). **(D)** Light micrograph from a second animal whose retina was detached for 5 days and reattached for 7 days (5d:7r). In this case the ROSs are approximately one-half normal length. The 3 COSs shown in the field (arrows) are also abnormally short (×800). **(E)** The photoreceptors and RPE in a retina detached for 8 days and reattached for 7 days (8d:7r). In this region there is a wide range of outer segment lengths. The outer segment tips are positioned along the perimeter of the scalloped surface, and that may account for the uneven spacing between outer segments. Displaced cone nucleus (arrow) (×800).

plasma membrane on the centric face of the connecting cilium (Fig. 15). Sheet-like RPE processes contact the shortened outer segments. Cilia with associated striated rootlets and basal bodies can sometimes be identified within these RPE processes. When the COS is not present or limited to only a few disorganized disc membranes, the processes ensheath the distal portion of the cone inner segment.

The distribution and number of organelles in such cones are also affected. Organelles that are usually segregated in the myoid and ellipsoid regions are distributed throughout a single, smaller compartment. Mi-

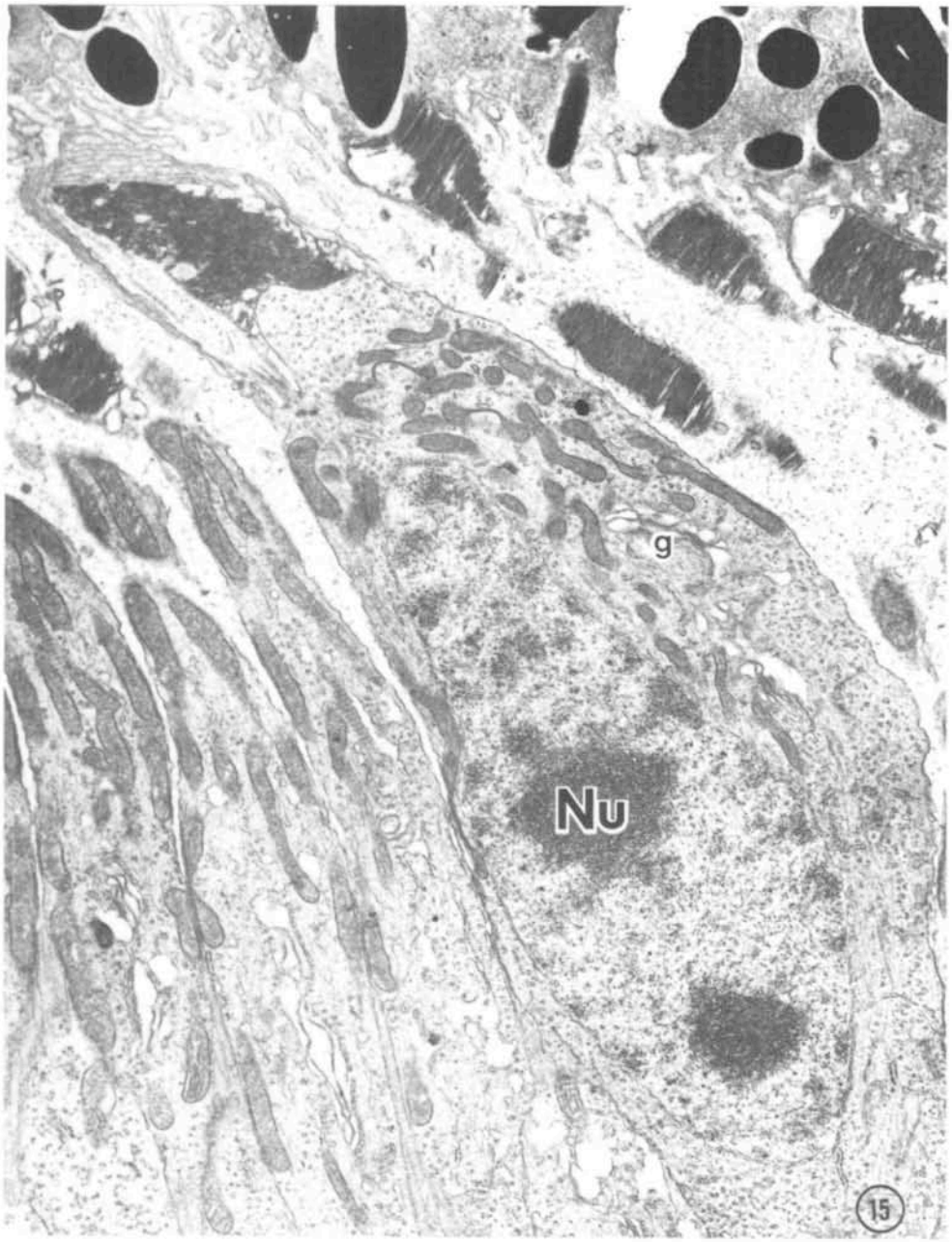

Fig. 15. Cone photoreceptor in ı retina detached for 7 days and eattached for 210 days. The COS is structurally normal, but t contains many fewer discs than ısual. The inner segment is omposed of a single compartnent that includes the nucleus Nu), the remaining mitochonlria of the ellipsoid, Golgi aparati (g) and rough endoplasmic eticulum (×7,000).

tochondrial density is much reduced, and many cone nuclei are displaced into the inner segment from their normal locations just vitread to the outer limiting membrane (Fig. 15). Electron microscope autoradiograms show that, 48 hr after intravitreal injection of ^{3}H-leucine, most of the labeling is found over the cone nuclei in these cells whereas cones from the control eye show most labeling over the disc membranes with very little nuclear labeling.

Discussion

Experimental studies of reattachment have been reported previously in the rabbit,[7] owl moneky,[8-9,11] and rhesus monkey[10] retinas. In the previous monkey studies, detachments were produced by injecting a solution of hyaluronidase into the vitreous cavity. After mechanical disruption and repeated aspiration of the vitreous, 0.2 cc of digested vitreous was injected with sufficient pressure to produce a retinal hole and local detachment, which usually progressed to a total detachment.[10] We used a different method to produce detachments. After a core vitrectomy, a glass micropipette was used to inject a dilute solution of sodium hyaluronate (Healon; 5 mg/ml, Pharmacia; Uppsala, Sweden) directly into the subretinal space. Hyaluronic acid is a naturally occurring component of the interphotoreceptor matrix,[21] and is commonly found in human subretinal fluid samples in a wide range of concentrations (0.01–33.5 mg/ml).[22] We cannot rule out the posibility that injected Na-hyaluronate influences

the process of reattachment and the resulting morphology. In some locations, it can be observed histologically in the subretinal space after brief detachment episodes. In reattachments, it was rarely identified by light or electron microscopy, so it is presumably metabolized or otherwise removed from the subretinal space during the detachment/reattachment interval. Therefore, Na-hyaluronate appears to be an appropriate substance to use where experimental control is required over detachment duration, size, and retinal location.

A comparison of previous studies with the present one yields a number of similarities as well as some interesting differences. The retinae of all species examined had reattached regions where the outer retinal layers appear folded or buckled. In the owl monkey, this occurred in animals that had been detached for long intervals (8–12 wk).[8-9] In the cat, we found no firm correlation between detachment duration and retinal folding. In the owl monkey, detachments of less than 7 days showed little RPE mounding upon reattachment.[8] Cat retinae detached for shorter intervals (ie, 3 days) show pronounced mounding that persists after reattachment. Irregularities in and "thinning" of the nuclear layers are present in the monkey reattachments detached for ≥8 wk.[8] In the cat reattachments, there is a definite reduction in the number of photoreceptor nuclei in retinas detached ≥1 month, in areas of folded retina, or in regions of RPE or Müller cell proliferation; intermittent areas of pyknosis occur at the shortest detachment intervals examined. In the owl monkey pyknotic nuclei were not identified after two days reattachment irrespective of the detachment interval.[8] Kroll and Machemer[10] observed disc membrane-like profiles within the distal inner segments of rhesus monkey rods and cones that they interpreted as an aberration of disc morphogenesis. In the cat we found no instances of disc membranes in the inner segment cytoplasm.

There is very little data on the rates of disc synthesis or disposal after reattachment. One study[11] suggests that the outer segments undergo a phase of acclerated disc synthesis within the first 24 hr, but the disc displacement rate could not be measured directly due to outer segment disruption. The same limitation applies to the cat retinae reattached for brief intervals. However, several relevant inferences can be drawn from the cat retinae reattached for 1 wk. ARGs from these animals show a band of labeled protein at the ROS bases at the normal time, 24 hr after injection of ^{3}H-amino acids. Estimates of ROS length 1 wk after reattachment are clearly less than those of the controls (see Table 1; Fig. 14C–E). This suggests that regenerating outer segments must take longer than 1 wk to reattain normal length. If ROSs were to maintain new disc synthesis at the normal rate (2.3 μm/day)[23] immediately after reattachment, the outer segments could achieve normal length (12–18 μm) about 1 wk later—but only in the absence of any disc shedding. Because maintenance of constant outer segment length is a function of both the disc synthesis and disposal rates,[24] regenerating outer segments probably take several weeks or longer before they achieve equilibrium. The fact that some outer segments are abnormally short many months after reattachment suggests that their reduced length may be sustained indefinitely.

Secondly, there is a surprisingly wide range of ROS lengths within individual retinae and between different retinae with similar detachment/reattachment times. This may reflect inherent variability in the detachment/reattachment process itself. Some areas may lag behind others in forming a close photoreceptor–RPE apposition. The extent of separation between the retina and RPE during detachment could also influence the recovery process.

The regenerative capacity of COSs may be inferior to that in rods, although the evidence is equivocal. Rhesus COSs, but not ROSs, reportedly show ultrastructural abnormalities one month after reattachment.[10] In the cat, COSs in early reattachments are substantially shorter than control outer segments in the same or opposite eye. In addition, nuclear displacement and the disruption of inner segment compartmentalization is limited almost exclusively to cones. This displacement of photoreceptor organelles is morphologically identical to the early stages of a phenomenon recently identified in normal rat and human retinae where photoreceptor cells are eventually displaced into the subretinal space.[25-26] It is possible that these changes are early stages in cell degeneration and, therefore, reflect a selective loss of cone photoreceptors in the reattached retina. We expect that reattachment studies currently underway in the primate macula will help resolve this issue.

Whether or not photoreceptors can recover completely from an episode of detachment has not been resolved. Previous studies in experimental animals reached different conclusions. Nakamura[7] reported limited and variable outer segment recovery in rabbit retinae that spontaneously reattached without requiring any surgical manipulations. In contrast, Kroll and Machemer[9-10] concluded that ". . . the prominent changes of a detached retina are reversible, so that a reattached retina can look normal after approximately 4 wk. This is true for all detachments of up to 12-wk duration." At the light microscope level, the reattached cat retina can sometimes appear histologically normal. However, ultrastructural morphology does not return to the pre-detachment state even after brief episodes of detachment coupled with prolonged recovery periods. The photoreceptor mosaic in the reattached cat retina is a literal microscopic patchwork of different

regions each with different characteristics. The fact that some rod and cone outer segments do not reattain normal length, even after prolonged reattachment periods, implies some maintained imbalance in the assembly and/or disposal phases of the outer segment renewal process.

The tendency of both RPE and Müller cells to proliferate after detachment results in similar detrimental effects after reattachment. Müller cell processes extending into the SRS act as a cellular barrier that prevents RPE-photoreceptor reapposition. Proliferated RPE cells also produce an abnormal layer of intervening cellular material. Outer segment recovery where Müller cell processes have invaded the SRS is invariably poor, but in areas of RPE proliferation the extent of recovery depends upon the configuration and surface polarity of the proliferated cells. Morphologically, subretinal proliferation is very similar in the cat and human retinas.[27-28] Future efforts aimed at suppressing proliferation and hypertrophy in these two cell types could lead to improved visual recovery after reattachment.

Re-establishment of the Photoreceptor–RPE Interface: Early Events

The initial molecular interactions that occur between the photoreceptors and RPE cells after reattachment remain obscure. The photoreceptors are not reapposed to their original loci on the apical RPE surface. How then is the ensheathment of individual rod and cone outer segments by the apical processes re-established and, secondly, how is the regrowth of the outer segments regulated? At present, the specific events in this process as well as the precise time course are matters for speculation. But our results in the reattached retina suggest a number of parallels between initial photoreceptor development and the recovery that ensues after reattachment.

Immediately after reattachment, the tips of the photoreceptor inner segments are juxtaposed to the mounded and undifferentiated apical surface of the RPE (see Fig. 14C). Undefined metabolic processes which promote RPE–photoreceptor adhesion tend to maintain that apposition.[29-30] The apposition of the detached retina must engage a sequence of molecular events that elicits and controls the re-differentiation of the RPE apical surface and the re-ensheathment of the photoreceptors by the apical processes. Because the ensheathment of rod and cone outer segments is different in many mammals,[31] there must be a mechanism for regulating the configuration of apical microvilli to the two photoreceptor classes. This is virtually the same process that occurs during initial development.

Results in the reattached retina, as well as in the developing retina,[32] confirm that the apical processes appear before the outer segments are present. They are longer and more numerous in the reattached compared to normal retina. The point at which the outer segments cease to elongate during the recovery phase must depend upon the net difference in the rates of disc production and disposal, just as it does in the developing retina.[24] In the reattached retina, however, persisting abnormalities in either or both of these processes may lead to substantial regional variation in outer segment lengths. Furthermore, regenerated outer segments are not oriented normally in a single parallel row. Instead, they appear irregularly oriented in longitudinal section because the topography of the apical RPE surface has been permanently altered during detachment.

Functional Implications

The morphological changes described above could have significant consequences for the recovery of vision after reattachment. A widespread loss of photoreceptor cells, especially cones, could have obvious and irreversible effects on acuity, color vision, photopic sensitivity, and stereopsis could be affected secondarily. Misalignment of regenerated outer segments could affect aspects of visual sensitivity.[17] Many of the morphological changes we observed affect underlying photoreceptor cells in many localized regions—perhaps producing small multiple defects in the visual field. In experimentally detached retinae, these phenomena tend to occur with limited frequency or in small circumscribed regions, especially at short detachment durations. Therefore, their functional effects may be similarly restricted to small retinal regions. Although reattached retinae may be interlaced with many such regions where photoreceptors and perhaps other retinal cells are functionally abnormal or degenerate, the deficits may be insignificant from the standpoint of overall visual capacity.

On the other hand, such changes could have profound effects on visual recovery if they occur within the macula. In humans, the area centralis has a diameter of 5.5 mm, and the foveola measures approximately 350 μm in diameter.[33] Retinal changes that might otherwise be inconsequential in the peripheral retina may be quite significant if they occur in this small, but highly specialized, retinal location.

The presence of a macular detachment is associated with persistently reduced acuity, metamorphopsia, and with chronic disturbances in color vision (primarily tritanomalous defects) after reattachment.[6,14,16,18-19] Recent evidence strongly suggests that most variation in final acuity is attributable to macular detachment duration.[19] Because different mammalian retinae respond similarly to experimental detachment, we expect that the morphological changes identified in the reattached cat retina also occur in the reattached primate

macula. Our initial results in the detached and reattached rhesus monkey fovea support that conclusion.* These changes at the photoreceptor–RPE interface begin to provide an explanation, at the cellular level, for the poor visual recovery associated with lengthy detachment durations, and for the lack of complete visual recovery after macular detachment in humans.

In summary, we conclude that a modified version of the normal intercellular relationship between the photoreceptors and the RPE cells is re-established after reattachment. The morphological abnormalities are not homogeneous across the retina. They range from subtle ultrastructural anomalies at the outer segment–RPE interface to dramatic degenerative and proliferative changes in the outer retina. The degree of recovery depends broadly upon detachment and reattachment parameters. The best recovery is associated with short detachment periods, but a return to completely normal retinal morphology is probably unattainable. If we assume that this modified relationship between the photoreceptors and the RPE also occurs after retinal reattachment in humans,* it is apparently sufficient to subserve near normal vision under ideal circumstances.

Key words: reattachment, detachment, photoreceptor, RPE, retina

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* Our unpublished results in the Rhesus monkey strongly suggest that virtually all of the changes described in the cat also occur in the reattached primate macula.