Investigative Ophthalmology & Visual Science, Vol. 30, No. 8, August 1989

Retinal Reattachment of the Primate Macula

Photoreceptor Recovery after Short-Term Detachment

Christopher J. Guérin, Don H. Anderson, Robert N. Fariss, and Steven K. Fisher

The macula of the neural retina from 12 adult rhesus monkeys (*Macaca mulatta*) was detached from the overlying retinal pigment epithelium (RPE) by subretinal injection of a balanced salt solution. Seven days later, the two layers were reapposed by draining fluid from the vitreous cavity and replacing it with a 3:1 mixture of sulphur hexafluoride gas and air. Animals were sacrificed at 1 hr, 2 days and 7 days after detachment, and at periods ranging from 3 to 14 days after reattachment. At 2–7 days prior to sacrifice, some eyes received an intravitreal injection of ^{3}H-L-fucose. The eyes were then fixed for light and electron microscopy (EM), and tissue sections were processed for autoradiography (ARG) or immunocytochemistry. During the 7-day detachment interval, rod outer segments (ROSs) and cone outer segments (COSs) degenerated, but inner segments remained intact and the rest of the retina appeared normal. The apical RPE surface dedifferentiated during the detachment interval. At 3 days after reattachment, a regrowth of rudimentary ROSs and COSs had occurred, but the disc stacking was clearly abnormal. ROSs and COSs both showed an increase in length and a tendency to return to their normal configurations with increasing time after reattachment. ROSs and COSs regained approximately 40% of their normal lengths after a 2-week reattachment period; however, persistent outer segment abnormalities were frequently found in otherwise well regenerated areas. Autoradiographic results confirmed that new disc membranes were synthesized subsequent to reattachment. Newly synthesized rod disc membranes were uniformly labeled using antibodies to bovine opsin. Regenerating outer segments interdigitated with newly formed apical RPE processes, and radiolabeled phagosomes were identified within the RPE cytoplasm by 1 week after reattachment. Proliferation of the RPE cell layer was identified at some locations in all animals, and was strongly correlated with a lack of underlying outer segment regeneration. Because of the short detachment interval, and the absence of underlying pathology or trauma, the recovery process described here probably represents an example of optimum recovery after retinal reattachment. Invest Ophthalmol Vis Sci 30:1708–1725, 1989

The outer segments of photoreceptor cells are organelles specialized for the absorption and transduction of light quanta into neuronal activity. Degeneration of these organelles is one of the most prominent morphological changes, and one of the most significant factors in the loss of vision, after retinal detachment. Conversely, the capacity of rods and cones to regenerate their outer segments and to reestablish their normal anatomical relationship with the retinal pigment epithelium (RPE) is an important factor in defining the limits of visual recovery after retinal reattachment.

From the Neuroscience Research Institute, University of California, Santa Barbara, California.

Supported by research grants EY-02082 and EY-00888 to DHA and SKF, respectively, from the National Eye Institute, National Institutes of Health, Bethesda, Maryland.

Submitted for publication: September 19, 1988; accepted March 8, 1989.

Reprint requests: Christopher J. Guérin, Neuroscience Research Institute, University of California, Santa Barbara, CA 93106.

Evidence from experimental studies of retinal reattachment is generally consistent in demonstrating that outer segments retain some capacity for regeneration after detachment.[1–4] In addition, the prevailing evidence strongly suggests that detachment duration is an important, and perhaps critical, variable in that process.[4] Precise identification of temporal parameters, however, has proven to be elusive due to small sample sizes and the variability inherent in the experimental detachment procedure. In studies of human retinal detachments it has been reported that visual acuity following reattachment can sometimes approach predetachment levels, although the prognosis is much less favorable if the macula is involved.[5–9] These results imply that the photoreceptor–RPE layer in the macular region either has a reduced capacity for recovery, or that the high degree of anatomical and functional specialization of that area leads to more severe consequences following injury.

Here we report our initial findings on morphological recovery of the primate macula after experimental

retinal detachment and reattachment, focusing on the early stages of recovery following a short detachment interval (7 days). The photoreceptor–RPE interface undergoes an extensive degree of "remodeling" in the first week following reattachment, with slower but continued recovery occurring over the next 7-day period. There is no indication that the macula is more or less susceptible to the effects of detachment than any other retinal region. The photoreceptor and RPE abnormalities, previously identified in the detached and reattached cat retina,[4,10] also occur in the primate macula. It is highly likely, therefore, that these abnormalities contribute to the lack of complete visual recovery usually associated with macular retinal detachments.

Materials and Methods

Unilateral retinal detachments were produced in 12 adult, domestically bred, rhesus monkeys whose average age was >10 years. Reattachments were performed after 7 days. The detachment and reattachment intervals, in days, are as follows with the number of animals at each time point given in parentheses: 1:0 (1), 2:0 (1), 7:0 (1), 7:3 (2), 7:5 (1), 7:7 (2), 7:14 (3), and 7:30 (1). The boundaries and other characteristics of each detachment were recorded on a standard ophthalmic detachment chart at the end of each surgery. The eyes were examined periodically using an indirect ophthalmoscope. The animals were killed by an overdose of barbiturate followed by cardiac perfusion of fixative. All animals were cared for according to the ARVO Resolution on the Use of Animals in Research.

Surgery

Unilateral retinal detachments were created using a variation of the technique we published previously.[4] In brief, animals were anesthetized initially with an intramuscular injection of Ketamine HCl (Bristol, Syracuse, NY) and Xylazine HCl (Haver Lockhart, Morris Plains, NJ), and surgical anesthesia was maintained with periodic injections of these agents. For local anesthesia a retrobulbar injection of 0.5 cc 2% lidocaine HCl (Vedco, Saint Joseph, MO) was given. An infusion canula was inserted into a 20-gauge incision made in the inferotemporal pars plana and connected to a bag of lactated Ringers solution (Baxter, Deerfield, IL). A second incision was made superior to the first and a complete vitrectomy was performed. Using the superior incision a micropipette (80–100 μm tip) was inserted into the vitreous cavity and advanced using a micromanipulator until it was just above the superior vessel arcade of the macula. Balanced salt solution (Alcon, Fort Worth, TX) was slowly injected through the pipette as it advanced through the retina. When the pipette reached the subretinal space (SRS) a small retinal detachment formed which could be enlarged to the desired size by regulating the amount of fluid injected. As the volume of injected fluid increased a bullous detachment formed which separated the neural retina from the RPE. In most of the animals the entire macula was detached, and in no case did the detachment include less than 50% of the macula. Initially the height of the detachment at its apex was at least several millimeters above the RPE surface, however, over the course of the next 7 days detachment height decreased spontaneously. At the end of the 7-day interval, detachments were not ophthalmoscopically visible. Postoperative antibiotics, consisting of gentamycin sulfate (Elkins-Sinn, Cherry Hill, NJ), cortiosporin ointment (Burroughs Wellcome, Triangle Park, NC) and penicillin (Bristol) were administered and all animals recovered without complications. Retinae were reattached by means of a simple fluid gas exchange with a mixture of 75% sulphur hexafluoride gas (Matheson, Cucamonga, CA) and 25% room air. Postoperative antibiotics were again administered as above. All animals recovered without complications. No attempt was made to seal the retinal hole and in no case was redetachment observed.

Light and Electron Microscopy

The fixation protocol and staining techniques employed in this study have been previously published.[10] Briefly, eyes were fixed by intracardiac perfusion of 1% gluteraldehyde and 1% paraformaldehyde in phosphate buffer, and then enucleated. Animals were entrained to a 12:12 light-dark cycle and euthanized 4 hr after light onset. After removal of the anterior segments, eyecups were immersed in the same fixative overnight. This was followed by post-fixation in osmium tetroxide, dehydration in graded ethanols and embeddment in Araldite 6005 resin. Sections (1 μm thick) were cut for light microscopy and stained with a mixture of methylene blue, azure II and toluidine blue in aqueous solution containing Na Borate, and then counterstained with basic fuchsin and photographed using a Zeiss photomicroscope III. Ultrathin sections for transmission electron microscopy were cut with a diamond knife, stained with uranyl acetate and lead citrate, and then carbon-coated prior to viewing.

Outer segment lengths were measured using a Zeiss Photomicroscope equipped with a ×100 oil immersion objective and an ocular micrometer. The protocol and criteria were essentially the same as those described previously.[11] Aligned rod and cone outer segments were measured in the maculas of normal control eyes and from the regions adjacent to the

Table 1. ^{3}H-fucose injection schedule

Detachment period (days)	*Reattachment period (days)*	*Labeling interval (hr)*
7*	3	72
7	7	168
7	14	2
7*	14	96

* Two animals were injected at these timepoints.

detachment in experimental eyes. Measurements from normal areas of experimental eyes, and normal areas from the opposite control eyes, were pooled and averaged together. Likewise, measurements taken from animals with identical detachment:reattachment intervals were also averaged together. Only outer segments that were well oriented for their full length were measured. At many of the earlier timepoints rudimentary outer segments were very disorganized or absent entirely; the lengths of very disorganized outer segments were not measured. Similarly, those photoreceptors with no apparent outer segment were not included in the measurements.

Autoradiography

^{3}H-L-fucose (500 μCi) was injected intravitreally at the timepoints listed in Table 1. Sections were processed for electron microscope autoradiography using the flat substrate method of Young and Droz[12] as outlined in Anderson, Fisher and Breding.[13] Thin sections (60–70 nm) were dipped in Ilford L-4 emulsion under Na vapor illumination and exposed for 4–6 weeks at 4°C. The autoradiograms were developed in complete darkness using either phenidon developer for 1 min at 15°C or Elon-ascorbic acid after gold latensification.[14]

Immunocytochemistry

For the immunocytochemical studies we employed the indirect, post-embedding technique described in Erickson et al.[15] Tissue specimens were fixed as described above, except that immersion fixation was for only 1 hr, the tissue was stained en bloc using 1% aqueous uranyl acetate, and dehydration was through a series of graded methanols. Tissue specimens were embedded in LR White resin polymerized at 52°C. Thin sections were placed on nickel grids and preincubated in normal goat serum for 15 min before overnight incubation in rabbit anti-bovine or rabbit anti-rat opsin diluted 1:400 in phosphate buffered saline containing 1% bovine serum albumin (PBS/BSA). After several washes in PBS/BSA, grids were incubated in goat anti-rabbit IgG-Au (5 nm) (Jannsen, Piscataway, NJ). After further washing grids were then stained briefly with osmium tetroxide vapors, Reynold's lead citrate and uranyl acetate, after which they were carbon-coated and viewed in a transmission electron microscope. Control sections were cut from nondetached areas of the experimental eyes as well as from the normal, contralateral eye.

Results

Changes after Experimental Detachment

Acute (ie, <1 hr) retinal detachments produced some disruption of the outer segment tips and the apical RPE surface in the detached area; this was more severe close to the injection site. Occasional red blood cells were apparent in the subretinal space but the detached retina surrounding the retinal hole remained intact. Figure 1A and B are low-power electron micrographs illustrating the morphological effects of separating the two cell layers using the detachment procedure outlined in *Materials and Methods*. Many outer segments remained intact and attached to their respective inner segments (Fig. 1B). Some large packets of lamellar material apparently detached from their inner segments as a result of the procedure and these were situated immediately above the intact outer segments. Pigment granules and associated membranous debris derived from the apical RPE processes were also present in this location. Very little outer segment debris was identified in the rest of the newly expanded subretinal space. The RPE cell monolayer was intact although the microvilli on the apical RPE surface were smaller, less numerous and shorter than normal (Fig. 1A). At the edge of the newly created detachment, there was an abrupt transition zone between detached and attached retina. The detachment height at the transition zone was quite shallow (ie, <1 retinal thickness) (Fig. 2). The adjacent attached area appeared normal.

At 2 days post-detachment, the appearance of the retina was similar to the acute detachment. Most outer segments were still present, but the normal parallel alignment of the disc membranes at the outer segment bases, the site of new disc assembly, was disrupted. The apical RPE surface had a scalloped profile and was populated by microvillous processes much shorter than those that normally interdigitate with photoreceptor outer segments.

After 7 days of detachment the region of detached retina that included the macula appeared flat ophthalmoscopically. Histologically, however, this region was still shallowly detached. The height of the detachment at 7 days ranged from tens of microns at its periphery (Fig. 2) to several hundred microns at its highest point. At this stage, the photoreceptor outer segments had degenerated almost completely, and the expanded subretinal space between the photore-

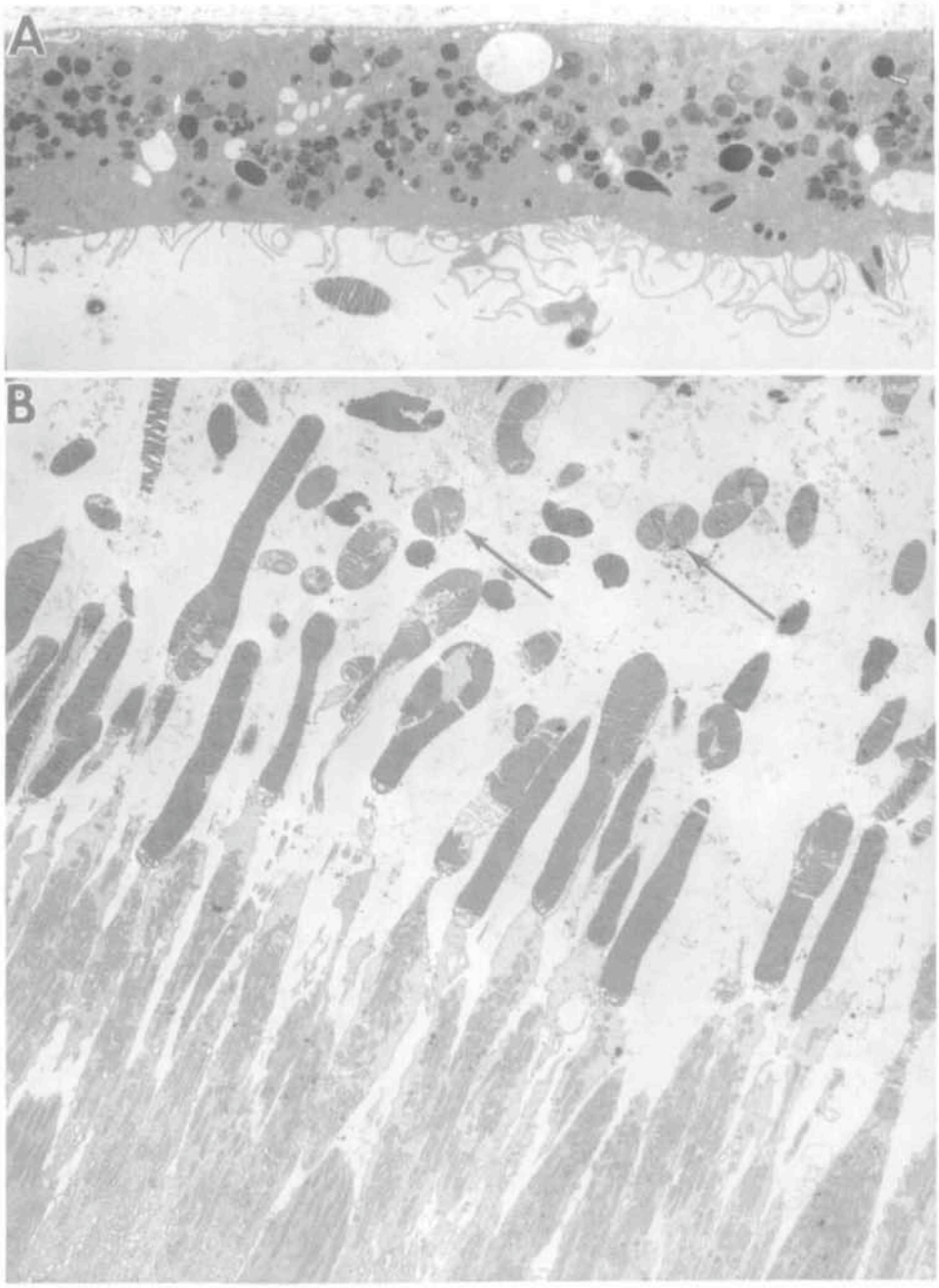


Fig. 1. Electron micrograph of the RPE (**A**) and photoreceptor layers (**B**) <1 hr after microinjection of balanced salt solution into the subretinal space. (**A**) The apical microvilli that interdigitate with the photoreceptor outer segments were shorter and less numerous after experimental detachment. Otherwise, the morphology of the RPE cell layer was normal. (**B**) The photoreceptor layer and the rest of the retina also remained intact after detachment. However, some outer segment fragments appeared in the subretinal space (arrows), while others remained attached to their respective inner segments. Membrane-bound vesicles and other cellular debris, presumably derived from damaged outer segments and RPE apical microvilli, were located in the interphotoreceptor space and immediately above the intact outer segments in the expanded subretinal space (A, ×2400, B, ×3625).

ceptors and the RPE was filled with lamellar debris (Fig. 2). Photoreceptor inner segments appeared intact, although the organelles usually concentrated in either the ellipsoid or myoid regions were often comingled in both cellular compartments. The number of mitochondria in the ellipsoid was reduced, individual mitochondria appeared distended, and photoreceptor nuclei were occasionally displaced from their usual positions into the inner segment. Phagocytic cells, filled with large amounts of membranous material, were prominent in the subretinal space near the distal inner segments. A small amount of disorganized membranous material was also evident at the distal tips of some of the inner segments. The morphology of the RPE monolayer was similar to its appearance at 2 days post-detachment.

Outer Segment Recovery after Reattachment

A light micrograph of a parafoveal control area from an experimental eye is shown in Figure 3A, illustrating the normal morphological relationship

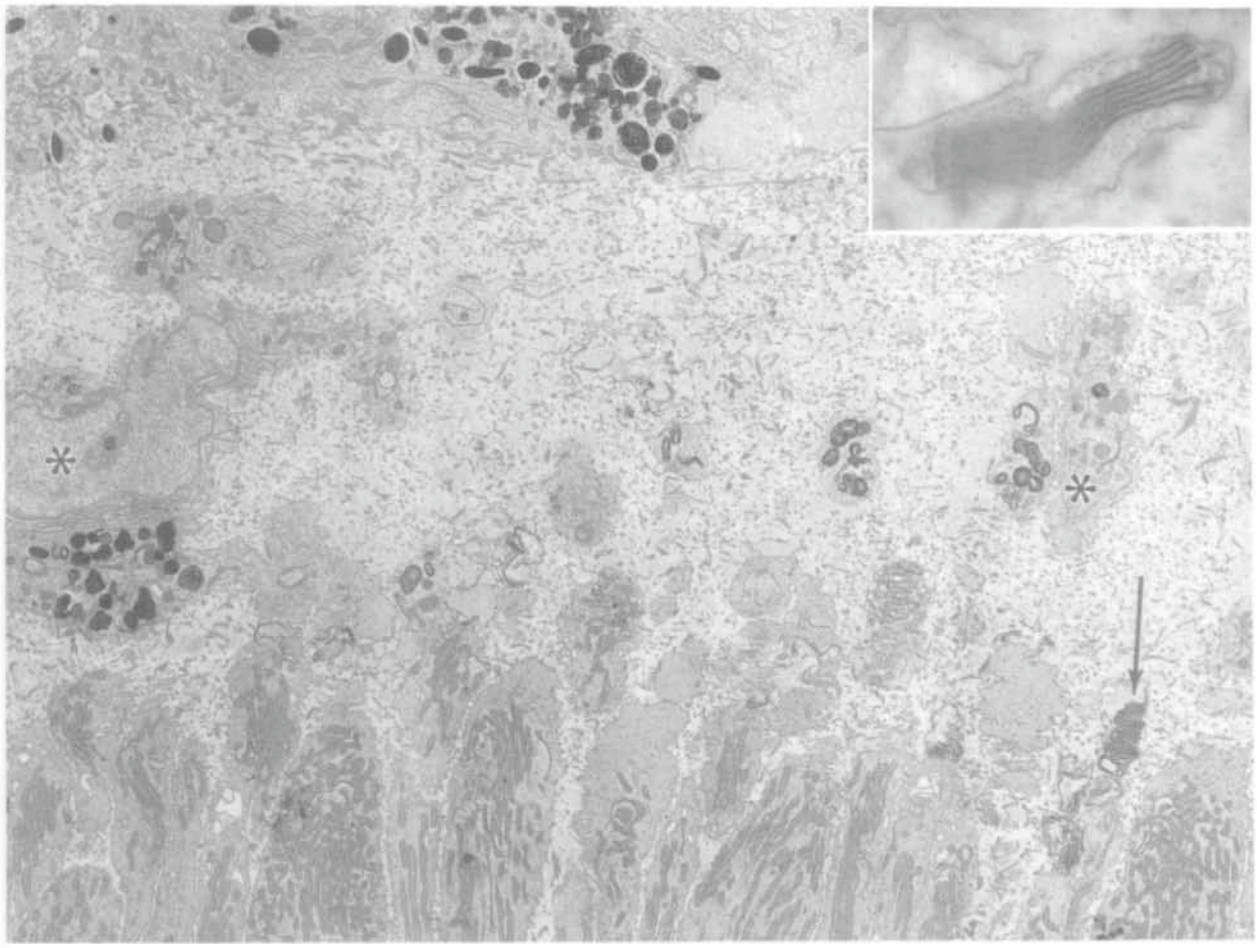


Fig. 2. The RPE–photoreceptor interface 7 days after experimental retinal detachment seen near the transition zone between attached and detached retina. Seven days after production of a macular detachment, the retina appeared flat by indirect ophthalmoscopy. However, histologically the retina remained shallowly detached. In this animal, the subretinal space was filled with lamellar debris (inset) almost certainly derived from degenerating photoreceptor outer segments. Only small disorganized stacks of membrane were apparent at the distal tips of some inner segments (arrow). Phagocytic cells of either RPE or hematopoietic origin were located in the subretinal space (asterisks) (×3300, inset ×39000).

between the photoreceptors and the RPE. The tips of the rod outer segments (ROSs) are enveloped by the RPE apical processes and are in close proximity to the RPE apical cell surface. Extrafoveal cone outer segment (COS) tips are recessed from the RPE apical surface and are ensheathed by an organized array of processes, the cone sheath, that terminates near the outer segment base. The cylindrical rod and cone outer segments are closely aligned, as are the myoid and mitochondrial-rich elipsoid regions. Pigment granules are positioned near the apical RPE surface and sometimes within the apical processes.

Figure 3B through E are a light microscopic survey of 7-day retinal detachments followed by 3, 5, 7 and 14-day reattachment intervals. Figures 4–7 show the corresponding electron microscopic appearance of these retinas at the same four reattachment timepoints. Overall, rod and cone outer segments gradually elongated and tended to regain their cylindrical configurations as the reattachment interval lengthened. At 3 days, the earliest reattachment timepoint sampled, many photoreceptors appeared to have no outer segment. The outer segments that were present had a mean length ≤ 3 μm and their shape was highly irregular (Figs. 3B, 4, 8A, B). The longest COSs identified were about 3.5 μm (Fig. 8B) and, at this stage, more cones than rods possessed rudimentary outer segments. At 5 days post-reattachment the majority of inner segments had accompanying outer segments (Figs. 3C, 5). COSs remained quite short ($\bar{x} = 3.2$ μm) and showed more irregularities in the organization of their outer segment membranes than did rods; mean ROS length more than doubled to 6.4 μm. At 7 and 14 days mean ROS length increased to 8.7 and 9.9 μm, respectively, whereas COS lengths were 6.5 μm and 7.2 μm at the same timepoints. The increase in

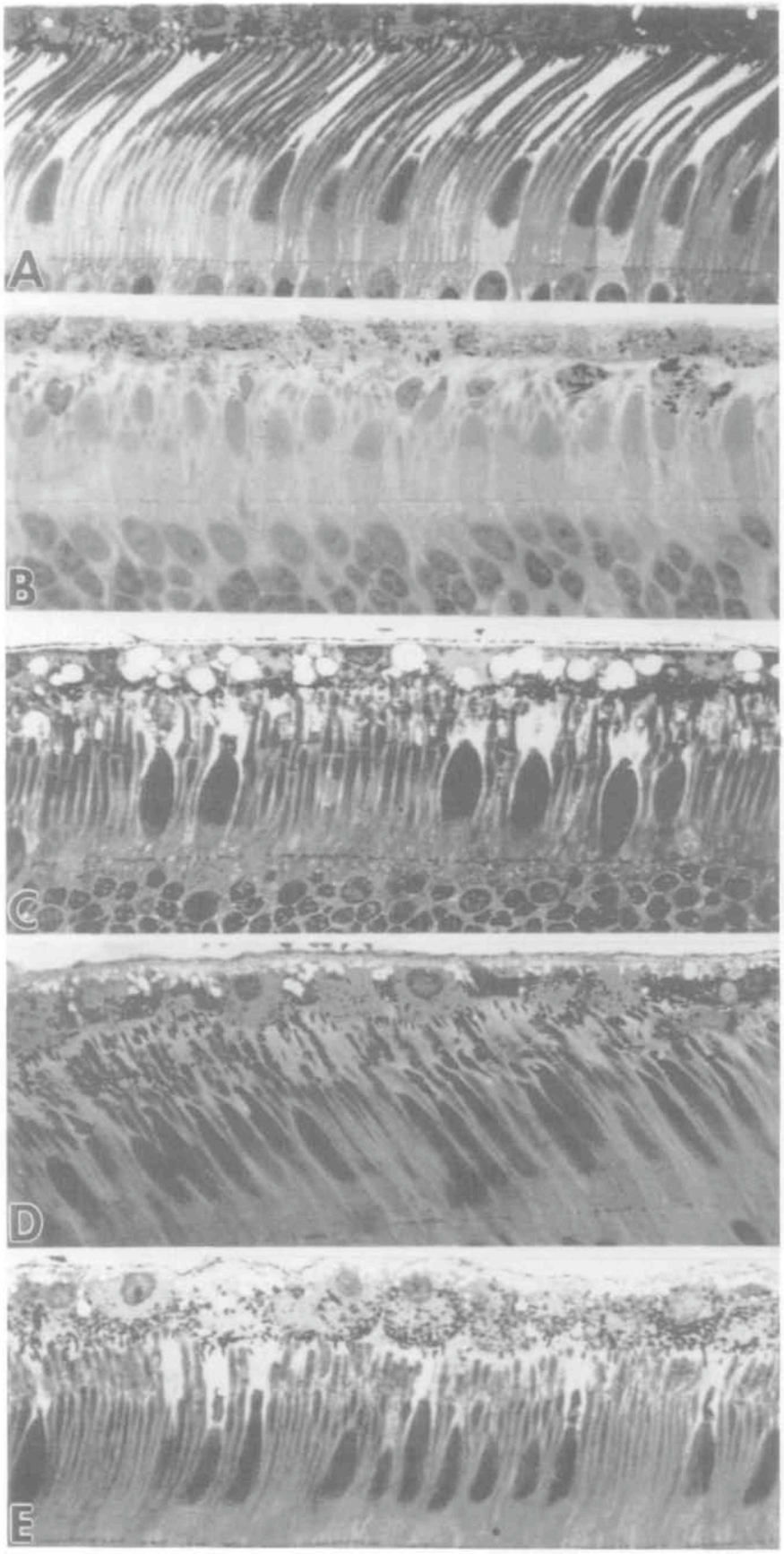


Fig. 3. Light microscopic survey of the photoreceptor–RPE interface after short-term reattachment. **(A)** A normal control region from an experimental eye located just nasal to the optic disc. The zone of detachment in this eye included the macula and a surrounding zone temporal to the optic disc. **(B)** Seven days' detachment: 3 days' reattachment. The outer segment layer is virtually absent, although small stacks of discs can be identified at the tips of some rod and cone inner segments. **(C)** Seven days' detachment: 5 days' reattachment. Most rod and cone inner segments have a short outer segment at this stage. Vacuolation at the basal surface of the RPE shown here, and to a lesser extent in (D) and (E), is an artifact attributable to the perfusion fixation. **(D)** Seven days' detachment: 7 days' reattachment. In this parafoveal region, the ROSs and COSs have elongated somewhat, but the disc stacks remain disorganized. The scalloped apical surface of the RPE (see E also) is characteristic of retinal regions that had been detached. **(E)** Seven days' detachment: 14 days' reattachment. After 2 weeks, ROSs and COSs remain substantially shorter than normal (A–E, ×1000).

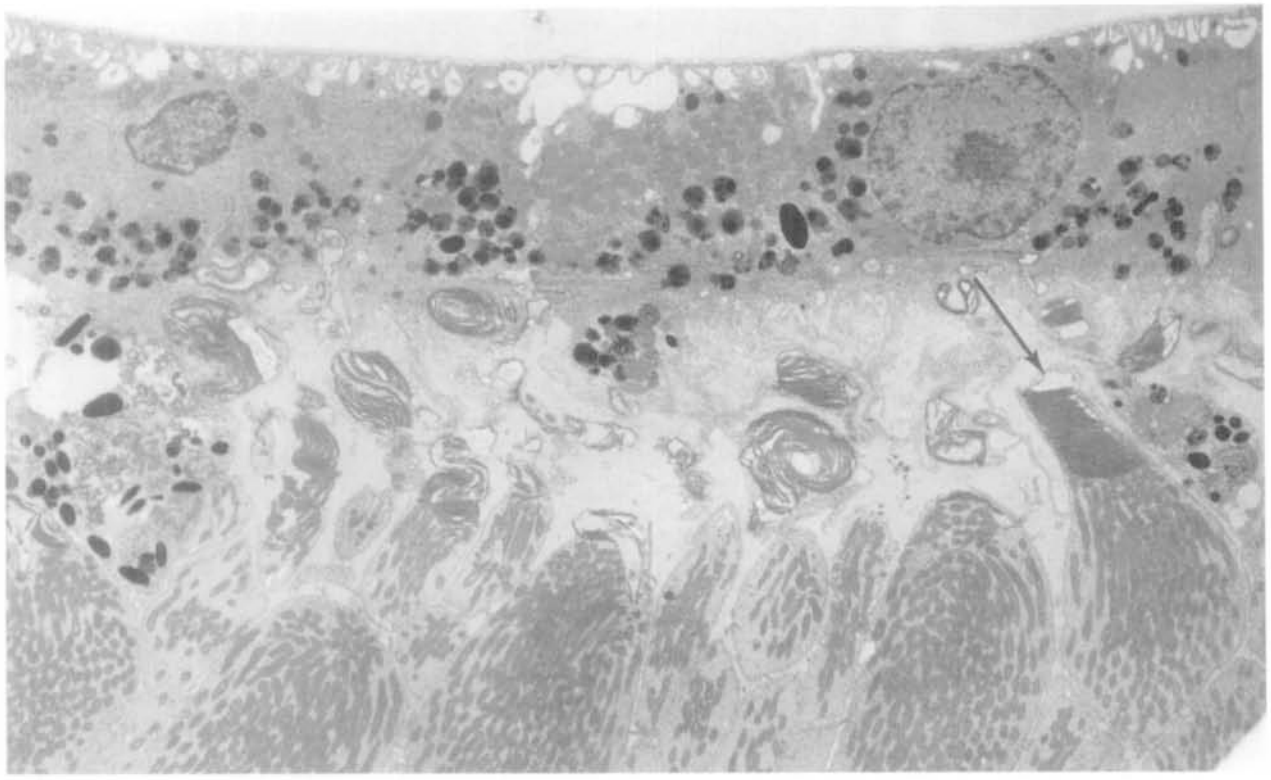


Fig. 4. Seven days' detachment: 3 days' reattachment. At the shortest reattachment interval examined, many inner segments have no associated outer segment. Of those that do, the disc membranes are usually highly disorganized. A small minority of photoreceptors have well organized stacks of disc membrane (arrow) although the rudimentary outer segments are much shorter than normal. RPE phagosomes are rare at this stage (×4200).

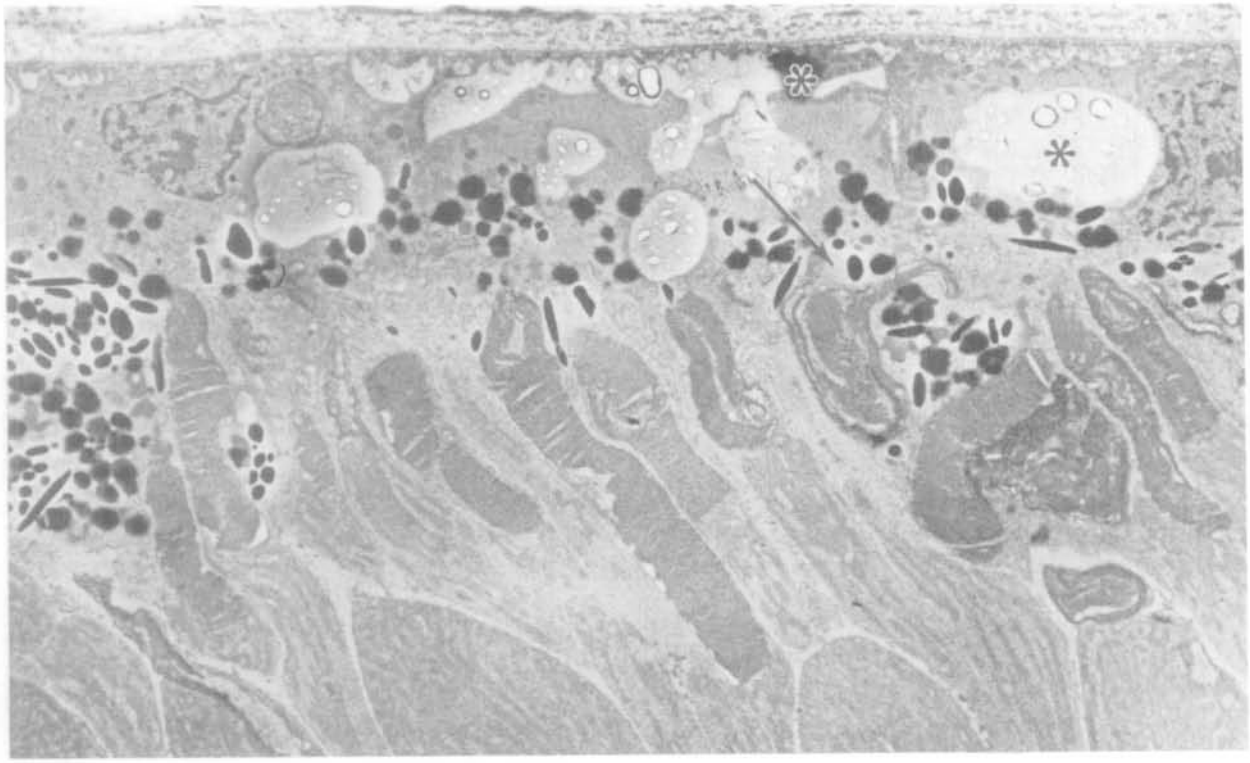


Fig. 5. Seven days' detachment: 5 days' reattachment. At 5 days more inner segments have an associated outer segment. Large whorls of outer segment membrane appear in the subretinal space (arrow). A few aligned ROSs are apparent. The phagosome content of the RPE is very low. The swelling at the basal surface of the RPE (asterisks) is artifactual (×4200).

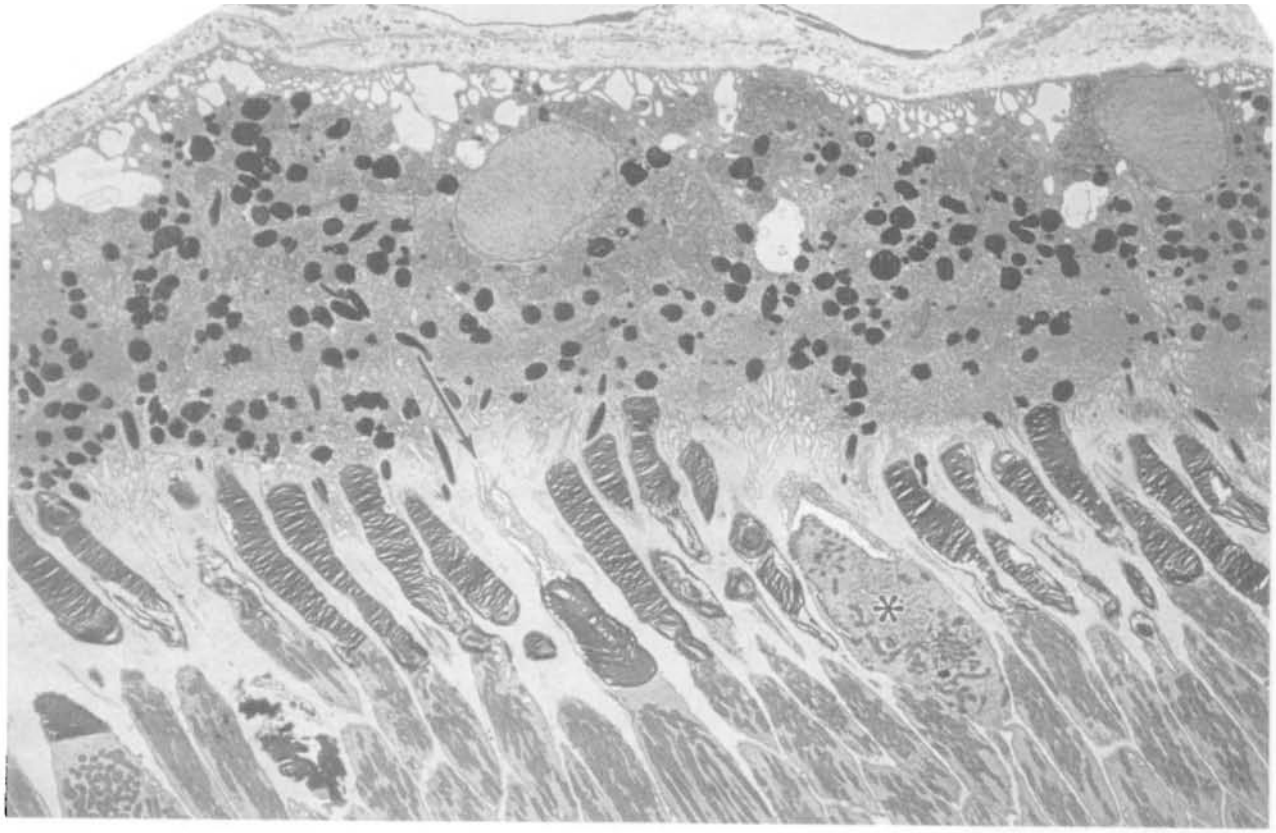


Fig. 6. Seven days' detachment: 7 days' reattachment. After 1 week some reattached regions have well aligned stacks of ROSs and COSs whose tips interdigitate with RPE apical processes. In this micrograph apical processes extend down to ensheath the tip of a COS (arrow). An adjacent cone (asterisk) has an abnormally low content of mitochondria in the inner segment and no apparent outer segment. Phagosome content in the RPE is still low (×2850).

ROS and COS mean length during the first week after reattachment was much greater than the increase shown during the second week (Figs. 3D–E, 6–8). In addition, the distribution of ROS and COS lengths broadened considerably at the 7- and 14-day time-points (Fig. 8). After 14 days reattachment both ROSs and COSs measured approximately 40% of their normal adult lengths.

In general, rods and cones in the reattached retinas showed radiolabeling patterns similar to those in normal control eyes after intravitreal injection with ^{3}H-fucose. Two hours after ^{3}H-Fucose injection, a concentration of labeled material was apparent in the myoid region of photoreceptors from both normal regions and regions reattached for 14 days. In the two experimental eyes where the retinas were reattached for 3 days, ^{3}H-fucose was injected intravitreally at the time of reattachment. Electron microscope autoradiograms indicated clearly that the outer segment material associated with both ROSs and COSs was heavily labeled (Fig. 9). No discrete band of radiolabeled protein could be identified in the disorganized ROSs at this time. However, in the 14-day reattachments, an advancing front of labeled protein was positioned approximately 6 μm from the ROS bases when ^{3}H-fucose was injected at reattachment day 10 and the animal was fixed 96 hr later (Fig. 10). The mean displacement in the opposite control retina was 9 μm over the same time period. Regenerating COSs were uniformly labeled. A band of radioactive protein was apparent at the distal tips of some ROSs 7 days after ^{3}H-fucose injection in an eye detached for 7 days and reattached for the same period of time. In addition, radiolabeled phagosomes were identified in the RPE cytoplasm.

In the two 14-day reattachments sampled, ROS disc membranes from both normal and regenerating ROSs were uniformly labeled using polyclonal antibodies to either rat or bovine opsin (Fig. 11). The antibody to bovine opsin cross-reacted weakly with normal and regenerating COS disc membranes.

Overall, regenerating COSs showed a much wider range of configurations than ROSs at the various reattachment intervals studied (Figs. 4–7, 12). Figure

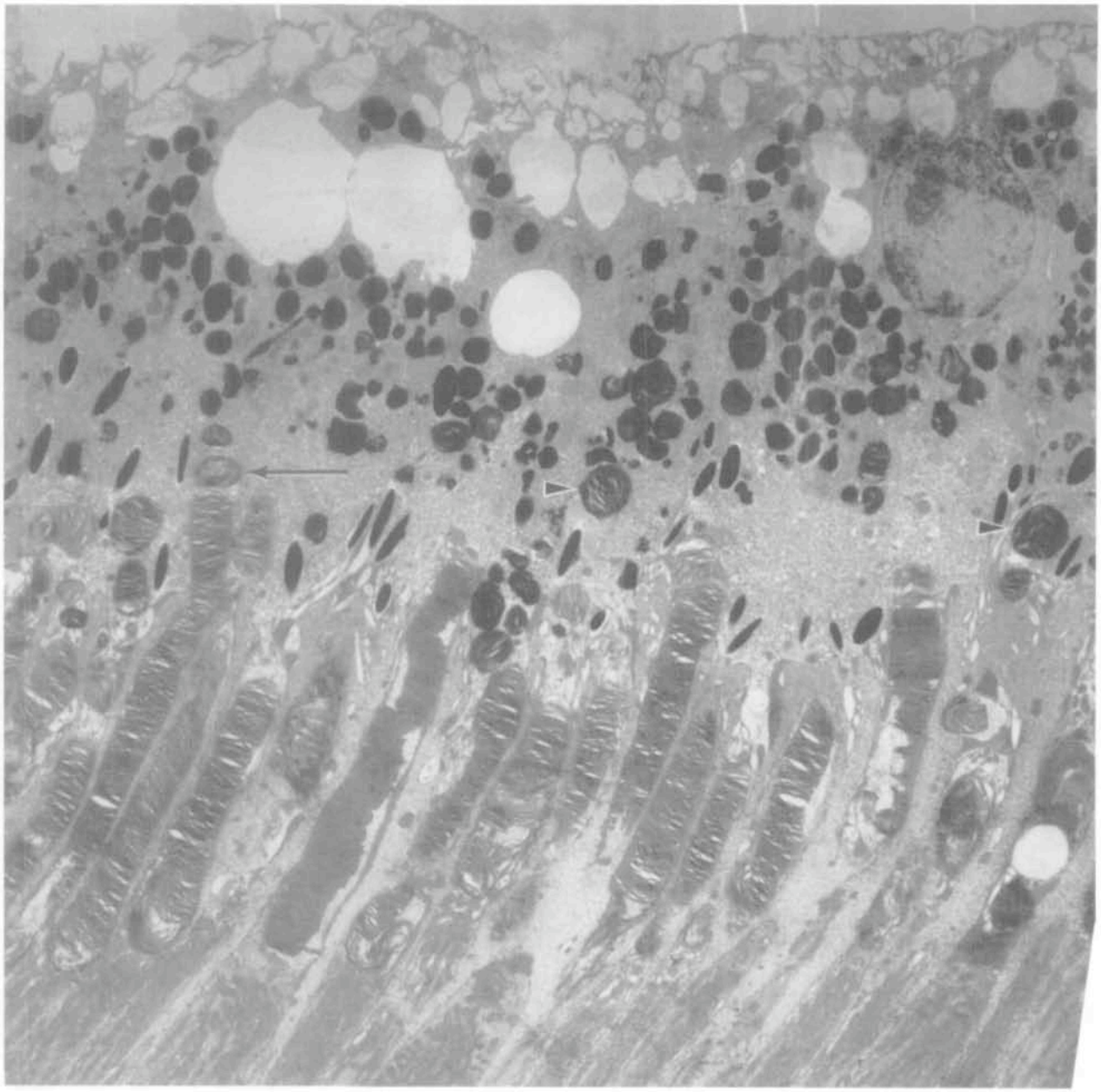


Fig. 7. Seven days' detachment: 14 days' reattachment. After 2 weeks, ROSs and COSs have elongated somewhat, and their tips abut the apical RPE surface. Disc packets in the process of detaching from the outer segment tips are clearly visible (arrow) as are freshly shed phagosomes (arrowheads) (×4125).

12A through D are representative COSs selected from the retinas reattached for 3, 5, 7 and 14 days, respectively. Although the degree to which they regained their normal morphology was highly variable, both ROSs and COSs tended to be more cylindrical at the 7- and 14-day intervals. At the earliest timepoints, the COSs consisted of only balloon-like extensions of the cilium (Fig. 12A); in other cases the outer segment was comprised of a short stack of closely apposed disc membranes (Fig. 12B). Stacks of outer segment disc membranes were frequently located at the lateral margin of inner segments instead of being positioned at their distal tips. When this occurred, the connecting cilium was displaced laterally from its normal location.

Recovery after Reattachment: The Photoreceptor–RPE Interface

At 3 days after reattachment, short microvillous processes were present on the apical RPE surface, but they did not interdigitate with the rudimentary outer

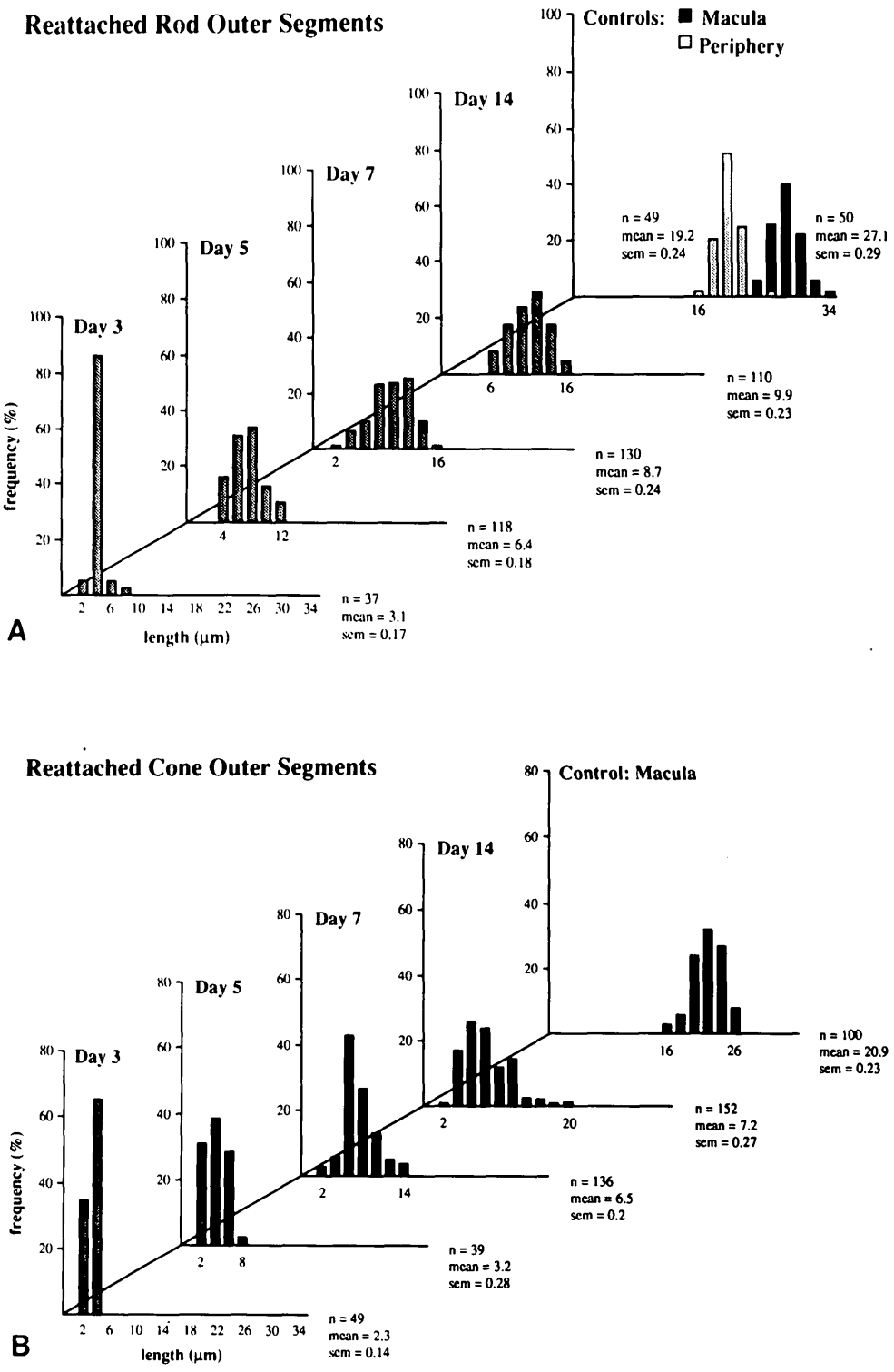
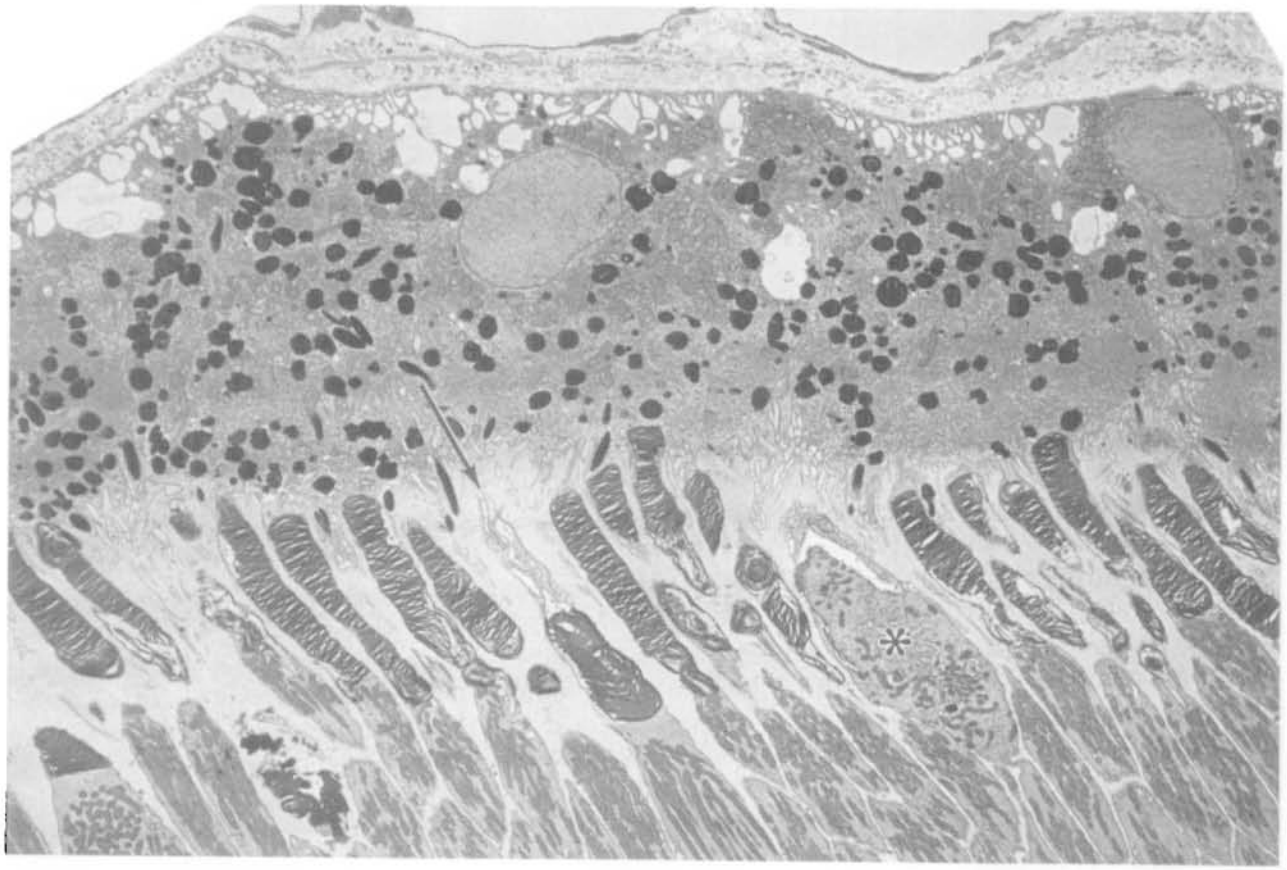


Fig. 8. Frequency histogram of ROS and COS length as a function of reattachment interval. (**A**) Reattached ROSs; (**B**) reattached COSs. ROS and COS lengths were measured in areas of reattachment using the protocol described in the text. Lengths were measured in normal control eyes and at 3, 5, 7 and 14 days after reattachment. Measurements were grouped into 2 μm bins and plotted as a percentage of total frequency. Inner segments with no apparent outer segment were not counted. Overall, both ROSs and COSs tended to elongate, and their distributions widened considerably with increasing time of reattachment. At the 3-day timepoint, both ROSs and COSs measured about 10% of their normal adult lengths. At the 2-week timepoint mean length of reattached macular ROSs was approximately 40%, and reattached macular COSs were about 35% of normal mean length.

segments (Fig. 4). Newly formed outer segments were attached to their respective inner segments by a connecting cilium (Fig. 12A). There was considerable lamellar debris in the subretinal space, but no evidence of phagosomes within the RPE cytoplasm. Pigment granules remained within the RPE cytoplasm. Numerous phagocytic cells filled with lamellar debris were positioned close to the photoreceptor inner segments.

Within the first week following reattachment, the RPE–photoreceptor interface underwent a significant reorganization (Figs. 4–6). Lamellar debris in the subretinal space decreased substantially, although there was no evidence that cells of the RPE monolayer were



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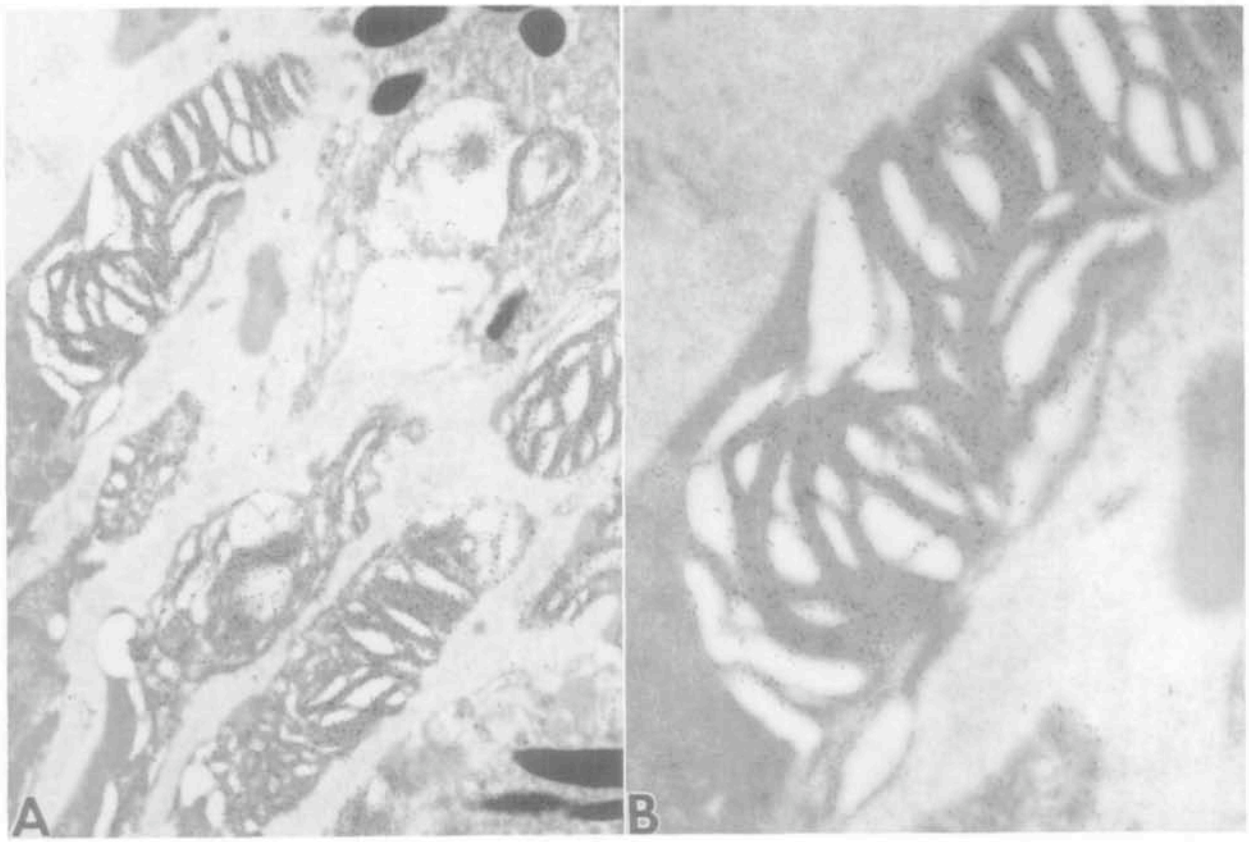

Fig. 11. Immunolocalization of opsin in regenerating ROSs. (**A**) Seven days' detachment: 14 days' reattachment. ROSs from reattached regions of several retinas were uniformly labeled with Au particles, suggesting that opsin was probably transported and inserted into new disc membrane during the reattachment interval. (**B**) Higher power view of disc membranes shown in (A). Opsin was localized to the disc membranes of reattached ROSs using the immunogold, post-embedding technique described in the text (A, ×10,741, B, ×25,187).

Persistent Outer Segment Defects

In areas where most outer segments had regained their typical cylindrical configurations, some photoreceptors showed persistent defects in outer segment alignment and orientation, ciliary location, and in disc alignment (Fig. 14A, B). In cones particularly, large disorganized whorls of membrane located immediately above the inner segments were sometimes the only indication of outer segment recovery.

Discussion

The experimental detachment procedure used in these experiments, direct microinjection of physiological saline into the subretinal space, produced a relatively atraumatic detachment of the macula and surrounding retina. In acute detachments, separation of the two tissue layers resulted in a significant expansion in the volume of the subretinal space and in the formation of a dome-shaped bleb, without producing substantial morphological damage to either the retina or RPE cells. The apical cell surface of the RPE cells changed abruptly after separation, but the cells remained viable and adherent to Bruch's membrane. Experimental detachment, followed by a 7-day waiting period, led to nearly complete degeneration of photoreceptor outer segments, but left inner segments and the rest of the photoreceptor cells intact. Thus, at the time of reattachment in these experiments, photoreceptors had either no outer segment at all or a rudimentary outer segment composed of a small amount of disorganized disc membrane. In addition, the specialized arrays of microvillous processes that normally ensheath the outer segments were absent.

The earliest phase of morphological recovery that we observed was 3 days following reattachment (7 days' detachment; 3 days' reattachment). At this stage the photoreceptor cells were closely apposed to the undifferentiated apical RPE surface. The subretinal space contained a large amount of membranous debris derived from the degenerated outer segments. Phagosomes were absent from the RPE cytoplasm. Outer segment regrowth was minimal but more cones possessed rudimentary outer segments than did rods. Electron microscopic autoradiograms strongly sug-

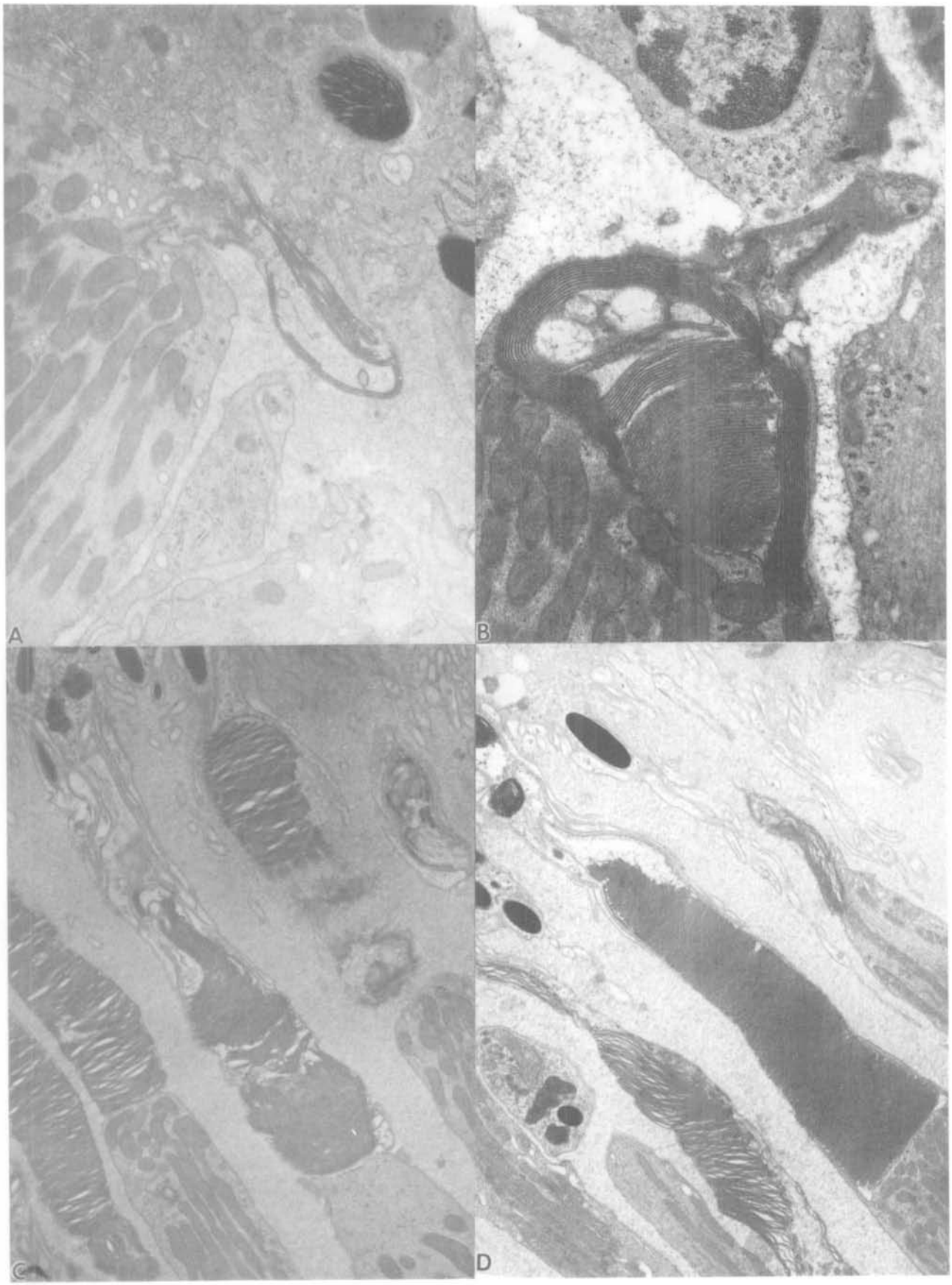
A
B
C
D

Fig. 12. Representative COSs at various reattachment intervals (**A**) seven days' detachment: 3 days' reattachment. Most COSs at 3 days consist of small stacks of apposed membranes that are connected to an adjacent cilium. (**B**) Seven days' detachment: 5 days' reattachment. Accumulations of disorganized membrane at the tips of cone inner segments were relatively common in the early stages of regrowth. (**C**) Seven days' detachment: 7 days' reattachment. (**D**) Seven days' detachment: 14 days' reattachment. After 1–2 weeks, some COSs had a well organized disc stack. Processes from the RPE ensheathed the OS tips (A, ×28,750, B, ×30,187, C, ×15,675, D, ×13,650).

gested that the formation of these rudimentary outer segments occurred during the 3-day reattachment interval. Regenerating rod outer segments, when present, tended to be more cylindrical than the cone outer segments, which often appeared as disorganized membranous stacks. These observations suggest that, although the initial regrowth of COS membrane may proceed more rapidly than in rods, cones do not reacquire their normal configurations as readily. The implication is that the mechanism by which rod and cone outer segments acquire and maintain their characteristic shapes is different. In light of the known differences between rod and cone outer segments in outer segment organization,[16,17] shape and extracellular milieu,[18] such a difference is not surprising.

As the recovery process proceeded, the initial outgrowth of outer segments coincided with a remodeling of the RPE's apical surface. At approximately 1 week after reattachment apical RPE microvilli were longer and more numerous and, for the first time, some processes interdigitated with the regenerating outer segments. Pigment granules were repositioned near the apical cell surface from their formerly random distribution within the cytoplasm, although they did not appear within the apical processes as is nor-

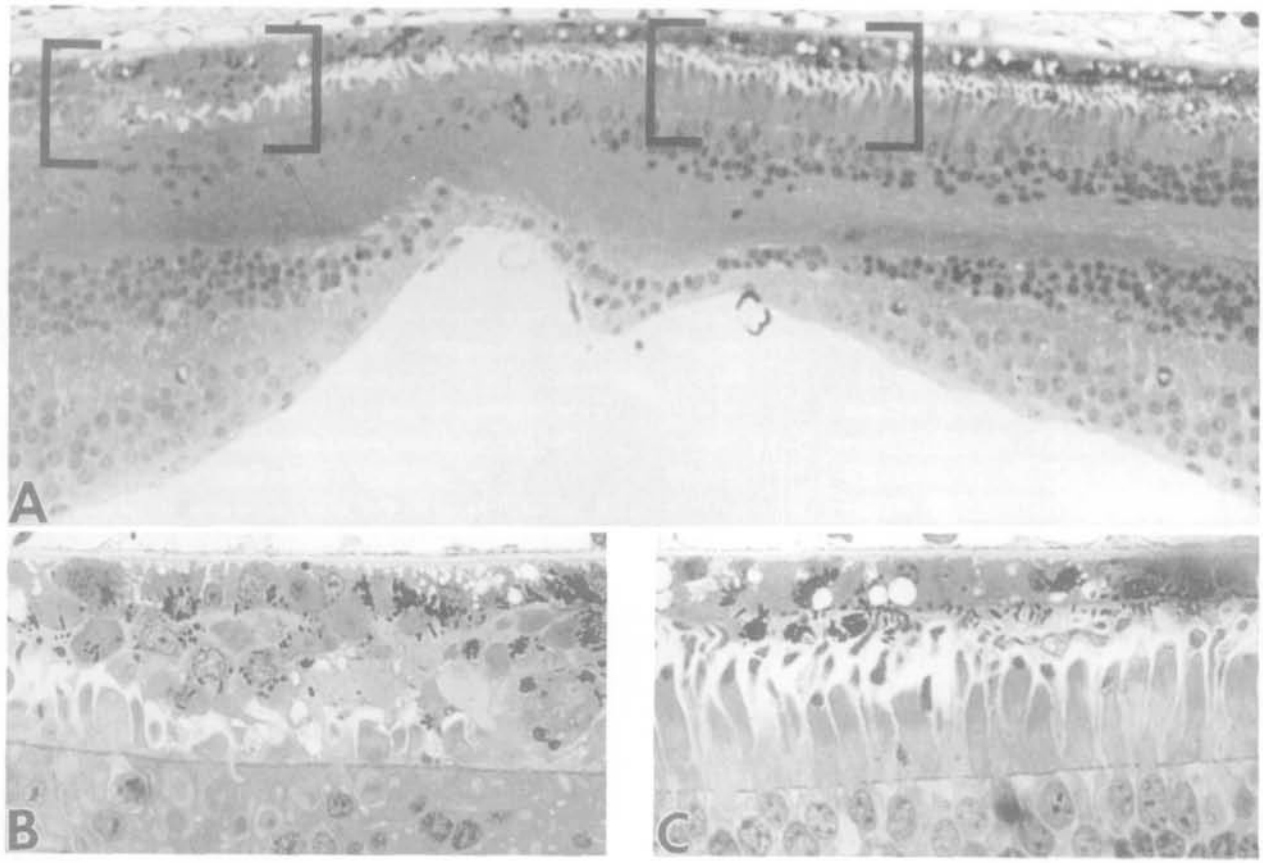

Fig. 13. RPE proliferation in the reattached fovea. Seven days' detachment: 30 days' reattachment. (**A**) Low-power light micrograph of the foveal pit. (**B**) Higher-power view of the region in (A), upper left, that is enclosed by brackets. An area of RPE proliferation immediately adjacent to the foveal pit. No COS regeneration has taken place in the region underlying the proliferated RPE cells. In addition, some thinning of the cone inner segments and nuclei appears to have occurred. (**C**) Higher-power view of the region in (A), upper right, enclosed by brackets. In the absence of RPE proliferation, some rudimentary COSs are identifiable; cone inner segments and nuclei are intact (A, ×72, B, C, ×480).

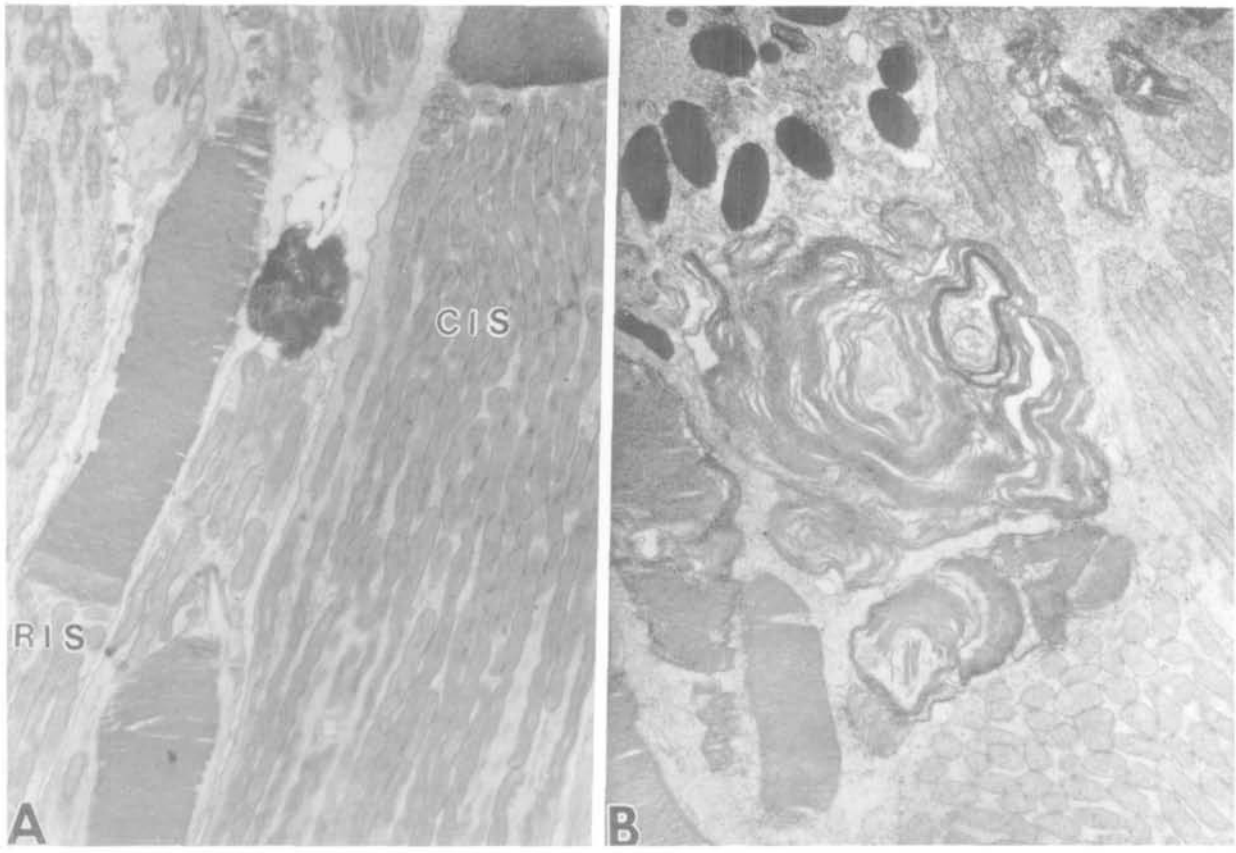

Fig. 14. Persistent abnormalities in photoreceptor recovery 2 weeks after reattachment (7 days' detachment: 14 days' reattachment) (**A**) Abnormalities in the normal orientation of photoreceptor outer and inner segments is a common feature after reattachment. Cone inner segment (CIS), rod inner segment (RIS). (**B**) Some COSs fail to reacquire their normal cylindrical configurations and appear instead as disorganized whorls of membrane (A, ×8225, B, ×13,750).

mally the case. In contrast to the earliest timepoints, membranous debris was no longer present in the subretinal space, having been phagocytosed by migrating phagocytes derived from two sources: RPE cells that detach from Bruch's membrane and blood-borne monocytes that invade the subretinal space from the choriocapillaris.[10] At this stage, there was a noticeable increase in the number of rod inner segments that had associated outer segments. ROSs were usually longer than COSs, although most outer segments remained <50% of normal length. The outer segments in most, but not all, regenerating rod and cone outer segments displayed the prototypical parallel alignment and cylindrical configuration.

In autoradiograms, the appearance of a discrete front of radiolabeled glycoprotein in regenerating ROSs, together with observations demonstrating that this front was displaced toward the tips at longer injection-fixation time intervals, indicated clearly that disc morphogenesis was proceeding after short-term reattachment. Similarly, the labeling pattern identified in cone outer segments was identical to the diffuse pattern of incorporation characteristic of normal cones,[19] suggesting that vectorial transport and incorporation of nascent protein into COSs had not been permanently impaired by detachment.

Recovery at the photoreceptor RPE interface after 2 weeks reattachment was marked by continued elongation of the cylindrical outer segments and their interdigitation with the newly formed microvilli that emerged from the apical RPE surface. There was less distinction between ROSs and COSs in their relationship to the apical processes than there was normally. Abnormalities in the orientation of individual outer segments, the location of the connecting cilium and the organization of the disc stack persisted in many recovering cells. As the regenerating outer segments elongated, phagosomes reappeared within the RPE cytoplasm and evidence of disc shedding from the outer segment tips was observed. These observations, together with the autoradiographic and immunocytochemical data, indicated clearly that after only 2 weeks' reattachment, the regenerating outer segments had reestablished the two primary processes

that account for outer segment renewal: biosynthesis of disc membrane at the bases and disposal of old membrane from the outer segment tips.[20]

There are parallels between the remodeling of the photoreceptor–RPE interface that occurs after reattachment and the maturation of these two cell layers during embryonic development. In the developing mammalian retina, the outer segments appear first as tubular extensions of the cilia and then become disorganized lamellar structures.[21,22] Only subsequently do they appear as well aligned stacks of disc membranes. The RPE lacks the dense carpet of apical microvilli at early developmental stages and only later, synchronous with the development of large numbers of balloon-like outer segments, do the apical processes increase in length and number and begin to ensheath the developing photoreceptors.[21] Similarly, our results show that in the detached retina the reformation of an aligned disc stack does not take place until the photoreceptors have been reapposed to the RPE. In addition, the redifferentiation of the apical surface occurs only after outer segment regrowth is initiated. The recovery process following reattachment, therefore, is aptly described as an example of mutual induction. In that respect, it is similar to the process described in the developing mammalian retina.[21–25]

Most of the morphological abnormalities identified in the reattached cat retina[4] were also observed in the reattached primate macula. In this study, where detachment duration was limited to 1 week, changes associated with longer-term detachment, such as subretinal gliosis, were conspicuously absent. However, areas of RPE proliferation were identified in reattached eyes at every stage of recovery and, in one case, directly within the fovea (Fig. 13). As in the cat, outer segment regeneration underlying such areas was uniformly poor. For the most part, however, regions of proliferation in the macula were sparse. These results are consistent with the conclusion that the frequency of morphological abnormalities is inversely related to detachment duration; and they imply that reattachment per se may be sufficient to retard or even arrest such changes after they have begun. There is now good evidence from studies of human reattachment patients that an exponential relationship exists between detachment duration and the recovery of acuity, with longer durations resulting in a progressive decline in final acuity.[9] Taken together, these studies provide a strong and compelling case for minimizing the time between detachment onset and reattachment of the retina.

The current results, along with results from earlier studies of experimental retinal reattachment in the owl monkey,[1,2] rhesus monkey,[3] and cat[4] indicate unequivocally that the photoreceptors and RPE retain a significant capacity for recovery after short-term retinal detachment that includes regrowth of outer segments and their interdigitation with newly formed apical processes from the RPE. In earlier studies[3] it was suggested that an initial, supranormal burst of disc morphogenesis occurred immediately after retinal reattachment, followed by elongation of outer segments to normal length after approximately 30 days. The results from the current studies provide no indication of an initial burst. Rather, they suggest that outer segment regrowth occurs steadily, but that the rate of disc morphogenesis is somewhat slower than normal at least in the first 2 weeks after reattachment. In normal rhesus monkey ROSs, the mean rate of disc displacement is reported to be approximately 2.5 μm/day.[19] Measurements of disc displacement obtained from electron microscope autoradiograms of normal control ROSs in this study confirm those estimates. At that rate regenerating ROSs could potentially reach normal length within 9–13 days in the absence of any disc shedding. As judged by the scarcity of phagosomes in the RPE, normal disc shedding does not appear to make a significant contribution to outer segment length at the earliest reattachment time points (3 and 5 days). Nevertheless, mean ROS length at both these timepoints (2.3 μm and 3.2 μm, respectively) is substantially below that which would be expected from assembly at the normal rate (7.5 μm and 12.5 μm) (see Fig. 8). The preliminary autoradiographic data are also consistent with this conclusion. Data obtained from a single animal injected with ^{3}H-fucose indicate that the mean displacement over a 4-day reattachment interval, from reattachment day 10 to day 14, was 6.0 μm or 1.5 μm/day (Fig. 10).

Extrapolation of the results gathered from this and previous studies of experimentally detached retinas to those encountered in human patients should be approached with caution. There is a high degree of similarity between the macular detachments produced in monkeys and the retinal detachments encountered in human patients. It is highly likely that this similarity extends to the recovery process as well. However, there are also some notable differences between experimental and clinical detachments that may lead to a certain degree of dissimilarity between the morphological recovery observed and that which occurs clinically. Experimental detachments are produced more rapidly (<1 min) than most detachments encountered in human patients where subretinal fluid tends to accumulate over a longer time, and often leads to a progressively larger detachment. Sec-

ond, several different types of fluid (eg, BSS, diluted Na hyaluronate, enzyme-treated vitreous, serum) have been used to produce experimental detachments[3,10,26] or to influence subretinal fluid resorption,[27] whereas in human detachments subretinal fluid consists of incarcerated vitreous and serum components. Third, detachments in human patients often involve large retinal tears or breaks, in conjunction with bleeding from damaged retinal vessels. Experimental detachments produced by microinjection result in virtually no intraretinal bleeding and produce an extremely small retinal hole which probably seals within a few minutes. The process described here applies to recovery that ensues after a macular detachment of approximately 1 week. Detachments of longer, or perhaps shorter, duration would be expected to affect the temporal sequence of recovery as well as the incidence of persistent abnormalities (see below). Finally, the variable of detachment height, the distance between the detached retina and apical RPE surface, needs to be taken into consideration. Previous experimental studies suggest that higher or more bullous detachments are correlated with more severe degenerative changes in the photoreceptor layer.[4,28] The detachments produced in this study were bullous initially, but over the course of the next 7 days, detachment heights diminished rapidly to the point where they were not ophthalmoscopically visible.

These factors lead us to conclude that the process described here should probably be regarded as an example of near optimum recovery. The RPE–photoreceptor layers are shallowly detached for most of the 7-day period; they are separated for a relatively short interval and in the absence of other, potentially confounding, factors. Human retinal detachments that occur as a result of trauma, those which are relatively bullous and/or persist for longer than 1 week, or those where there is other coexistent pathology would be expected to have a higher frequency of morphological abnormalities in the macula that would, in turn, result in a reduced capacity for visual recovery.

The RPE–photoreceptor interface in the primate macula retains a remarkable capacity for morphological recovery following a retinal detachment of short duration (≤7 days). ROS and COS regrowth begin immediately or very soon after reapposition of the photoreceptors to the apical RPE surface and within 2 weeks after reattachment outer segments regain approximately 40% of their adult length, reacquire their cylindrical configurations and restart the process of disc shedding. The process of outer segment regrowth is analogous to that which occurs during initial development where outer segment elongation is a function not only of the rate of disc morphogenesis but also of disc disposal through periodic shedding.[22] Even under optimal experimental conditions and in the absence of underlying pathology, abnormalities in outer segment structure and other morphological defects persist and are probably implicated in the relatively small, but consistent diminution in visual capacity associated with macular detachments of short duration.

Key words: retinal reattachment, retinal degeneration, retinal pigmented epithelium, retina, photoreceptors

Acknowledgments

We are indebted to Coopervision, Inc. (Irvine, CA) for their gift of the Ocutome microsurgical system. Additionally we would like to express our appreciation to Geoffery Lewis for performing some of the surgical procedures and assisting in others; to Kenneth Linberg for critical reading of the manuscript and for helpful suggestions; and to Drs. Dean Bok, Brian Matsumoto and Terry Schuster for providing opsin antisera.

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