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CHAPTER

123

Cellular effects of detachment on the neural retina and the retinal pigment epithelium

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THE INTERFACE BETWEEN RETINA AND RETINAL PIGMENT EPITHELIUM

Early in development of the eye, the neural retina and the retinal pigment epithelium become closely apposed and remain so throughout the normal lifespan of an individual. During very early development of the human retina (until sometime after the eighth week of development), the undifferentiated cells of the neural retina are connected to the differentiating cells of the pigment epithelium by both adhering and gap junctions.[21] In other cell types the latter allow a free transfer of small molecules (less than about 1500 daltons) between cells.[3] The significance of coupling these two cell layers is not known, but as the neural retina begins to differentiate, these intercellular junctions disappear. Shortly thereafter, the apical surface of the retinal pigment epithelium elaborates numerous villous and sheetlike processes that interdigitate with the photoreceptor outer segments in the adult eye, and the rod and cone outer segments grow into the extracellular region that becomes the interphotoreceptor space.

The mature retinal pigment epithelium is a polarized monolayer of neuroepithelial cells that consists of a fenestrated basal surface lying immediately adjacent to a basal lamina (Bruch's membrane) and the choroidal vasculature. The components of this conventionally organized basal lamina are synthesized and secreted, at least in part, by the retinal pigment epithelial cells themselves.[14,42,44,52] At their lateral margins, each retinal pigment epithelial cell is connected to its neighboring cells by a junctional complex composed of adhering, gap, and tight junctions.[25]

The apical surface of the epithelium and its relationship to differentiated photoreceptors is an anatomically complex one. There are no actual cellular junctions between the two layers in the mature eye. Rather, photoreceptor outer segments are ensheathed by specialized arrays of microvilli and microplicae that are organized differently for primate rods and cones.[5,49] In human and rhesus monkey retinas, the apical surface of the retinal pigment epithelium extends villouslike processes toward the photoreceptor outer segments, where some of them expand into cytoplasmic sheets or lamelli that surround the outer segments.[5,50] For rods, these surround the distal portion of the outer segment with two or three partially overlapping layers. Foveal and extrafoveal cones are usually ensheathed with more layers of apical processes than are the rods, and the ensheathment usually extends further along the outer segment, about two thirds of the distance to its base. The greatest difference between the ensheathment of these primate photoreceptors is the existence of a substantial "supracone space" above the cone outer segments. Typically, cone outer segments do not reach the apical surface; for extrafoveal cones, the apical processes may traverse 10 to 20 μm before reaching the distal tip of the outer segment. The apical processes that will ensheath a single cone, however, group together in the supracone space, where they are easily recognizable by either scanning or transmission electron microscopy. The anatomic arrangement of the apical processes surrounding cone outer segments is known as the cone sheath.[50] Outside the fovea they are so distinctive that they are easily recognizable by light microscopy, as first described by

The photoreceptor/retinal pigment epithelium interface

The retinal pigment epithelial apical surface. One of the earliest effects of detachment occurs at the retinal pigment epithelial apical surface. The long and elaborate sheetlike and villous processes that normally ensheath the outer segments are lost within a few hours of detachment and replaced by a "fringe" of short microvilli (Fig. 123-2). Also occurring within the first 24 hours of detachment is a change in the overall surface morphology of these cells so that they begin to show a rounded contour with cytoplasm protruding past the normal limits of the apical surface into the subretinal space (Figs. 123-2, 123-3).[8] In addition, the nucleus is often displaced from its normal central location in the retinal pigment epithelial cell to a more apical location. This response has been termed "mounding" of the apical surface and recent experiments have shown that it begins within minutes of producing a detachment in rabbit retina.[26] On the basis of similar observations in cultured retinal pigment epithelial cells, it has been suggested that this effect is caused by a change of tension in the circumferential ring—bundles of actin-containing filaments at the apical border that ring the outer margin of the retinal pigment epithelial cell bodies.[40]

The proliferation of retinal pigment epithelial cells. If ^{3}H-thymidine, a specific precursor of DNA synthesis, is injected into the vitreous cavity and sections of retinal tissue are processed for autoradiography, silver grains appear over nuclei where ^{3}H-labeled molecules have been incorporated into DNA. Using this technique, labeled nuclei appear within the retinal pigment epithelial cell layer within 24 hours after production of a detachment.[7] Thus the retinal pigment epithelial cells, which are normally quiescent, enter a stage of stimulated DNA synthesis and proliferation soon after the two cell layers are detached (Fig. 123-4). This proliferative response can result in the transformation of the retinal pigment epithelium's uniform monolayer into a more heterogeneous morphology in which strands of cells extend from the original monolayer into the subretinal space. In long-term detachments (30 to 50 days) the proliferative response may induce the formation of mul-

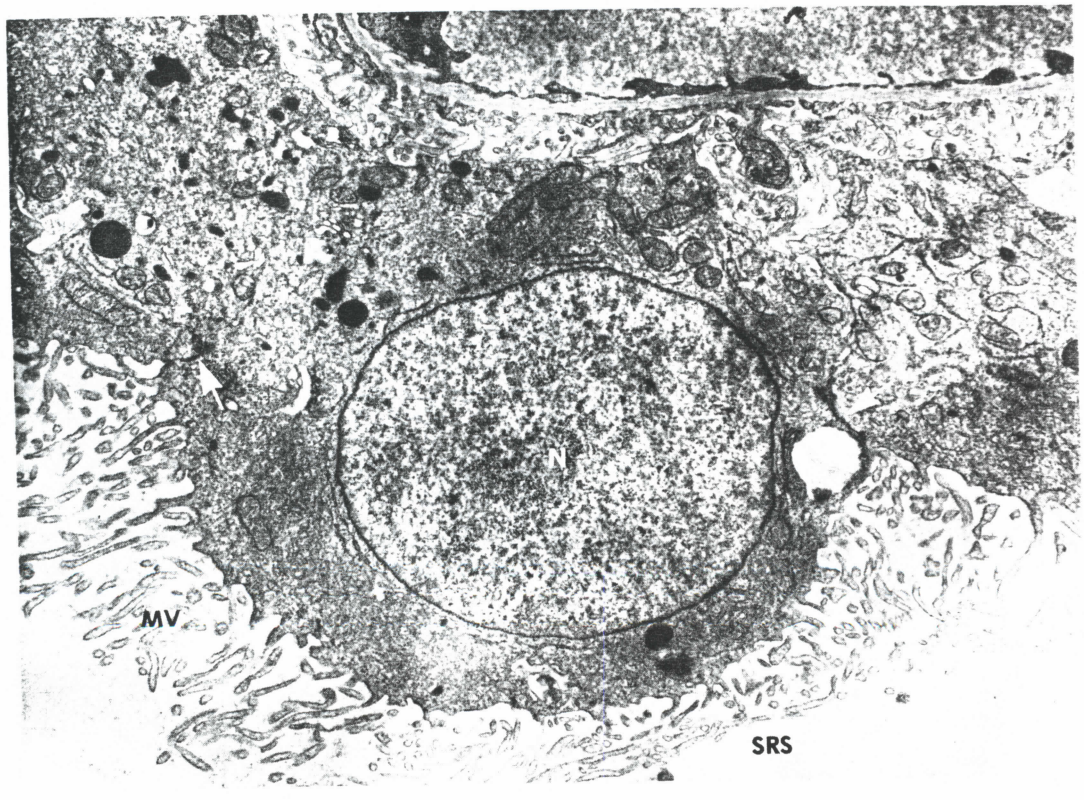


Fig. 123-2. An electron micrograph of the retinal pigment epithelium 1 day after production of a retinal detachment. In comparison with normal retinal pigment epithelial cells, the apical surface is mounded. The sheetlike apical projections that normally ensheath the outer segments have been replaced by a homogeneous fringe of short, microvillous processes *(MV)*. In this particular cell, the nucleus *(N)* is displaced into the mounded region. The cell's lateral junctions are indicated by arrows. *SRS,* Subretinal space. (Magnification × 6750.) *(From Anderson, DH et al.: Invest Ophthalmol Vis Sci 24:909, 1983.)*

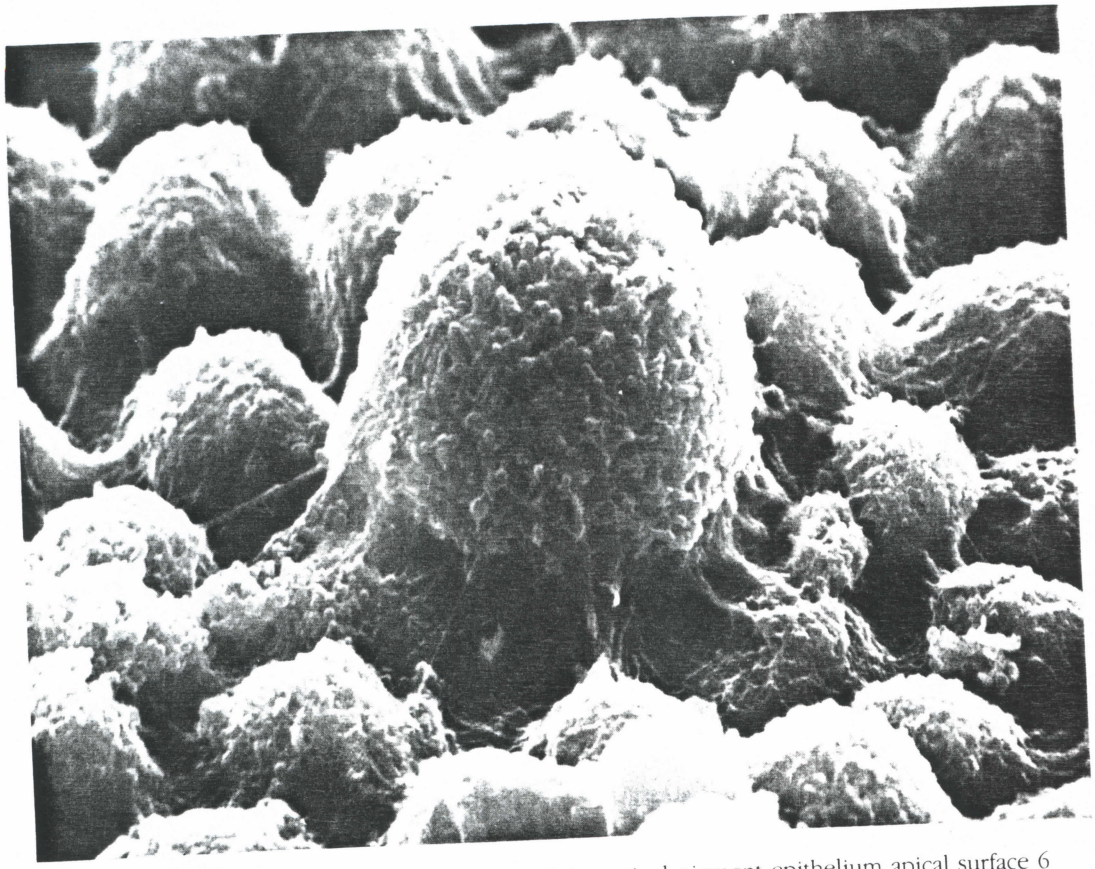


Fig. 123-3. Scanning electron micrograph of the retinal pigment epithelium apical surface 6 weeks after production of an experimental detachment. Demonstrates the pronounced mounding response of the epithelial cells. (Magnification × 4800.) *(From Anderson, DH et al.: Invest Ophthalmol Vis Sci 24:910, 1983.)*

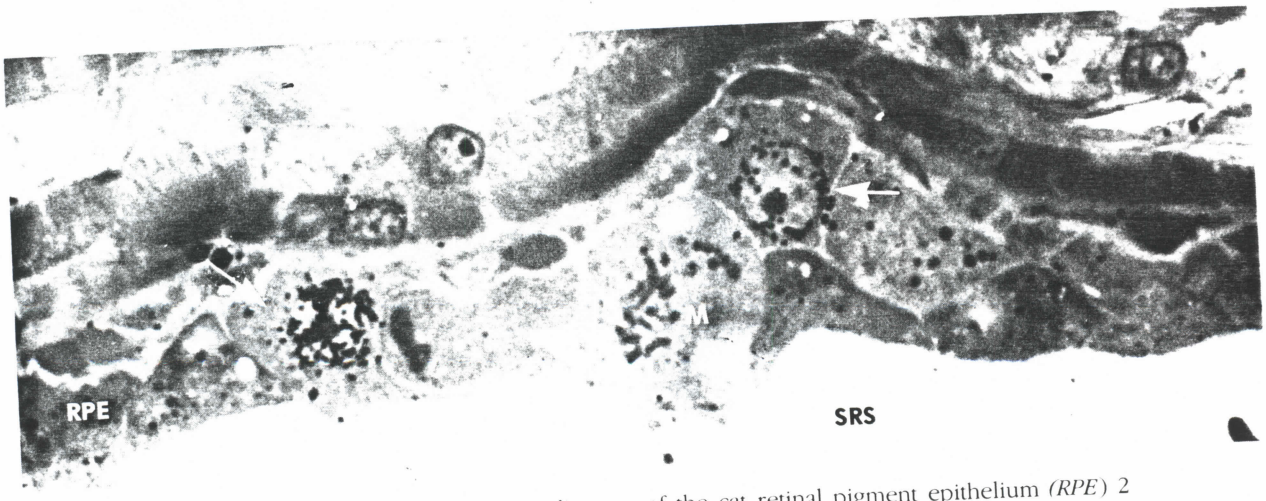


Fig. 123-4. Light microscopic autoradiogram of the cat retinal pigment epithelium *(RPE)* 2 days after detachment. Tritiated-thymidine was injected intravitreally 3 hours before fixation. Two labeled nuclei *(arrows)* and an adjacent mitotic figure *(M)* indicate that some retinal pigment epithelial cells are proliferating at this stage. *SRS*, Subretinal space. (Magnification × 850.) *(From Anderson, DH et al.: Invest Ophthalmol Vis Sci 24:911, 1983.)*

tiple layers of cells whose polarity does not necessarily match that of the original monolayer (Fig. 123-5). It is important to note that the molecular stimulus for DNA synthesis and proliferation is unknown, but its effect is limited to the region immediately underlying the detached retina; attached regions in the same eye remain mitotically quiescent. These data strongly suggest that attachment of the retinal pigment epithelium and neural retina acts to keep the retinal pigment epithelium mitotically inactive and its apical surface highly differentiated. In the absence of the apposing photoreceptor cells, the apical retinal pigment epithelial surface faces a lumen. As such, retinal pigment epithelial cells in the detached state are similar to cultured retinal pigment epithelial cells, and they display many of the morphologic characteristics of confluent cell cultures.

From long-term experiments it does appear that the proliferative response of the retinal pigment epithelium is self-limiting. In experimental detachments of both the owl monkey and the cat retina, proliferation (measured by ^{3}H-thymidine incorporation) is at very low levels after long detachment intervals (e.g., 12 to 14 months).[8,35] Proliferation of the retinal pigment epithelium during detachment is of clinical significance. The proliferation of retinal pigment epithelial cells in the subretinal space is probably the basis of pigmentary changes often observed during ophthalmoscopic examination of retinal detachments in humans. It is likely that the demarcation lines noted in human retinal detachments represent zones of proliferated retinal pigment epithelium occurring at transitions between detached and attached regions of the eye. Current experimental evidence also suggests that such proliferation may be one of the factors adversely affecting photoreceptor outer segment regeneration after reattachment.[6,8]

The migration of cells into the subretinal space. In the mammalian retina, the interphotoreceptor space is normally free of cells. Within 24 hours of an experimental detachment, PAS-positive cells appear in the subretinal space. These cells are also found in the choroidal and retinal capillaries and have the overall morphology of polymorphonuclear neutrophils and monocytes in different maturational stages. Monocytes are sometimes found between adjacent retinal pigment epithelial cells, apparently in the process of migration from the choriocapillaris into the subretinal space (Fig. 123-6). These cell types appear to mature into tissue macrophages within the subretinal space, where they then phagocytose cellular debris (including membrane from degenerating outer segments).

Retinal pigment epithelial cells can also be found free within the subretinal space after a detachment. Studies by electron microscopy show that by about 72 hours after a detachment, some retinal pigment epithelial cells begin to lose their attachment to their neighbors and

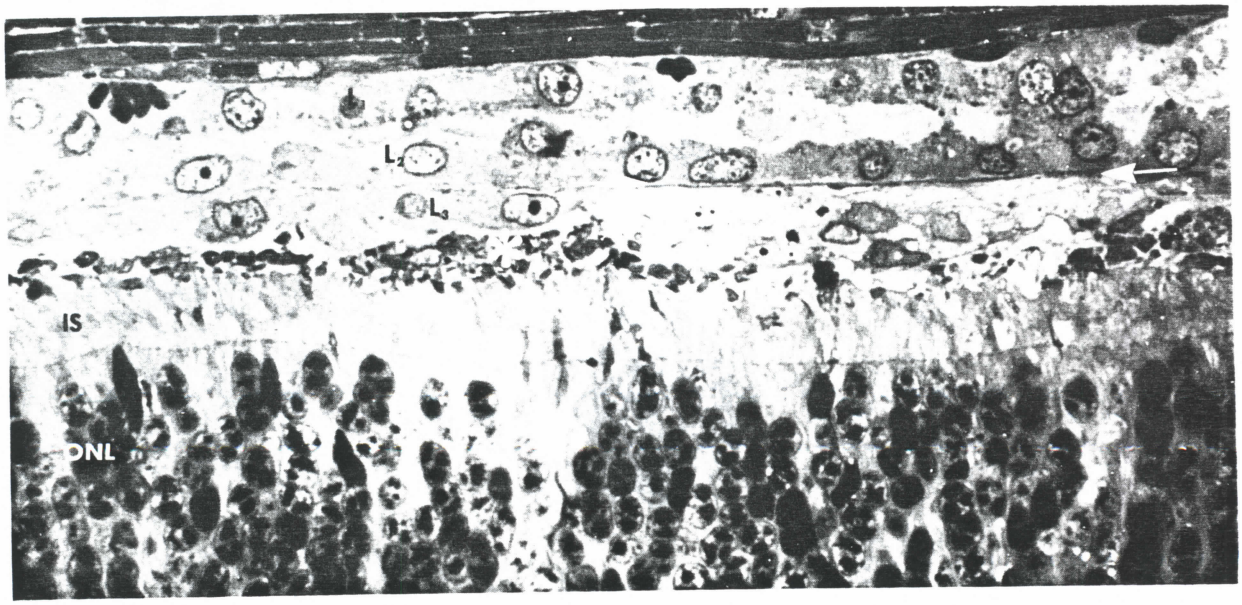


Fig. 123-5. A light micrograph of an area of retinal pigment epithelial cell proliferation in a cat retina detached for 14 days and reattached for 30 days. Three monolayers of retinal pigment epithelial cells are present *(L_1, L_2, L_3)*, each displaying different surface polarity. The apical surfaces 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 *(IS)* tips. *ONL*, Outer nuclear layer. (Magnification × 800.) *(From Anderson, DH et al.: Invest Ophthalmol Vis Sci 27:174, 1986.)*

migrate into the subretinal space (Fig. 123-7). These cells also contain large packets of outer segment debris, indicating their probable role in scavenging material from the degenerating photoreceptors. Thus there are at least two different sources for cells that move into the subretinal space and phagocytose cellular debris. Some are of hematopoietic origin, whereas others are derived from the retinal pigment epithelial layer; the proportion of cells derived from each source has not been determined.

Identifying the tissue of origin of subretinal cells cannot be done solely on the basis of morphologic criteria. Antibodies to cell-type specific proteins can be used, however, as immunochemical probes to distinguish between different cell types. For example, cellular retinaldehyde-binding protein is found only in retinal pigment epithelial and Müller cells.[11] An antibody to this protein, coupled indirectly to an appropriate marker molecule such as colloidal gold, can be used to identify cells expressing this particular protein. Cells with the morphologic features of tissue macrophages appear within the subretinal space in the detached cat retina. However, some of these cells are labeled by antibodies to cellular retinaldehyde protein using immunohistochemical methods and, as such, can be positively identified as retinal pigment epithelial cells (Fig. 123-8).

The degeneration of photoreceptor outer segments. Like the retinal pigment epithelial cells in the detached retina, the photoreceptor outer segments also project into the expanded subretinal space. Under experimental conditions, many outer segments appear relatively normal up to 12 hours after the detachment; but some outer segments are almost certainly damaged by the injection of fluid into the interphotoreceptor space. Indeed, mechanical damage seems likely in the

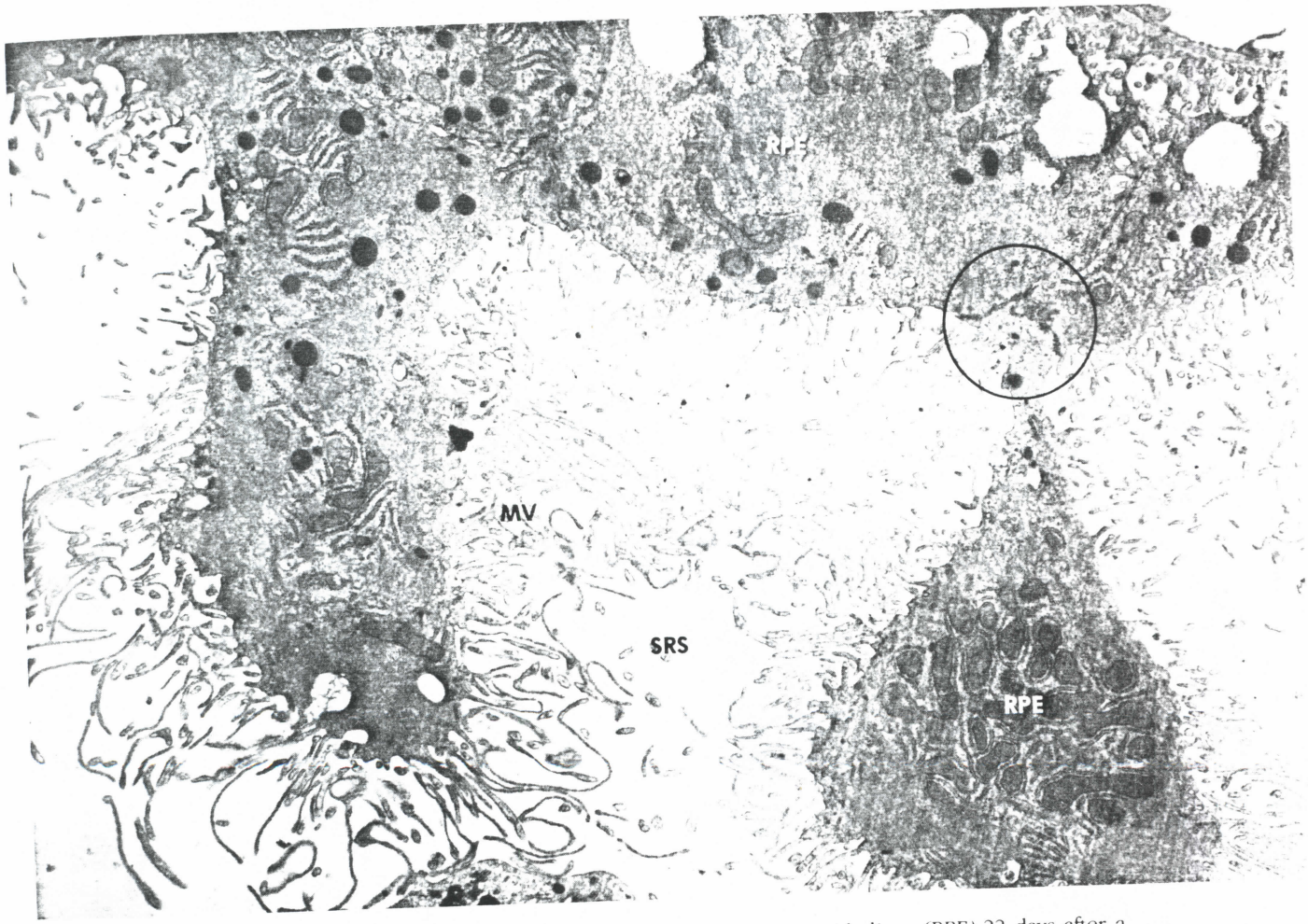


Fig. 123-6. An electron micrograph of the retinal pigment epithelium *(RPE)* 22 days after a detachment. The cell on the right has nearly separated from the monolayer. A narrow tail of cytoplasm retains junctions to neighboring cells *(circle)*. The apical processes *(MV)* of these highly mounded cells are longer than those found at earlier detachment times. *SRS*, Subretinal space. (Magnification × 6250.) *(From Anderson, DH et al.: Invest Ophthalmol Vis Sci 24:920, 1983.)*

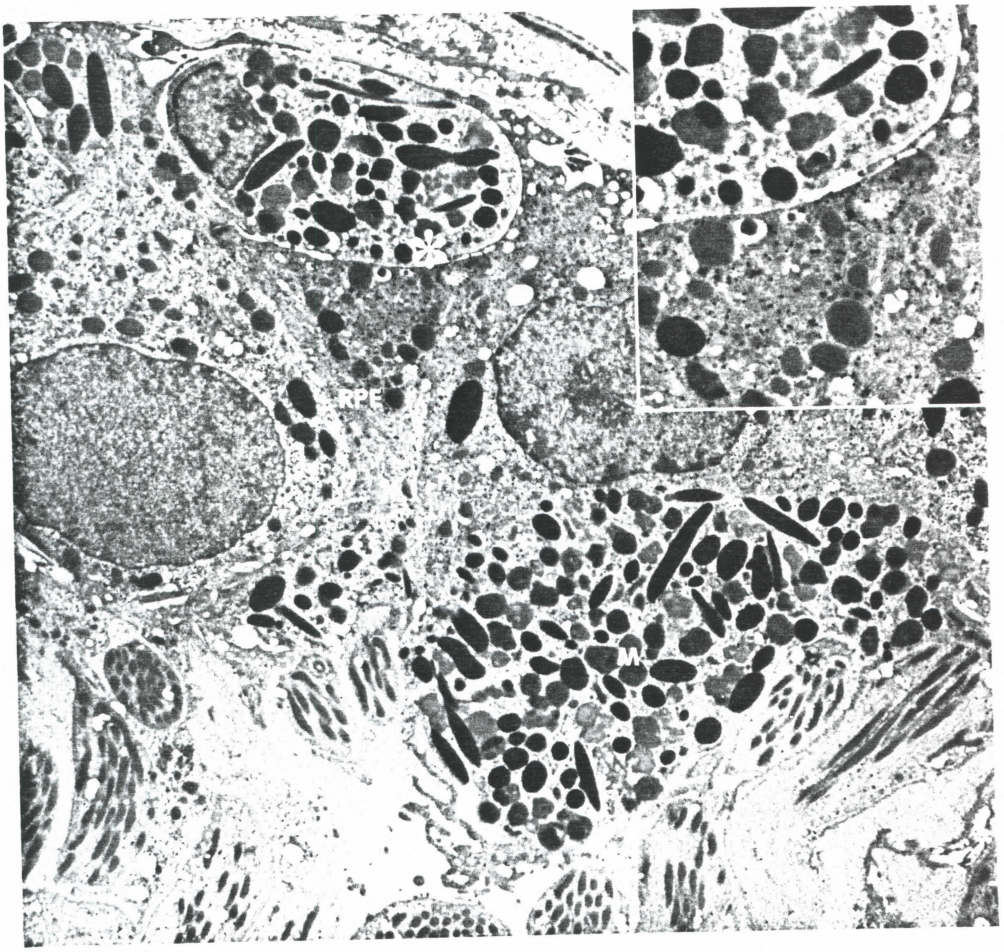


Fig. 123-7. An electron micrograph from a reattached monkey retina. The tissue sections were incubated with an antibody to cellular retinaldehyde–binding protein and then labeled with a secondary immunoglobulin-gold conjugate. The protein is expressed only in retinal pigment epithelial and Müller cells. The small black spheres scattered throughout the cytoplasm of the retinal pigment epithelium *(RPE)* indicate the presence of the protein (see inset). The two unlabeled cells *(M)* are almost certainly invading cells from the circulation. The inset comes from the area marked by the asterisk. (Magnification × 53,000; inset, magnification × 96,000.)

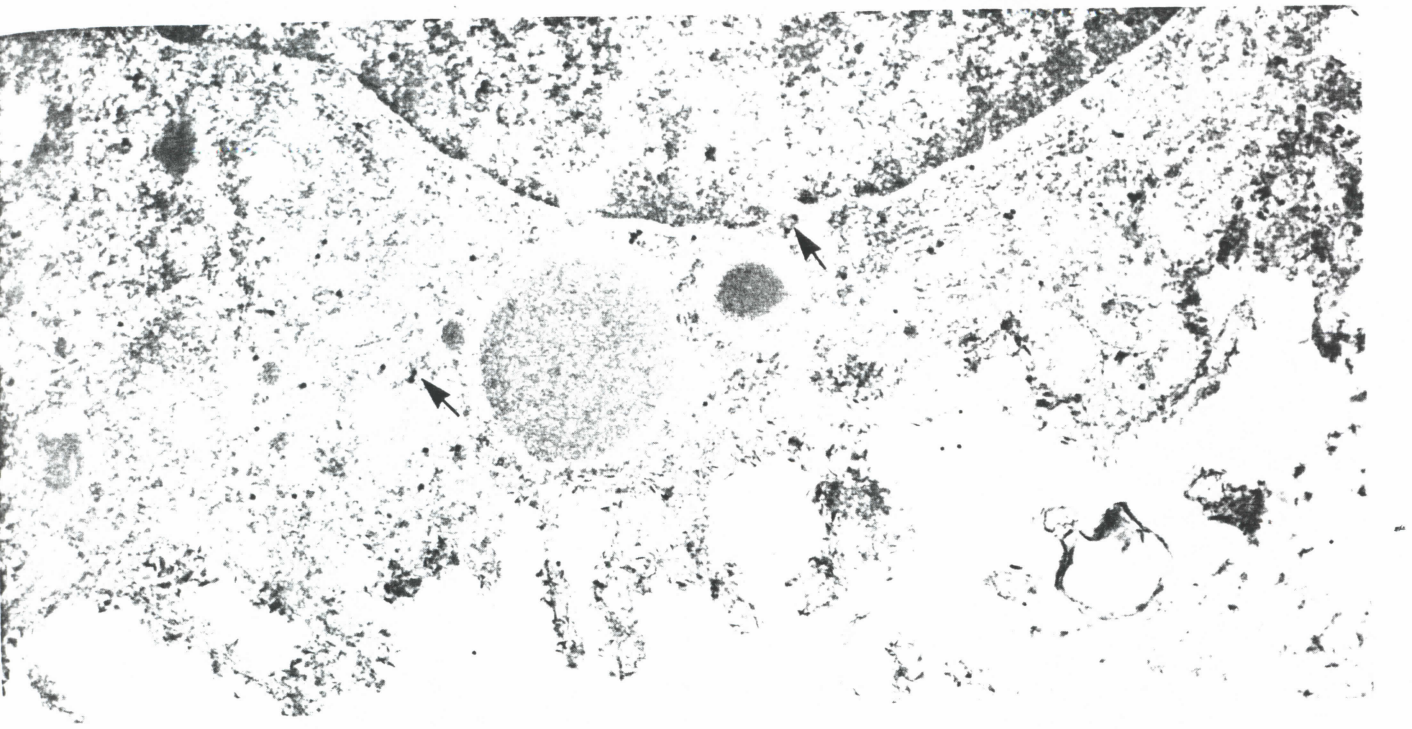


Fig. 123-8. An electron micrograph of a cell in the subretinal space in a cat retina detached for 2 months. The tissue sections were incubated as described in Fig. 123-7. The presence of gold spheres *(arrows)* scattered in the cytoplasm indicates that this subretinal cell is derived from the retinal pigment epithelium. (Magnification × 33,000.)

detachment of any mammalian retina, whether it is experimental or clinical. The retinal pigment epithelial-photoreceptor interface constitutes a complexly organized intercellular compartment that is quite susceptible to disruption. Although the outer segment bases are ensheathed by a basket of fine calyceal processes, their only connection to the inner segment is by a single ciliary stalk.[34] As previously mentioned, the apices of the outer segments are enveloped by arrays of apical retinal pigment epithelial processes (Fig. 123-9, *A*). The interphotoreceptor matrix has long been presumed to play a role in maintaining the structural integrity of these elements. In any case, the measured adhesion between the two cell layers is reported to be strong,[61] and any force that separates them will probably produce some damage to this anatomically complex interface.

The most common damage reported during the first 12 hours of detachment is a vacuolation or distortion of the distal end of the outer segment. Between 24 and 72 hours after detachment, both rod and cone outer segments show evidence of damage. Although many are still attached to their connecting cilium, they appear greatly distorted, with discs improperly oriented within a truncated outer segment. The degeneration of outer segments continues until the few remaining in the zone of detachment appear only as empty sacs of membrane attached to the connecting cilium (Fig. 123-9, *B*). During this same time, outer segment debris appears within the subretinal space. This is an abnormal process because in the normal eye the shedding of discs from the photoreceptor distal end is accompanied by immediate phagocytosis by the adjacent retinal pigment epithelial cell.

Photoreceptor outer segment renewal. Both rod and cone outer segments are renewed by a continual process of disc addition at the base and loss at their tips.[4,24,51,60] Thus any process that interrupts either the production of new discs or their shedding and subsequent phagocytosis at the distal end will affect the integrity of the outer segment. Many questions remain about the regulation of disc morphogenesis and shedding during a period of detachment. There is experimental evidence that when photoreceptors are detached from the retinal pigment epithelium, disc shedding cannot be induced by treatments that stimulate massive shedding in normal retinas.[55] It also appears from ultrastructural studies that the process of disc morphogenesis must slow down considerably while the retina is detached. In long-term detachments, ciliary stalks with a few rudimentary discs or membrane evaginations are present at the distal inner segment tips. These disc membranes are found long after membranes synthesized before detach-

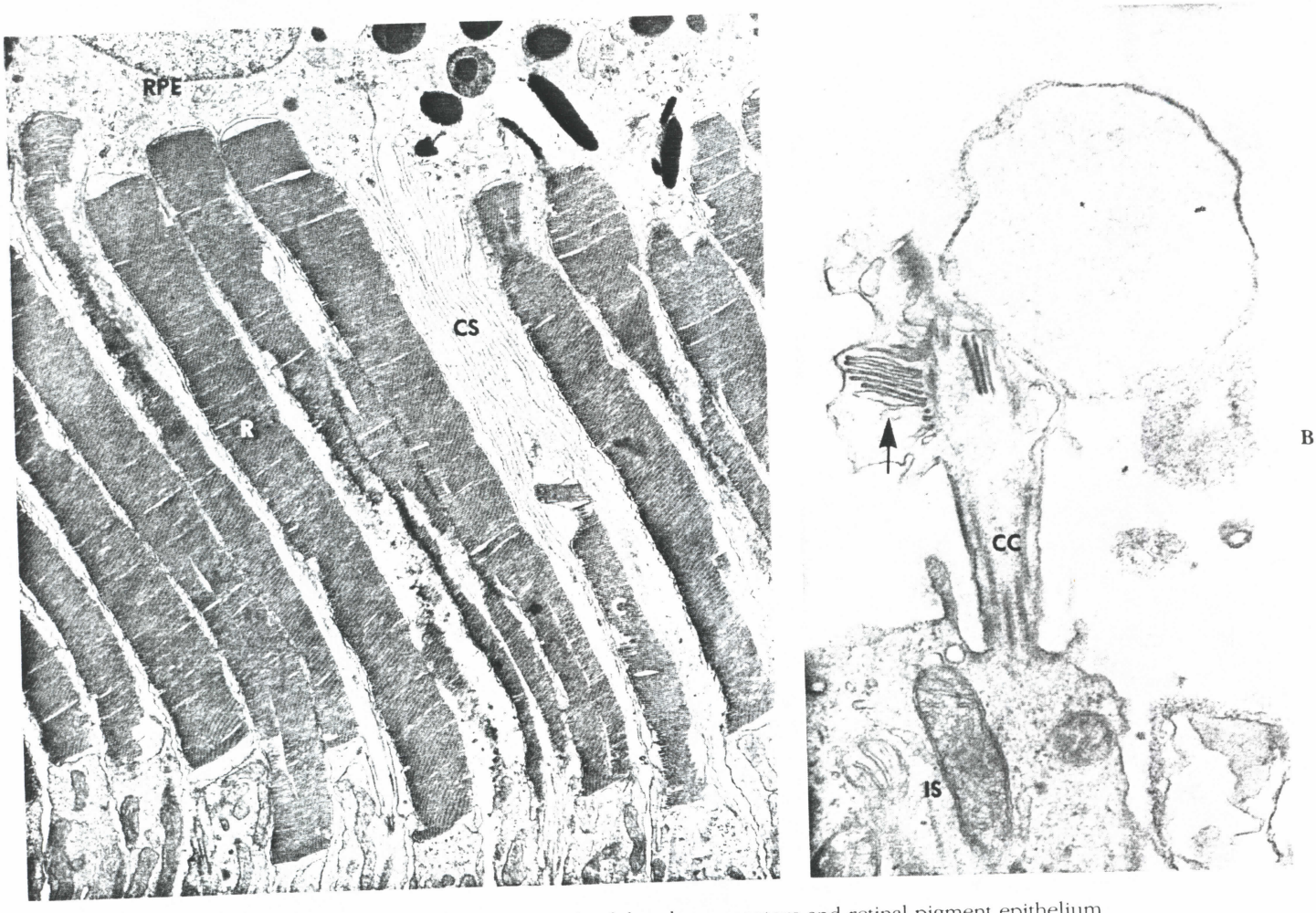


Fig. 123-9. A, An electron micrograph of the photoreceptors and retinal pigment epithelium *(RPE)* in a normal cat retina. The outer segments are oriented along their longitudinal axes in a uniform, densely packed row. Cone outer segments *(C)* are shorter than those of rods *(R)* and are ensheathed by a complex array of apical processes known as the cone sheath *(CS)*. **B,** An electron micrograph showing the photoreceptor outer segment structures that remain in a cat retina detached for 13 days. *IS,* Photoreceptor inner segment. *CC,* Photoreceptor connecting cilium. Arrow indicates a few disclike structures in the degenerated outer segment. (Magnification × 28,000.) *(**A** from Anderson, DH et al.: Invest Opthtbalmol Vis Sci 27:173, 1986.)*

ment would be expected to persist. Immunolocalization experiments indicate that these discs are labeled with opsin antibodies (Fig. 123-10). These results suggest that disc membrane formation and opsin biosynthesis do not cease completely, even after lengthy detachment episodes. But it is not known if visual pigment, i.e. opsin, synthesis is down-regulated during detachment. Disorganized lamellar debris rather than discrete packets of discs is found in the subretinal space in long-term detachments. This provides additional evidence that discs are not shed in the normal manner. Thus it appears likely that both the normal shedding process and disc formation are adversely affected by the separation of the two layers. Once the two layers are reapposed and outer segments begin to regenerate, a normal balance between disc addition and disc shedding must be reestablished if the outer segments are going to reattain normal length.

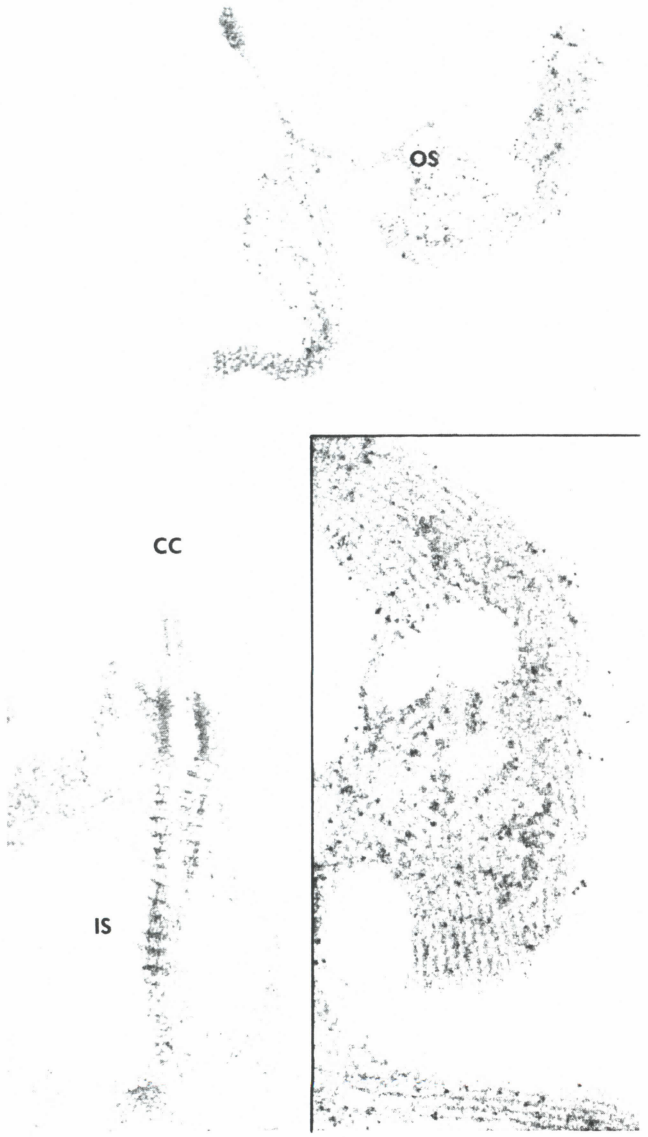


Fig. 123-10. An electron micrograph demonstrating immunolabeling of an outer segment remnant with an antibody to opsin, the major membrane protein in photoreceptor outer segments. The retina was detached for 60 days. *IS,* Photoreceptor inner segment. *CC,* Connecting cilium. *OS,* Outer segment remnant. (Magnification × 32,500.) The inset shows a portion of the outer segment at higher magnification. The electron-dense gold spheres signify the areas where opsin is present. (Magnification × 73,000.)

The effects of detachment on the photoreceptor/retinal pigment epithelial interface are rapid and extensive. The retinal pigment epithelial apical surface morphology changes abruptly, and cells begin to proliferate and then migrate into the subretinal space. Other cells of hematopoietic origin also enter the subretinal space. All these probably function to some degree as phagocytic cells, which serve the purpose of removing debris left from degenerating photoreceptors. The photoreceptor outer segments degenerate, leaving most photoreceptors with only a rudimentary cilium that may have a few disorganized discs associated with it.

The inner retina

Although the earliest and most obvious effects of detachment are seen at the retina/retinal pigment epithelial interface, they are by no means limited to that region of the retina. A number of changes occur in the retina proximal to the outer segments, especially in detachments lasting more than a few days. There are species differences in the ability of the outer nuclear layer to survive retinal detachment, and these differences probably relate to the degree of vascularization of the retina. Rabbits, which have an "avascular" retina, show extensive and rapid degenerative effects in the layer of photoreceptor nuclei, with changes occurring as quickly as 3 days after detachment. Dogs, monkeys, humans, and cats have capillaries within the neural retina, and in these species the neural retina is apparently spared from total degeneration during detachment.

Photoreceptor inner segments. Changes in inner segment morphology do not take place as rapidly as in the outer segments. During the first day of a detachment the inner segments appear essentially normal, but between the first and third days of detachment they begin to show signs of degeneration. The most common signs of inner segment degeneration are swelling, disruption, and loss of mitochondria in the ellipsoid region; loss of ribosomes and of rough endoplasmic reticulum from the myoid region; and, in about 2 weeks, an overall size reduction of the inner segment. It is interesting to note that the cilium that normally connects the inner segment to the outer segment is retained even in severely

affected inner segments in long-term detachments. This is a crucial point from the standpoint of visual recovery after reattachment, because the connecting cilium is almost certainly essential for morphogenesis of the disc membranes that make up the outer segment. The loss of mitochondria also has the potential to significantly affect the photoreceptors' ability to regenerate, because the metabolic rate in these cells is among the highest of any in the body.

Outer nuclear layer and outer plexiform layer. The outer nuclear layer contains the cell bodies of the photoreceptor cells. These cells extend a process toward the outer plexiform layer where they form the characteristic synaptic terminals of rods (spherules) and cones (pedicles).[45] The outer plexiform layer also contains the processes of second-order neurons whose cell bodies lie in the inner nuclear layer. These processes synapse with each other, as well as with the photoreceptors.

The photoreceptor cell bodies and synaptic terminals show a rapid response to detachment of the feline retina. By 3 days after detachment the cell bodies and terminals show extensive vacuolization and can also show distension of the nuclear envelope and endoplasmic reticulum. The cells often show a loss of cytoplasmic ground substance and degeneration of their mitochondria. Multivesicular bodies occurring in both the cell body and synaptic terminals are also indicators of degeneration in these cells. Not all photoreceptor cells degenerate at the same rate. Some show extensive signs of degeneration, whereas adjacent cells look relatively intact. It does appear that rod cell bodies react more quickly to detachment than do those of cones. In a region in which nearly all of the rod cell bodies show signs of degeneration and even necrosis, neighboring cone cell bodies may look relatively intact. Consistent with this observation, the rod spherules appear to be particularly susceptible to the effects of detachment. These synaptic terminals are normally filled with synaptic vesicles and contain one or two large presynaptic ribbons. When the retina has been detached for 3 days, many of these terminals appear depleted of vesicles, except for a few that remain as a halo around a greatly truncated ribbon. Similar to the effect at the level of the photoreceptor cell bodies, the cone synaptic terminals seem to survive the early effects of detachment better than do the rod terminals. They retain their characteristic shape and remain filled with synaptic vesicles; the most striking change is a population of abnormally large (85 nm to 100 nm) vesicles. Vacuolization also occurs in some of the outer plexiform layer processes from second order neurons in 3 day detachments.[19] These processes probably undergo further degeneration in longer term detachments and the cell bodies of second order neurons may be affected as well, although there is virtually no information available on this process. In very long-term detachments (50 days), photoreceptor terminals can no longer be identified by light microscopy and ultrastructural signs of synaptic contact between photoreceptors and second order neurons are rarely found.

Proliferation of cells in the inner retina. During the first 3 days of detachment there is also a proliferative component to the changes in the inner retina. Within 12 hours of the detachment, pericytes, capillary endothelial cells, and microglia are labeled by ^{3}H-thymidine autoradiography. By 2 days after the detachment, some Müller cell nuclei are labeled, and they are frequently translocated from their normal positions on the vitreal border of the inner nuclear layer into the outer plexiform and outer nuclear layers.

Müller cell hypertrophy. As detachment proceeds beyond 2 weeks, there is a significant hypertrophy of Müller cell processes within the outer plexiform layer and outer nuclear layer, presumably filling the spaces left by degenerating neurons. Müller cell bodies are now routinely found within these two layers. The cytoplasm of these cells is filled with 10 nm diameter (intermediate) filaments that, by immunochemical criteria, contain glial fibrillary acidic protein[20] and vimentin (Fig. 123-11). By 30 days of detachment, Müller cell processes sometimes extend into the subretinal space through localized disruptions in the outer limiting membrane. The extension of Müller cell processes onto the retinal surface becomes more commonplace as detachment time lengthens beyond 30 days, so that they eventually form a multilayered "glial scar" within the subretinal space, separating the neural retina from the retinal pigment epithelium. Similarly, within the outer plexiform layer the hypertrophy of Müller cell processes in long-term detachments can form a glial scar between the synaptic endings of the photoreceptors and the processes of the second order neurons. The proliferation and growth of astroglial cells, accompanied by the synthesis of intermediate filaments by the cells, is a common reponse to central nervous system injury, for example in spinal cord axotomy or neurectomy.[30] In this case, the resulting glial scar is thought to block the regeneration of spinal cord axons. The fibrillogenic response in Müller cells is even more dramatic than in brain or spinal cord astrocytes because the Müller cells normally have only a small population of intermediate filaments within their end-feet, on the border of the vitreous cavity. Within a few days of detachment these filaments begin to appear elsewhere in the cells, until they occur throughout the inner plexiform, inner nuclear, and outer plexiform layers.

The presence of Müller cell processes within the subretinal space appears to inhibit the regeneration of pho-

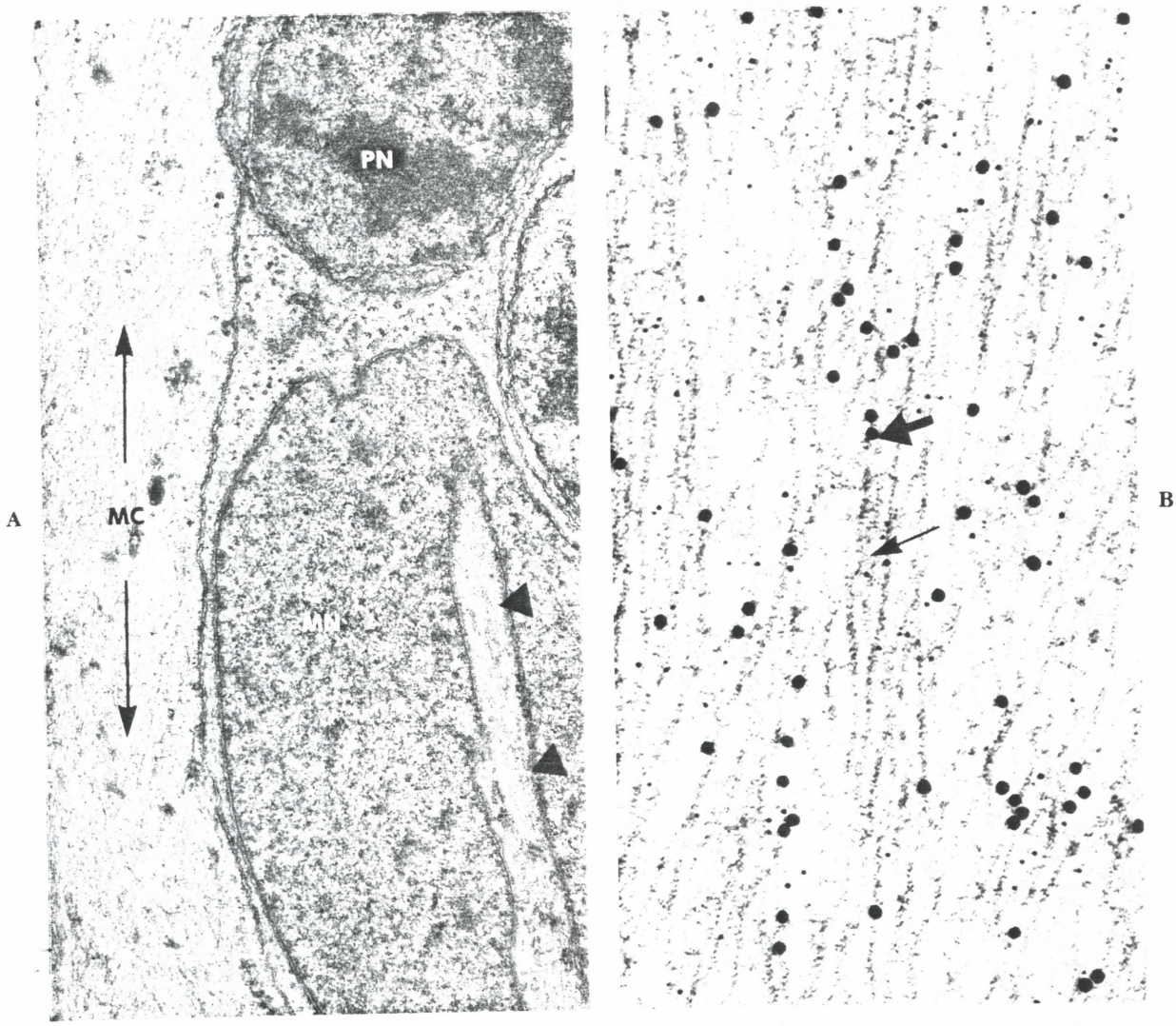


Fig. 123-11. A, An electron micrograph showing a Müller cell nucleus *(MN)* that has migrated to the outer plexiform layer in a retina detached for 30 days. There are numerous intermediate filaments near the Müller cell nucleus *(arrowheads)* and in the cytoplasm of the adjacent Müller cell *(MC)* process. Arrows indicate an adjacent Müller cell process containing numerous intermediate filaments. *PN,* Photoreceptor nucleus. (Magnification × 10,000.) **B,** An electron micrograph showing the immunolocalization of glial fibrillary protein *(small arrow,* 5 nm gold spheres), and vimentin (*large arrow,* 15 nm gold spheres) to the 10 nm diameter filaments found in Müller cell cytoplasm. (Magnification × 113,000.) *(***A** *from Erickson, PA et al.: Exp Eye Res 44:41, 1987.)*

toreceptors after retinal reattachment (see below), and their presence within the outer plexiform layer may well be expected to interfere with the reestablishment of any synaptic connections lost between photoreceptors and second order neurons during a period of detachment.

The loss of photoreceptor cells. The loss of cells from the photoreceptor layer occurs by necrosis and by the extrusion of photoreceptor cell bodies past the outer limiting membrane into the subretinal space. The mechanism of the latter event is not understood, but these cells have clearly lost all of their specialized features. They appear as rounded cells with very little cytoplasm surrounding their nucleus. In the cat retina, there is a significant decrease in the number of photoreceptor cells by 1 month after detachment, and a continued decline until the outer nuclear layer loses about 80% of its cell population by 90 days after detachment[19] (Fig. 123-12). In regions severely affected by photoreceptor degeneration, the outer nuclear layer can be reduced in thickness to one or two cell layers. Cell death within the retina is species-dependent. Of the species used in experimental detachments, the rabbit is the most severely affected, with the retina being reduced to a single layer of cells in detachments of 4 months duration.[37] Cell death was not reported in the outer nuclear layer of experimentally detached owl and rhesus monkey retinas, even in detachments of 14 weeks duration.[31,32,34] Based on data from the monkey studies, it might be concluded that cell death in the outer nuclear layer may not be a factor in detachments of the human retina. However, a recent histopathologic study by Wilson and Green[56] of retinal detachment in postmortem eyes showed that atrophy of the photoreceptor layer occurred in 26.5% of the retinas examined. Thus it appears that cell death in the photoreceptor cell layer could be a significant factor in the recovery from detachment, particularly in those of more than a few days duration.

Metabolic changes during detachment

There must be substantial metabolic changes in retinal cells during the period of degeneration after detachment or during regenerative changes that occur after reattachment, but few of these have been specifically identified. As discussed above, Müller cells greatly increase

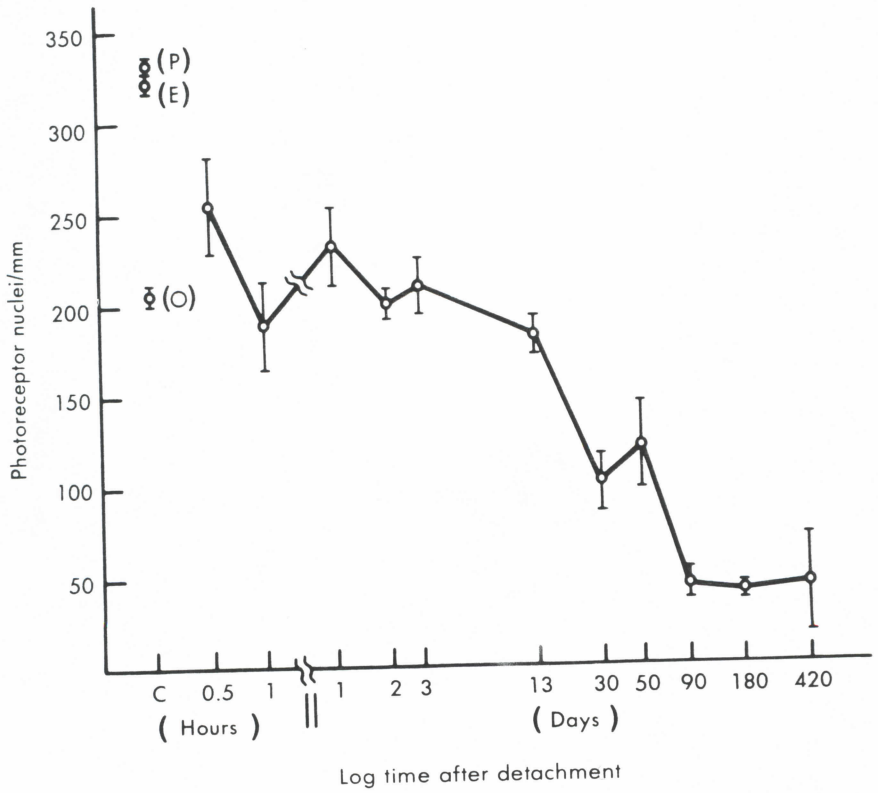
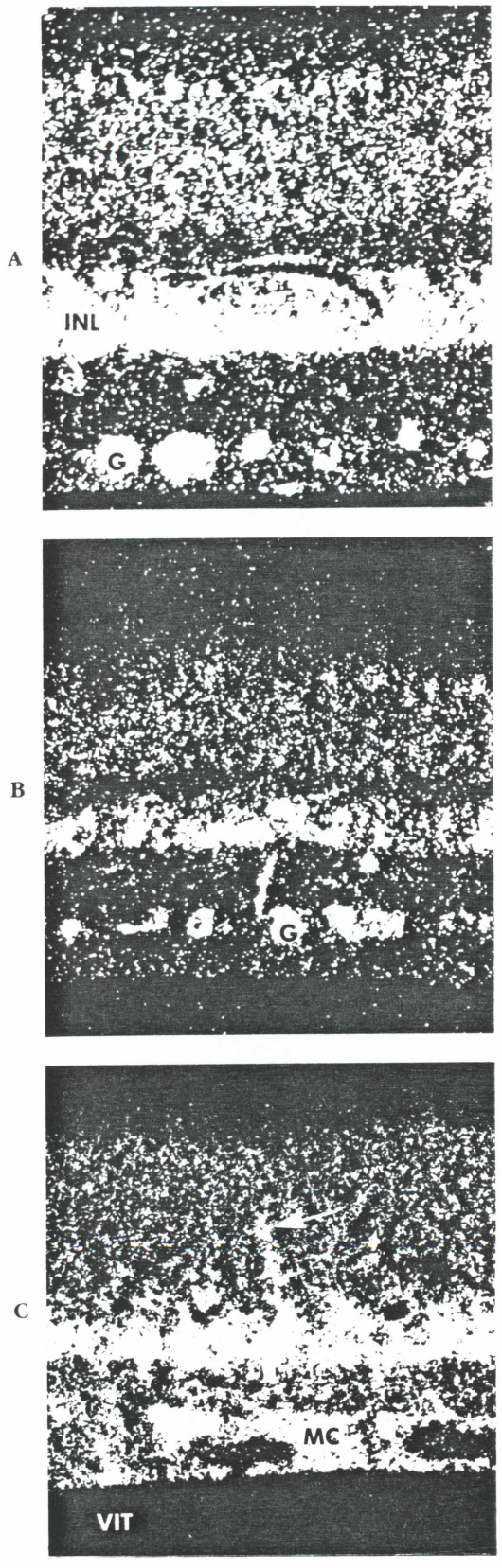


Fig. 123-12. A graph relating the number of photoreceptor nuclei in the outer nuclear layer to the time after retinal detachment *(error bars,* ± 1 standard deviation from the mean). The points indicated by *C* (control, attached retina) on the ordinate are from posterior retina *(P),* equatorial retina *(E),* and from between 1 to 2 mm posterior to the ora serrata *(O).* All counts from retinal deachment are from equatorial or posterior retina. *(From Erickson, PA et al.: Invest Ophthalmol Vis Sci 24:938, 1983.)*



their production of intermediate filaments. The only other change that has been documented is a change in RNA synthesis. It has been known for many years that neurons alter their levels of RNA synthesis after injury (usually axotomy). In tissue containing a mixed population of cells, the only way to determine relative changes in RNA synthesis in different cell types is to use tissue autoradiography after labeling with the ribonucleoside, ^{3}H-uridine, a specific precursor of RNA. Synthesis of RNA can be quantified in autoradiograms by counting exposed silver grains over individual cells, individual nuclei, or per unit area. Because an experimental detachment does not necessarily encompass the complete retina, the detached regions can be compared with attached regions of the same eye. When feline eyes detached for 24 hours are given an intravitreal or intravenous injection of ^{3}H-uridine and the eyes are then fixed 24 hours later, there is significantly less labeling in the photoreceptor and Müller cells in the region of detachment as compared with the adjacent, attached retina (Fig. 123-13). This could represent an overall depression of metabolic activity or a specific effect on RNA synthesis in the detachment zone. Labeling of the retina 48 hours after detachment produces the same decreased level of labeling in the photoreceptors, but a dramatic increase in the labeling of Müller cells (Fig. 123-13). If the retina is labeled 2 weeks or 30 days after detachment, the pattern and labeling levels are similar to those observed 24 hours after detachment. Because these changes occur in the region of detachment but do not occur in the adjacent attached region, they suggest a localized effect of the detachment on RNA biosynthesis. Although the RNA species that increases in the Müller cells 48 hours postdetachment is not known, it is correlated with a dramatic increase in intermediate filament density, which also is localized to the region of detachment. Müller cells in immediately adjacent attached retina do not show an increase in intermediate filaments in their cytoplasm.

Another metabolic change that occurs in Müller cells after detachment is a decrease in the level of immunostaining with antibodies to different proteins. In feline retinas detached for 2 months, there is a significant decline in the labeling intensity of the Müller cells when

Fig. 123-13. Light microscope autoradiographs (dark-field exposures) of retinas detached for 1 **(A, B)** or 3 **(C)** days and labeled for 24 hours with tritiated-uridine. **A** is from an attached region adjacent to the area of detachment shown in **B.** Note the decreased intensity of labeling in the outer and inner nuclear layers in the detached region. **C** shows the change in the pattern of labeling that occurs by 3 days after detachment. There is heavy labeling of the Müller cell bodies in the inner nuclear layer, the Müller cell end-feet *(MC)* near the vitreous *(VIT),* and strands of Müller cell cytoplasm extending into the outer retina *(arrow).* (Magnification × 325.)

stained with an antibody to the retinoid binding protein known as cellular retinaldehyde protein (Fig. 123-14, *A,B*). Similar immunolabeling experiments show a dramatic decrease in the levels of two Müller cell enzymes, carbonic anhydrase C and glutamine synthetase, during detachment. Thus detachment of the retina seems to produce changes in the metabolism of retinal glia. It is likely that the large increase in RNA synthesis seen in Müller cells represents a specific phase of altered transcription and translation that is a direct or secondary response to detachment.

EXPERIMENTAL RETINAL REATTACHMENT

Visual recovery after retinal reattachment

The fact that retinal reattachment restores at least partial vision in most human patients implies that many of the adverse effects of retinal detachment can be successfully reversed. Understanding the cellular changes that accompany this recovery and acquiring information that will promote maximal recovery is the ultimate goal of experimental studies of reattachment. Discovery of the cellular mechanisms underlying the recovery process and the variables that affect the extent of the recovery must depend almost entirely on animal models because retinal tissue from human reattachment patients is rarely obtainable.

Most studies of visual recovery in human reattachment patients have been restricted to basic acuity measurements as a function of detachment duration. In general, these studies indicate that detachment duration is inversely related to acuity recovery, with the longest durations correlated with the least recovery. A few studies clearly demonstrate that improvements in color vision, visual sensitivity, and other visual parameters are also an integral part of the recovery process.[15,22] In the case of detachments involving the macula, there is no general

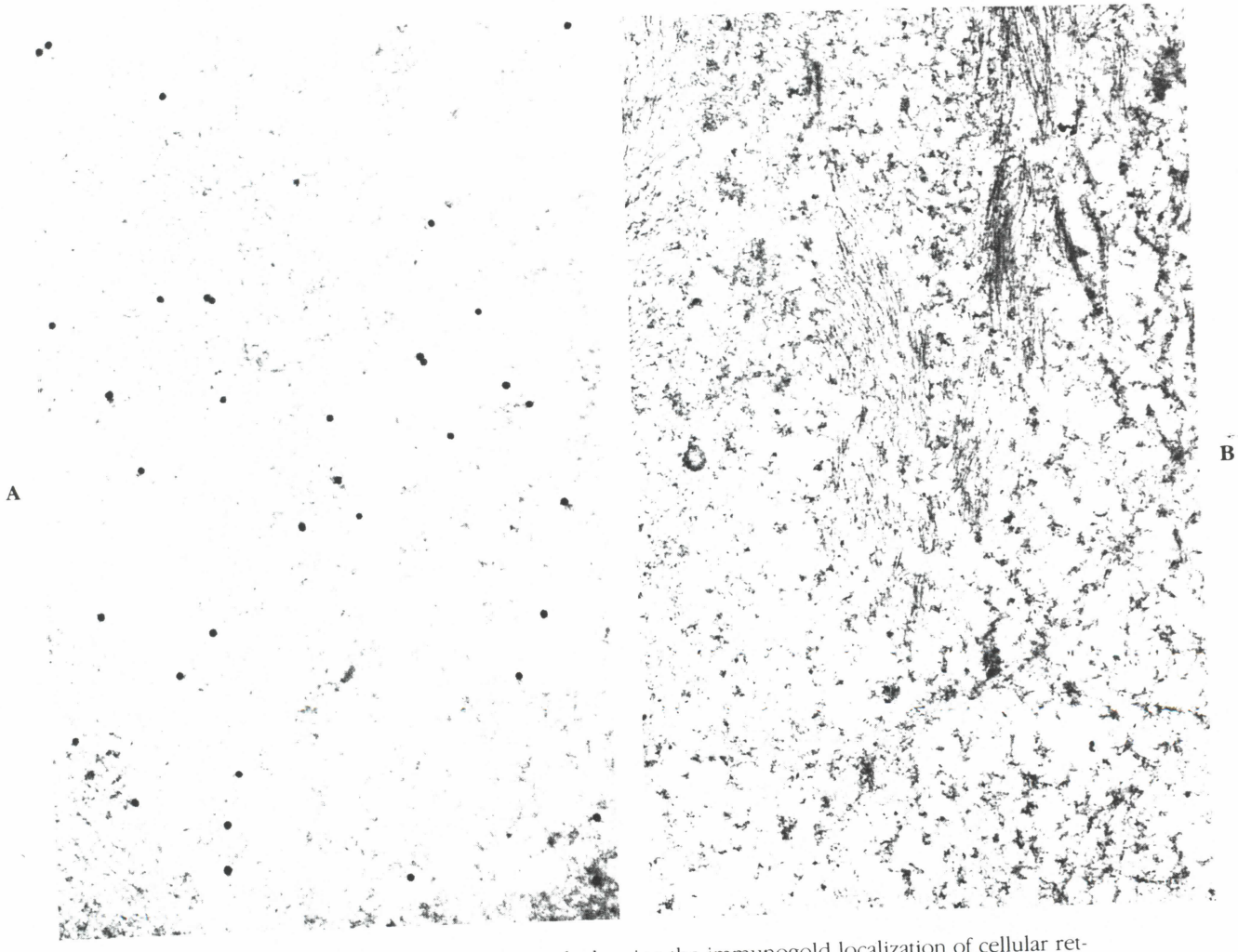


Fig. 123-14. A, An electron micrograph showing the immunogold localization of cellular retinaldehyde–binding protein in the cytoplasm of normal Müller cells. Gold spheres are 15 nm. (Magnification × 61,000.) **B,** Labeling is absent from the Müller cells in a zone of detached retina (60-days duration) adjacent to the attached region shown in **A.** (Magnification × 33,000.)

consensus in identifying a critical period beyond which the prognosis for recovery dims. However, one study[13] provides compelling evidence that visual recovery declines exponentially as a function of macular detachment duration. This conclusion conforms with the morphologic results obtained from experimental models of reattachment.

Reestablishing the photoreceptor–retinal pigment epithelial interface

It is evident that successful reestablishment of the photoreceptor–retinal pigment epithelial interface is the most important aspect of morphologic recovery after reattachment. The molecular interactions that must occur between the photoreceptors and retinal pigment epithelial cells after reattachment are not known. But some relevant concepts have emerged from the experimental analysis: (1) The photoreceptors are not reapposed to their original sites of interaction with the retinal pigment epithelium when the retina is reattached. Therefore site-specific interactions between these two cell types can be virtually ruled out as a factor in recovery. (2) Rods and cones in many mammals, including humans, have different ultrastructural relationships with the retinal pigment epithelial apical surface (see p. 165). It is still unclear whether these specific relationships are reestablished after reattachment and, moreover, whether this has implications for the successful regrowth of rod vs. cone outer segments. (3) The cell-cell interaction that must occur after reapposition of the two layers is analogous to the interaction that takes place during retinal pigment epithelial and photoreceptor development. Early in development, the photoreceptor inner segments are apposed to a mounded and undifferentiated apical retinal pigment epithelial surface. Unknown metabolic and molecular processes that promote retinal pigment epithelium–photoreceptor adhesion tend to maintain this apposition as the photoreceptor outer segments interdigitate with newly forming apical retinal pigment epithelial processes and eventually attain their mature configurations. Reattachment of the retina must induce a similar series of molecular events that controls the redifferentiation of the retinal pigment epithelial apical surface and the reensheathment of the regenerating outer segments. (4) The photoreceptors and retinal pigment epithelium must also reestablish a functional relationship. The transport of ions and molecules between the retina and retinal pigment epithelium is affected to an unknown degree when the two cell layers are separated from each other. For example, retinoids (vitamin A isomers), coupled to their binding proteins, must be transported back and forth between the neural retina and the retinal pigment epithelium as part of the visual cycle.[11] It is not known whether this transport is affected by detachment or reattachment processes.

Photoreceptor outer segment regeneration

Information on the rate of outer segment regrowth after reattachment is very sparse and difficult to obtain. In experimentally reattached retinas, regenerating outer segments often appear shortened and misaligned with respect to each other. The stacking of the disc membranes is usually abnormal. In addition, there is a high degree of variability in outer segment length from one reattached region to the next, which contributes to the impression that regeneration is not a homogeneous process across the retina (see below). All of these factors add to the difficulty of quantifying the rate of outer segment regrowth.

It has been reported that owl monkey rods undergo a period of accelerated membrane synthesis within the first 24 hours of reattachment.[33] In the cat and rhesus monkey, we have been unable to confirm any deviation from the normal rate of disc membrane turnover.[6,23] In cat retinas detached for between 5 and 10 days and reattached for 1 week, a band of radioactive protein appears near the base of the outer segment 24 hours after an intravitreal injection of ^{3}H-amino acids, the same location as in control eyes. Cat rod outer segments are renewed at a rate of about 2.3 μm/day, so they could theoretically regenerate to normal length (12 to 18 μm) in about 1 week in the absence of any disc shedding. Since the normal renewal process represents a balance between disc assembly and disc shedding, regenerating outer segments would be expected to take somewhat longer than a week to reattain their normal lengths. In fact, many rod outer segments are shorter than normal several months after reattachment, suggesting that they may never regain their normal size.[6]

"Patchwork regeneration," the result of proliferative changes. What is striking in reattachment studies is the variability in outer segment length within an individual retina or between different retinas with the same detachment and reattachment intervals. This may reflect inherent variability in the detachment and reattachment processes themselves. Retinas have detached regions that are deep or shallow and, hence, may suffer more or less severe photoreceptor degeneration. In addition, certain regions may regain close apposition to the retinal pigment epithelium more rapidly and begin the regenerative process more quickly than do adjacent regions.

In the experimentally reattached retina,[6,8] photoreceptor regeneration occurs in a "patchwork" way, with areas of near-normal appearance adjacent to areas showing virtually no regeneration. Only some of the mechanisms producing this result are understood. Two of them clearly result from proliferative events that occur during detachment. The proliferation of either retinal pigment epithelial cells or Müller cells into the subreti-

nal space is nearly always associated with poor or nonexistent photoreceptor recovery (Figs. 123-15 and 123-16). In the case of retinal pigment epithelial proliferation, recovery seems to depend on the degree of differentiation and the polarity of the proliferated cells. In many instances, the apical-basal surface morphology of proliferated retinal pigment epithelial cells bears little resemblance to that of normal retinal pigment epithelium. In others, additional layers of proliferated cells may have their polarity reversed, with the *basal* surface facing the photoreceptors (see Fig. 123-5). In either situation, regeneration is much poorer. Similarly, if the photoreceptors are apposed to glial (Müller) cell processes in the subretinal space, they are unable to regenerate an outer segment. Suppression of proliferation of these two cell types may lead to improved visual recovery after reattachment.

A MOLECULAR BASIS FOR RETINAL ADHESION

Although the major cellular changes that occur after retinal detachment and reattachment are now known in some detail, a clear understanding of why the retina adheres to the pigment epithelium remains elusive. It is widely believed that bulk flow of fluid moving through the retina in the vitreous to choroid direction constitutes the adhesive force that maintains the normal apposition between the retina and retinal pigment epithelium. The retina is presumed to offer resistance to this fluid movement and is consequently suctioned onto the apical surface of the epithelium. However, such theories are hard-pressed to account for the fact that the retina remains adherent after enucleation of the globe and removal of the anterior segment.[36] Similarly, the retina usually remains adherent even in the the presence of a retinal tear or hole that negates the "suction force" by presenting a path of no resistance to fluid flow. Thus it appears unlikely that the movement of fluid accounts for all aspects of retinal adhesion.[61] Cell-cell and/or cell-matrix interactions could be of equal importance in maintaining adhesion at the interface between these two cell layers.

Cell-cell adhesion

Current theories of cell-cell adhesion focus on a limited number of cell adhesion molecules (CAMs) that are present in a variety of different tissues.[18] CAMs are in-

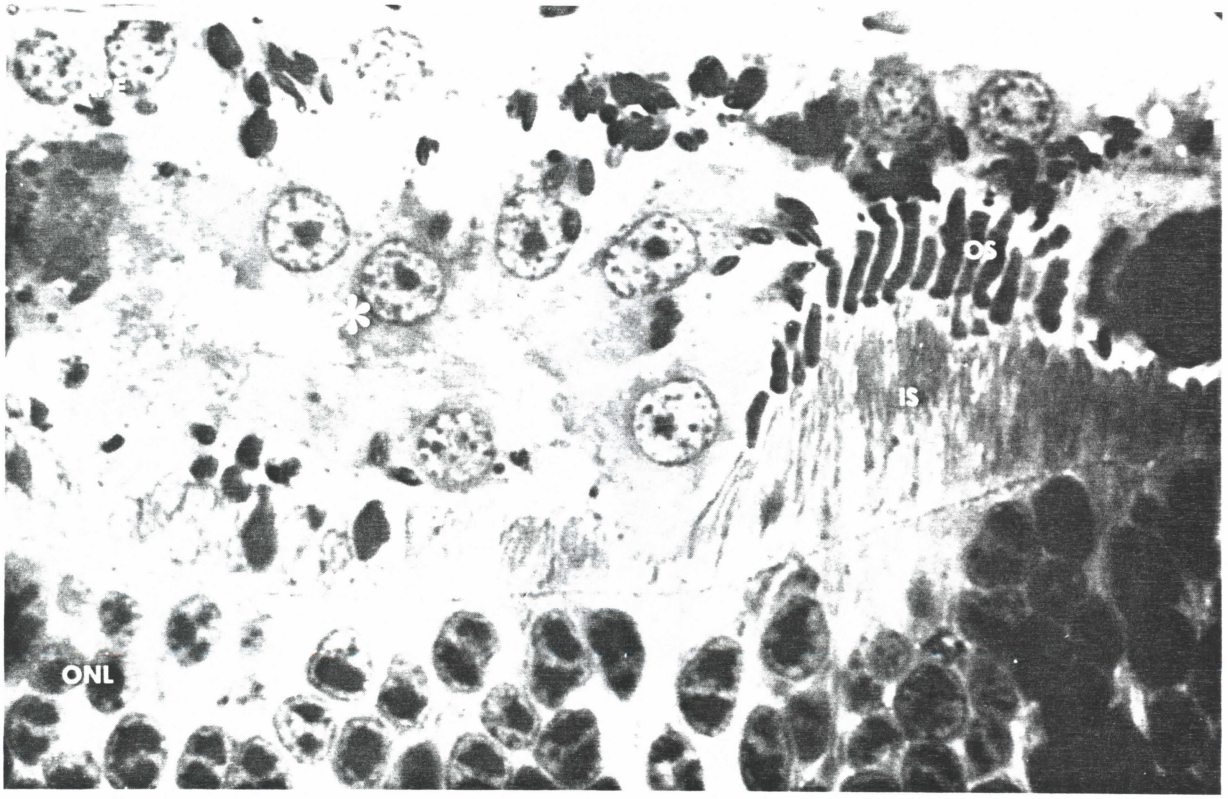


Fig. 123-15. A light micrograph showing an area of retinal pigment epithelial *(RPE)* cell proliferation in a reattached retina. Only a few outer segment remnants remain under the cluster of proliferated cells *(asterisk)*. The dimensions of the outer segments *(OS)* adjacent to the zone of proliferation are nearly normal. *IS,* Photoreceptor inner segment. *ONL,* Outer nuclear layer. (Magnification × 2200.) *(From Anderson, DH et al.: Invest Ophthalmol Vis Sci 27:173, 1986.)*

trinsic membrane glycoproteins that mediate cell-cell adhesion by direct molecular binding between CAMs on apposing cells. CAM-mediated retinal adhesion would require direct binding of CAMs on the plasma membranes of pigment epithelial microvilli to CAMs on the outer segment plasma membranes (Fig. 123-17). CAMs, or their equivalents that show a capacity for homophilic binding, have yet to be identified at the photoreceptor–pigment epithelial interface, although they are present throughout the inner retina.[46]

Cell-matrix adhesion

Alternatively, retinal adhesion could arise from cell-matrix interactions in the interphotoreceptor space. The mechanism by which cells in culture adhere to their substrate is now known to be a transmembrane process involving both matrix and cytoskeletal-related proteins. Cell surface molecules, such as fibronectin, laminin, collagens, and proteoglycans, interact with subplasmalemmal proteins such as vinculin and alpha-actinin, at sites of cell-substratum adhesion.[17] In that context, retinal adhesion could be hypothesized as a case of cell-substratum-cell attachment. Extracellular matrix molecules in the interphotoreceptor space would bind to specific ligands on the outer segment epithelial plasma membranes, which, in turn, would interact with subjacent cytoskeletal elements (Fig. 123-17). In fact, there is some fragmentary evidence consistent with this matrix-mediated hypothesis.

Fibronectin (*fibre*, fiber + *nectere*, to bind) is a candidate retinal adhesion molecule. It is a major component of blood plasma, cell surfaces, and extracellular matrices and has been implicated in a wide variety of adhesion and migration-related cellular events.[57] Although it is not detectable by biochemical analysis as a soluble compartment of the interphotoreceptor matrix,[1] it has been localized extracellularly on the apical microvilli of the rat pigment epithelium by immunoelectron micro-

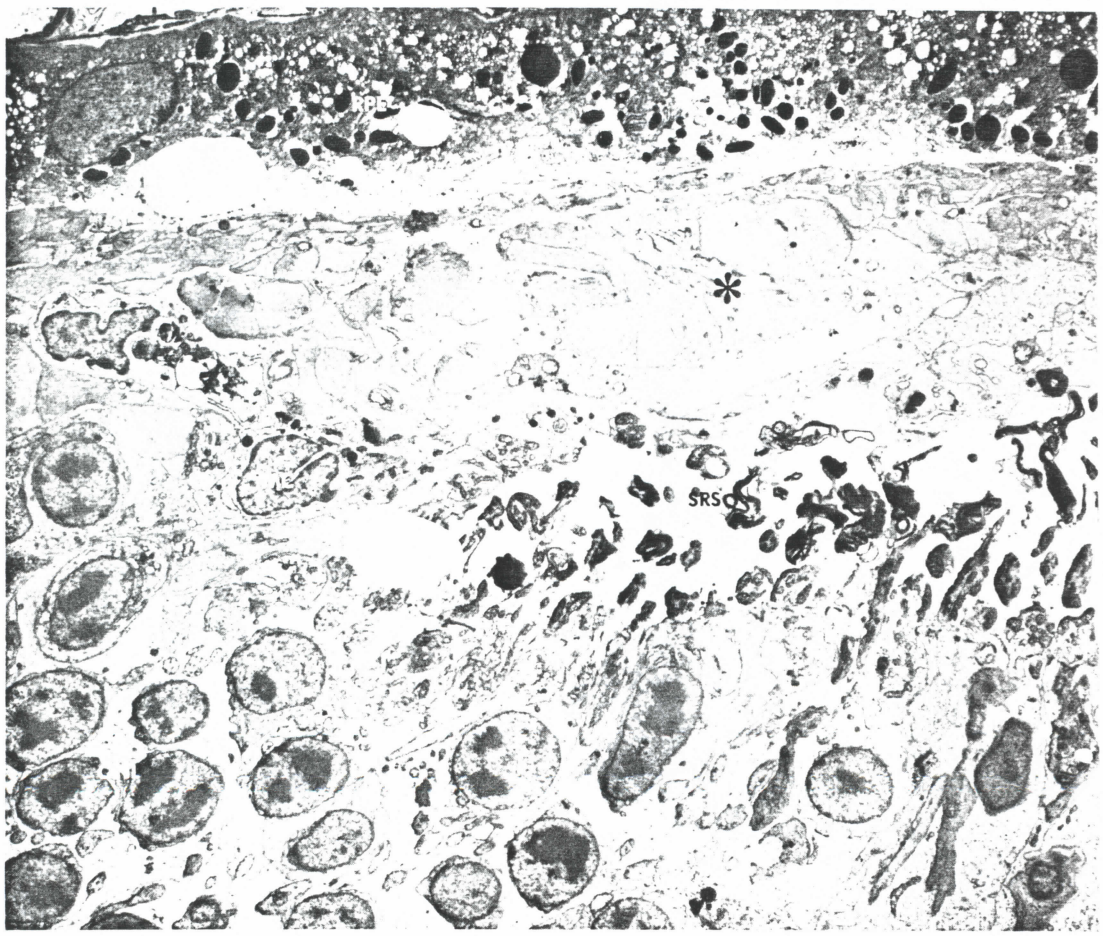


Fig. 123-16. An electron micrograph showing extensive Müller cell hypertrophy in the subretinal space in a retina that was detached for 6 weeks and reattached for 4 weeks. The Müller cell processes form a barrier between the retinal pigment epithelium *(RPE)* and the photoreceptor cell layer. *ONL,* Outer nuclear layer. *Asterisk,* Müller cell processes in the subretinal space *(SRS). MC,* A Müller cell body in the subretinal space. (Magnification × 2800.)

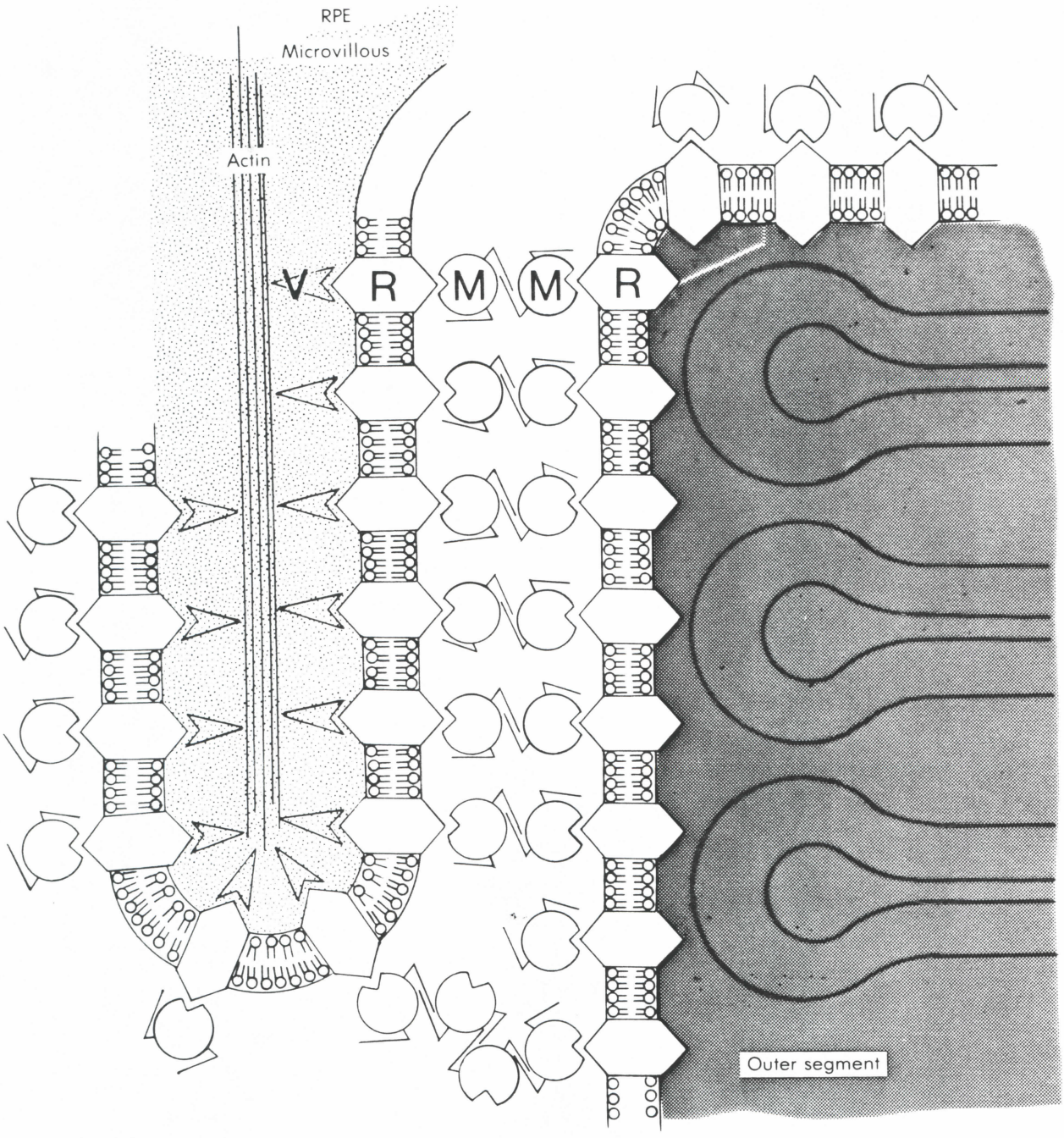


Fig. 123-17. A molecular model of matrix-mediated retinal adhesion. In this hypothetical model, aggregates or assemblies of matrix molecules in the interphotoreceptor space mediate binding to ligands on the plasma membranes of RPE cell apical microvilli and photoreceptor outer segments. The ligands are depicted here as the extracellular domains of membrane proteins that act as surface receptors *(R)* for matrix molecules *(M)*. The membrane receptors could be the same or they could be different for the two cell types. The cytoplasmic side of the receptors interacts with cytoskeletal and subplasmalemmal proteins *(V)*. Corresponding interactions with cytoskeletal elements of the outer segments may also be inferred but they are not shown. The alternate hypothesis described in the text requires direct molecular binding between identical cellular adhesion molecules on the microvillar and outer segment plasma membranes.

scopy.[41] Using immunofluorescence microscopy, it is not detected on the apical surface of pigment of epithelial explants in the chick,[40] but we have recently corroborated and extended the results in rat retina to the cat and rhesus monkey pigment epithelium, using similar immunocytochemical methods (Fig. 123-18). However, further work will be required to determine whether fibronectin or other photoreceptor matrix molecules are implicated in retinal adhesion.

The matrix sheath that envelops cone outer and inner segments in many species[10,27,28] could also play a role in retinal adhesion. The sheath extends beyond the outer segment tips and is apparently continuous with a glycosaminoglycan-rich region on the apical surface of the pigment epithelium.[53] Although the relationship of these matrix domains to the photoreceptor and pigment epithelial cell membranes is unknown, their locations are suitable for the type of cell-matrix-cell linkage alluded to above.

THE LIMITS OF MORPHOLOGIC RECOVERY

The evidence for complete photoreceptor recovery, even after a brief episode of detachment, is conflicting. Rabbit retinas that spontaneously reattached were described as showing limited and variable outer segment recovery.[38] In cat retinas that were detached and reattached for variable periods of time it was concluded that, ". . .ultrastructural morphology does not return to the pre-detachment state even after brief episodes of detachment coupled with prolonged recovery periods"[6] (Fig. 123-19). On the other hand, Kroll and Machemer[32] concluded that owl or rhesus monkey retinas detached for up to 12 weeks can appear relatively normal after approximately 4 weeks reattachment. A recent study in the macula of rhesus monkeys detached for 3 or 7 days and reattached for 3, 7, 14, or 30 days demonstrated a progressive increase in both rod and cone outer segment length and a tendency to return to their normal configurations[23] (Fig. 123-20). There is general agree-

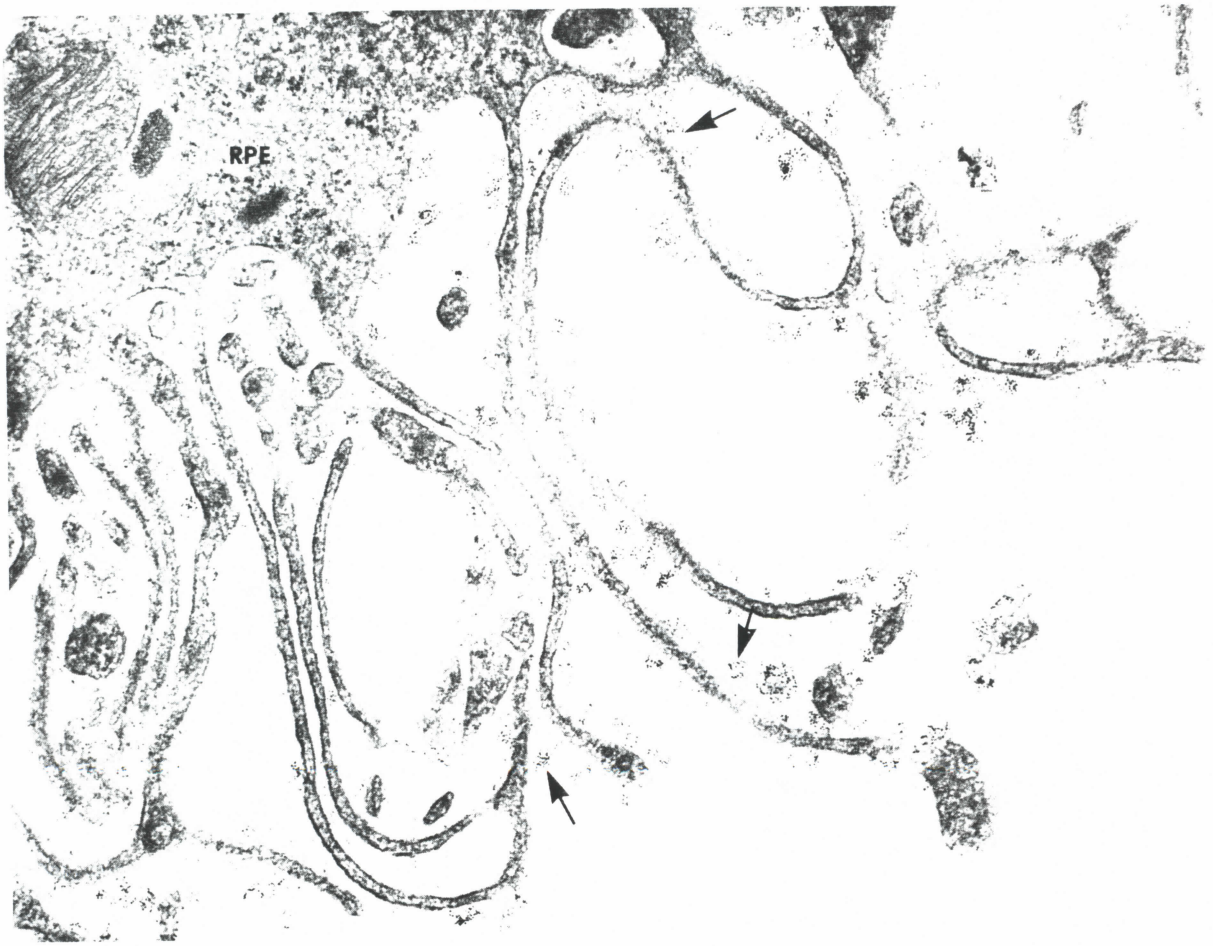


Fig. 123-18. An electron micrograph showing the immunolocalization of fibronectin to the apical surface of the cat pigment epithelium *(RPE)*. Clusters of ferritin particles (three are indicated by arrows) indicate the presence of fibronectin molecules adjacent to the plasma membrane of the apical microvilli. (Magnification × 44,000).

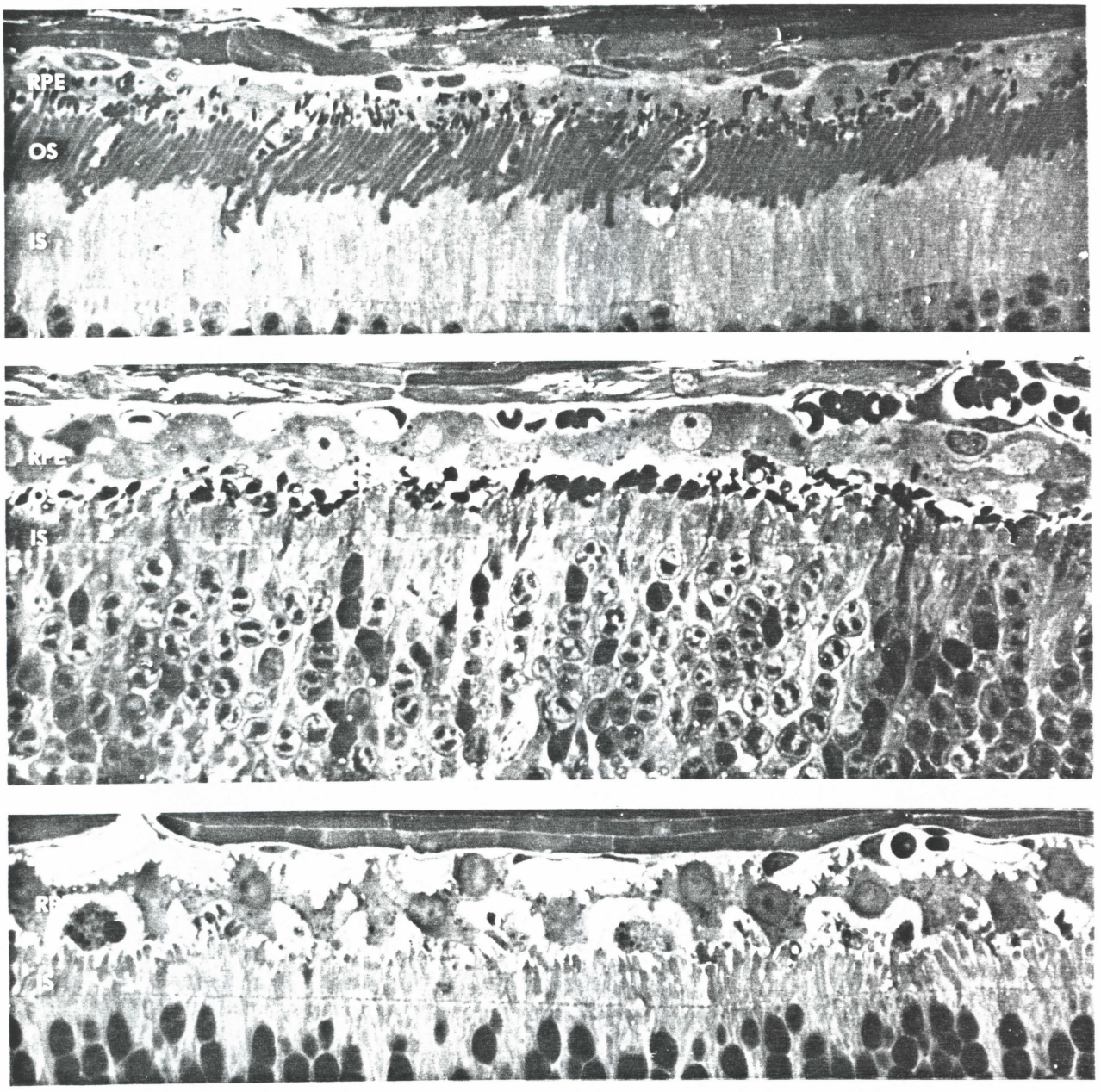


Fig. 123-19. Light micrographs of retinas detached and reattached for various intervals. **A,** The photoreceptor/retinal pigment epithelial interface after a 3-day detachment and 1-month reattachment. After short detachment intervals the outer segments can reattain normal length in a relatively short time frame. Discrete areas of disruption, however, can still be identified. **B,** In contrast to **A,** the lengthy detachment interval of 42 days coupled with a 30-day reattachment period results in distinctly inferior outer segment regeneration. The dark-staining nuclei in the outer nuclear layer are pyknotic cells. **C,** In a retina detached for 5 days and reattached for 7 days, there is virtually no evidence of outer segment regrowth in this region. The apical processes of the pigment epithelium extend down to the inner segments. This probably represents one of the earliest stages in recovery.

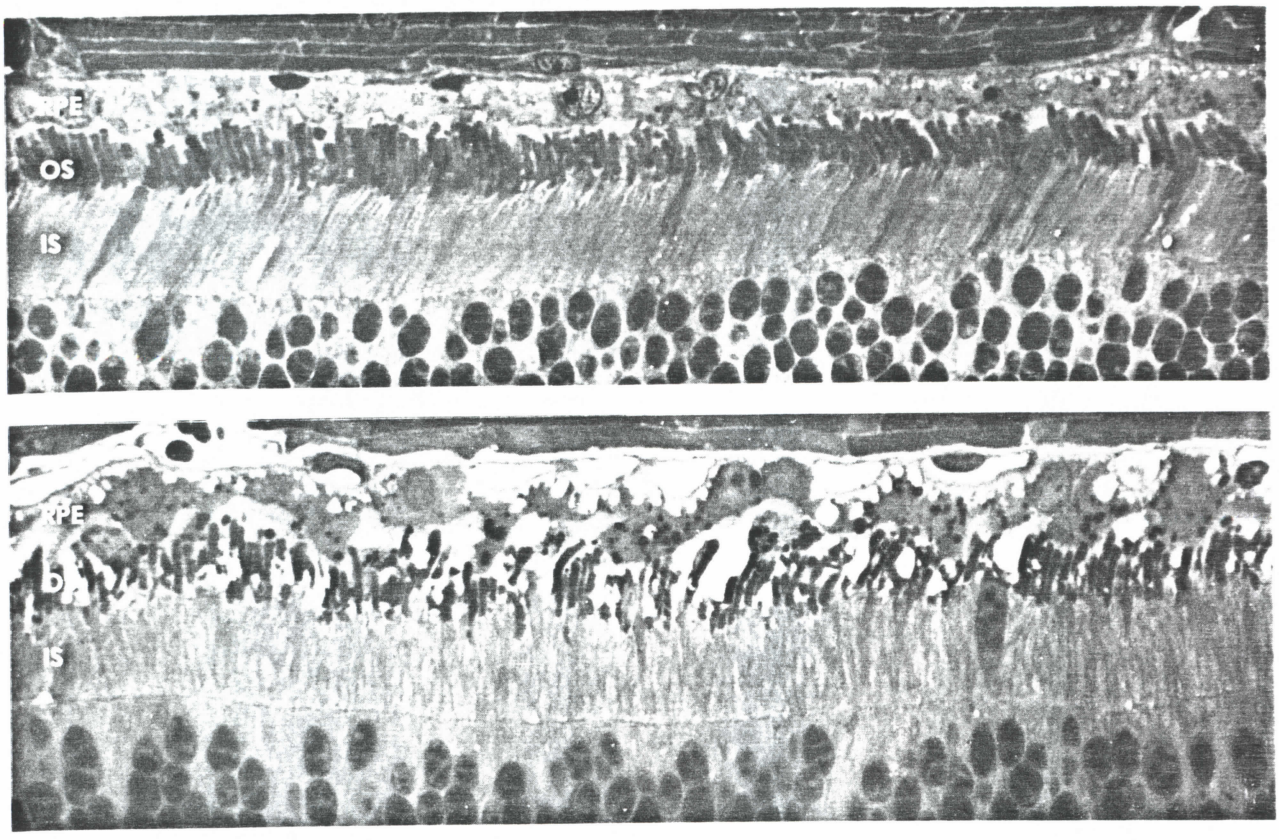


Fig. 123-19, cont. D, An example from a second animal whose retina was detached for 5 days and reattached for 7 days. In this case the rod outer segments are approximately half the normal length. Cone outer segments are also abnormally short. **E,** In a retina detached for 8 days and reattached for 7 days, there is a wide range of outer segment lengths. The outer segment tips are positioned along the perimeter of the mounded epithelial surface, and that may account for the uneven spacing between outer segments. *RPE,* Retinal pigment epithelium. *OS,* Layer of photoreceptor outer segments. *IS,* Layer of photoreceptor inner segments. (Magnification × 800.)

ment that after reattachment the outer segments do retain *some* capacity for regrowth and for reestablishing a relationship with apical retinal pigment epithelial processes. Moreover, the prevailing evidence also indicates that detachment duration is an important, if not critical, variable in determining the eventual extent of morphologic recovery. This clarity is lost, however, when attempts are made to define precisely the temporal parameters that govern recovery, as well as the absolute limits that may be imposed on morphologic recovery by detachment per se. At present, it seems reasonable to conclude that a return to *completely* normal retinal morphology is unattainable even after brief episodes of detachment.[6] But it also appears that incomplete morphologic recovery may actually be sufficient to subserve near normal vision under ideal circumstances.

This raises the issue of whether the neural retina, like other organ systems, has sufficient resilience to sustain an anatomic injury such as detachment without resulting in measurable or perceived deficits in function. At present, it simply is not known whether some of the ultrastructural abnormalities that persist indefinitely after reattachment have measurable effects on visual capacity. It may be that they do not, unless a particularly vulnerable retinal location, such as the fovea, is involved.

Many of the effects described in experimental reattachments occur in small localized regions, especially when the detachment interval is short. This may have no significant effect on the return of vision unless it occurs within the macula, where even a small disruption of retinal structure may have a profound effect. The presence of a macular detachment is known to produce disturbances in acuity, metamorphopsia, and disturbances of color vision that persist long after the retina is reattached. The loss of even a few photoreceptors from the fovea may have an impact on acuity. Although Curcio and coworkers[16] have shown substantial individual variability in the packing density of photoreceptors within the primate macula, the reduction in number in an al-

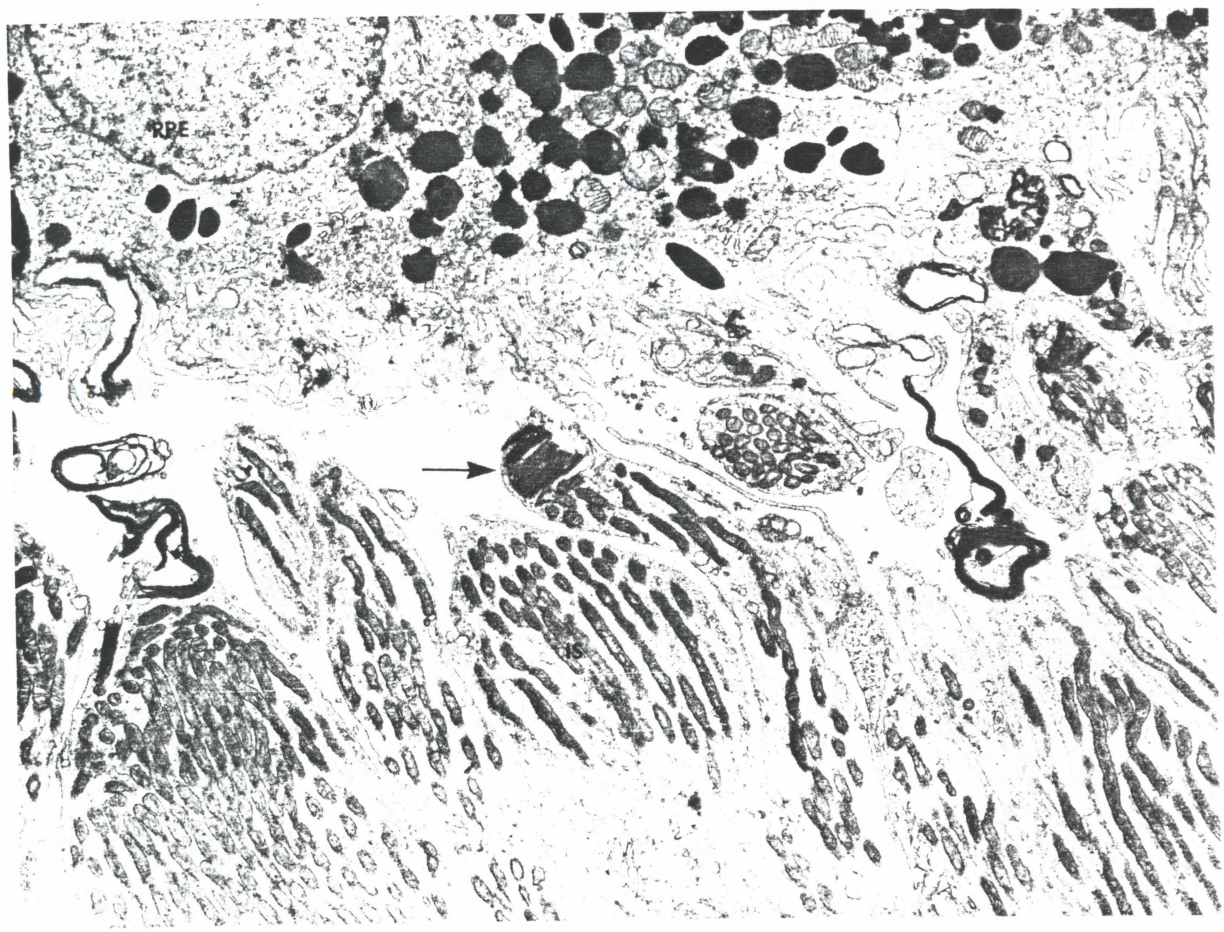


Fig. 123-20. An electron micrograph of the photoreceptor–retinal pigment epithelial *(RPE)* interface in the macula of a monkey retina detached 7 days and reattached for 3 days. Even after short reattachments intervals some regenerating outer segments *(arrow)* can be identified. *IS,* Photoreceptor inner segment. (Magnification × 7100.)

ready established mosaic may have an effect on the return of vision. Because of lack of plasticity, the adult visual system may not be able to compensate for the loss of photoreceptors in the mosaic.

SUMMARY

Recent experimental evidence suggests that retinal reattachment results in the establishment of a modified relationship between the neural retina and the retinal pigment epithelium.[8] Reattachment of detached retinas results in heterogeneity across the retina, so that regions of relatively normal retina may lie adjacent to areas of severe degeneration. Indeed, the range of structural abnormalities may range from relatively subtle changes in the retinal pigment epithelial apical surface to complete photoreceptor degeneration and the presence of subretinal gliosis. Degenerative changes extend to the inner retina in a long-term detachment; as yet unidentified subcellular changes may occur at the level of synaptic interactions, even in short-term detachments.

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REFERENCES

1. Adler, AJ, and Klucznik, KM: Proteins and glycoproteins of the bovine interphotoreceptor matrix: composition and fractionation, Exp Eye Res 34:423-434, 1982
2. Adler, AJ, and Severin, KM: Proteins of the bovine interphotoreceptor matrix: tissues of origin, Exp Eye Res 32:755-769, 1981
3. Alberts, B, Bray, D, Lewis, J, Raff, M, Roberts, K, and Watson, J: The molecular biology of the cell, New York, 1983, Garland Publishing
4. Anderson, DH, and Fisher, SK: Disc shedding in rodlike and conelike photoreceptors of tree squirrels, Science 187:953-955, 1975
5. Anderson, DH, and Fisher, SK: The relationship of primate foveal cones to the pigment epithelium, J Ultrastruct Res 67:23-32, 1979
6. Anderson, DH, Guerin, CJ, Erickson, PA, Stern, WH, and Fisher, SK: Morphological recovery in the reattached retina, Invest Ophthalmol Vis Sci 27:168-183, 1986
7. Anderson, DH, Stern, WH, Fisher, SK, Erickson, PA, and Borgula,

GA: The onset of pigment epithelial proliferation after retinal detachment, Invest Ophthalmol Vis Sci 21:10-16, 1981
8. Anderson, DH, Stern, WH, Fisher, SK, Erickson, PA, and Borgula, GA: Retinal detachment in the cat: the pigment epithelial-photoreceptor interface, Invest Ophthalmol Vis Sci 24:906-926, 1983
9. Berman, ER, and Bach, G: The acid mucopolysaccharides of cattle retina, Biochem J 108:75-88, 1968
10. Blanks, JC, and Johnson, LV: Specific binding of peanut lectin to a class of retinal photoreceptor cells: a species comparison, Invest Ophthalmol Vis Sci 25:546-557, 1984
11. Bunt-Milam, AH, and Saari, JC: Immunocytochemical localization of two retinoid-binding proteins in vertebrate retina, J Cell Biol 97:703-712, 1983
12. Bunt-Milam, AH, Saari, JC, Klock, IB, and Garwin, GG: Zonulae adherens pore size in the external limiting membrane of the rabbit retina, Invest Ophthalmol Vis Sci 26:1377-1380, 1985.
13. Burton, TC: Recovery of visual acuity after retinal detachment involving the macula, Trans Am Ophthalmol Soc 80:475-497, 1982
14. Campochiaro, PA, Jerdan, JA, and Glaser, BM: The extracellular matrix of human retinal pigment epithelial cells in vivo and its synthesis in vitro, Invest Ophthalmol Vis Sci 27:1615-1621, 1986
15. Chisholm, IA, McClure, E, and Foulds, WS: Functional recovery of the retina after retinal detachment, Trans Opthalmol Soc UK 95:167-172, 1975
16. Curcio, CA, Sloan, KR, Jr, Packer, O, Hendrickson, AE, and Kalina, RE: Distribution of cones in human and monkey retina: individual variability and radial asymmetry, Science 236:579-582, 1987
17. Damsky, CH, Knudsen, KA, and Buck, CA: Integral membrane glycoproteins in cell-cell and cell-substratum adhesion. In Ivatt, RJ: The biology of glycoproteins, New York, 1984, Plenum Press
18. Edelman, GM: Cell adhesion molecules, Science 219:450-457, 1983
19. Erickson, PA, Fisher, SK, Anderson DH, Stern, WH, and Borgula, GA: Retinal detachment in the cat: the outer nuclear and outer plexiform layers, Invest Ophthalmol Vis Sci 24:927-942, 1983
20. Erickson, PA, Fisher, SK, Guerin, CJ, Anderson, DH, and Kaska, DD: Glial fibrillary acidic protein increases in Müller cells after retinal detachment, Exp Eye Res 44:37-48, 1987
21. Fisher, SK, and Linberg, KA: Intercellular junctions in the early human embryonic retina, J Ultrastruct Res 51:69-78, 1975
22. Fitzgerald, CR, Enoch, JM, Birch, DG, Benedetto, MD, Temme, LA, and Dawson, WW: Anomalous pigment epithelial photoreceptor relationships and receptor orientation, Invest Ophthalmol Vis Sci 19:956-966, 1980
23. Guerin, CJ, Anderson, DH, Fariss, RN, and Fisher, SK: Regeneration of rod and cone outer segments in the primate macula, Invest Ophthalmol Vis Sci 28(suppl):341, 1987
24. Hogan, MJ, Wood, I, and Steinberg, RH: Phagocytosis by pigment epithelium of human retinal cones, Nature 252:305-307, 1974
25. Hudspeth, AJ, and Yee, AG: The intercellular junctional complexes of retinal pigment epithelia, Invest Ophthalmol Vis Sci 12:354-365, 1973
26. Immel, J, Negi, A, and Marmor, MF: Acute changes in RPE apical morphology after retinal detachment in rabbit: a SEM study, Invest Ophthalmol Vis Sci 27:1770-1776, 1986
27. Johnson, LV, Hageman, GS, and Blanks, JC: Restricted extracellular matrix domains ensheath cone photoreceptors in vertebrate retina, Prog Clin Biol Res 190:33-44, 1985
28. Johnson, LV, Hageman, GS, and Blanks, JC: Interphotoreceptor matrix domains ensheath vertebrate cone photoreceptor cells, Invest Ophthalmol Vis Sci 27:129-135, 1986
29. Johnson, NF, and Foulds, WS: Observations on the retinal pigment epithelium and retinal macrophages in experimental retinal detachment, Br J Ophthalmol 61:564-572, 1977
30. Kerns, JM, and Hinsman, EJ: Neuroglial response to sciatic neurectomy. II. Electron microscopy, J Comp Neurol 151:255-280, 1973
31. Kroll, AJ, and Machemer, R: Experimental retinal detachment in the owl monkey. III. Electron microscopy of retina and pigment epithelium, Am J Ophthalmol 66:410-427, 1968
32. Kroll, AJ, and Machemer, R: Experimental retinal detachment and reattachment in the rhesus monkey: electron microscopic comparison of rods and cones, Am J Ophthalmol 68:58-77, 1969
33. Kroll, AJ, and Machemer, R: Experimental retinal detachment in the owl monkey. VIII. Photoreceptor protein renewal in early retinal reattachment, Am J Ophthalmol 72:356-366, 1971
34. Machemer, R: Experimental retinal detachment in the owl monkey. II. Histology of retina and pigment epithelium, Am J Ophthalmol 66:396-410, 1968
35. Machemer, R, and Laqua, H: Pigment epithelium proliferation in retinal detachment (massive periretinal proliferation), Am J Ophthalmol 80:1-23, 1975
36. Marmor, MF, Abdul-Rahim, AS, and Cohen, DS: The effect of metabolic inhibitors on retinal adhesion and subretinal fluid resorption, Invest Ophthalmol Vis Sci 19:893-903, 1980
37. Nakamura, S: Morphological observation of experimental detachment of the retina. Part I. Acta Soc Ophthalmol Jpn 69:1329-1356, 1965
38. Nakamura, S: Studies on experimental retinal detachment. Morphological observations. Part II. Further observations of experimentally detached retina as well as observations of reattached retina, Acta Soc Ophthalmol Jpn 71:520, 1967
39. Negi, A, and Marmor, MF: Experimental serous retinal detachment and focal pigment epithelial damage, Arch Ophthalmol 102:445-449, 1984
40. Philp, NJ, and Nachmias, VT: Components of the cytoskeleton in the retinal pigmented epithelium of the chick, J Cell Biol 101:358-362, 1985
41. Pino, RM: Immunocytochemical localization of fibronectin to the retinal pigment epithelium of the rat, Invest Ophthalmol Vis Sci 27:840-844, 1986
42. Pino, RM, Essner, E, and Pino, LC: Location and chemical composition of anionic sites in Bruch's membrane of the rat, J Histochem Cytochem 30:245-252, 1982
43. Porrello, K, and LaVail, MM: Histochemical demonstration of spatial heterogeneity in the interphotoreceptor matrix of the rat retina, Invest Ophthalmol Vis Sci 27:1577-1586, 1986
44. Robey, PG, and Newsome, DA: Biosynthesis of proteoglycans present in primate Bruch's membrane, Invest Ophthalmol Vis Sci 24:898-905, 1983
45. Rodieck, RW: The vertebrate retina, San Francisco, 1973, WH Freeman and Co
46. Rutishauser, U, Thiery, J-P, Brackenbury, R, and Edelman, GM: Adhesion among neural cells of the chick embryo. III. Relationship of the surface molecule CAM to cell adhesion and the development of histotypic patterns. J Cell Biol 79:371-381, 1978
47. Steinberg, RH: Research update: report from a workshop on cell biology of retinal detachment, Exp Eye Res 43:695-706, 1986
48. Steinberg, RH, and Wood, I: Pigment epithelial cell ensheathment of cone outer segments in the retina of the domestic cat, Proc Roy Soc London (Biol) 187:461-478, 1974
49. Steinberg, RH, and Wood, I: The relationship of the retinal pigment epithelium to photoreceptor outer segments in the human retina. In Zinn, KM, and Marmor, ME, eds: The retinal pigment epithelium, Cambridge, Mass, 1979, Harvard University Press
50. Steinberg, RH, Wood, I, and Hogan, MH: Pigment epithelial ensheathment and phagocytosis of extrafoveal cones in human retina, Phil Trans Roy Soc Lond (Biol) 277:459-471, 1977
51. Steinberg, RH, Fisher, SK, and Anderson, DH: Disc morphogenesis in vertebrate photoreceptors, J Comp Neurol 190:501-518, 1980
52. Stramm, LE: Synthesis and secretion of glycosaminoglycans in cul-

tured retinal pigment epithelium. Invest Ophthalmol Vis Sci 28:618-627, 1987
53. Varner, HH, Rayborn, ME, Osterfeld, AM, and Hollyfield, JG: Localization of proteoglycan within the extracellular matrix sheath of cone photoreceptors. Exp Eye Res 44:633-642, 1987
54. Walls, GL: Human rods and cones: the state of knowledge. Arch Ophthalmol 12:914-930, 1934
55. Williams, DS, and Fisher, SK: Prevention of rod disk shedding by detachment from the retinal pigment epithelium. Invest Ophthalmol Vis Sci 28:184-187, 1987
56. Wilson, DJ, and Green, WR: Histopathologic study of the effect of retinal detachment surgery on 49 eyes obtained postmortem. Am J Ophthalmol 103:167-179, 1987
57. Yamada, KM, and Akiyama, SK: The interactions of cells with extracellular matrix components. In Elson, E, Frazier, W, and Glaser, L, eds: Cell membranes, methods and reviews, vol 2. New York, 1984, Plenum Press
58. Young, RW: The renewal of photoreceptor cell outer segments. J Cell Biol 33:61-72, 1967
59. Young, RW: Shedding of discs from rod outer segments in rhesus monkey. J Ultrastruct Res 34:190-203, 1971
60. Young, RW: Visual cells and the concept of renewal. Invest Ophthalmol 15:700-725, 1976
61. Zauberman, H: Adhesive forces between the retinal pigment epithelium and sensory retina. In Zinn, KM and Marmor, ME, eds: The retinal pigment epithelium. Cambridge, Mass, 1979, Harvard University Press