Chapter 119

Cellular Effects of Detachment on the Neural Retina and the Retinal Pigment Epithelium

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INTERFACE BETWEEN RETINA AND RETINAL PIGMENT EPITHELIUM

Early in development of the eye, the neural retina and the retinal pigment epithelium (RPE) become closely apposed and remain so throughout the normal life span 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.[30] In other cell types the latter allow a free transfer of small molecules (less than about 1.5 kDa) between cells.[3] The significance of coupling these two cell layers is not known, but as the neural retina begins to differentiate, the junctions disappear. Shortly thereafter, the apical surface of the RPE 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 RPE is a polarized monolayer of neuroepithelial cells that rests on Bruch's membrane.[16] At their lateral margins, each RPE cell is connected to its neighboring cells by a junctional complex composed of adhering, gap, and tight junctions.[41]

The relationship of the apical surface of the RPE to differentiated photoreceptors is anatomically complex. There are no actual cellular junctions between the two layers in the mature eye. Rather, photoreceptor outer segments are ensheathed by specialized arrays of RPE cell microvilli and microplicae that are organized differently for primate rods and cones.[5,71] In human and rhesus monkey retinas, the apical surface of the RPE extends villouslike processes toward the photoreceptor outer segments, where some of them expand into cytoplasmic sheets or lamellae that surround the outer segments.[5,70,71] 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 farther 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 ensheathe 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.*[70] Outside the fovea they are so distinctive that they are easily recognized by light microscopy, as was first described by Walls[76] in 1934. The apical projections of the RPE participate in the phagocytosis of membranous disc packets periodically shed from the apical tips of the rod and cone outer segments.[4,73,79-81]

The extracellular space between the apical surface of the RPE, the photoreceptor cells, and the apical microvilli of Müller's glia defines a specific anatomic compartment known as the *interphotoreceptor,* or *subretinal, space.* In this context, we use the term *subretinal space* only when the retina is detached from the RPE. The interphotoreceptor space contains both aqueous soluble and aqueous insoluble compo-

nents,[1,2,11] only a few of which have been characterized to date. Recent evidence, first obtained from lectin-binding studies, indicates that some of the insoluble components are not distributed homogeneously within the matrix. The extracellular compartment surrounding cone outer segments, termed the *cone matrix sheath,* is biochemically distinct from the rest of the interphotoreceptor matrix[45,75]; additional, specialized domains may also be associated with rod outer segments[25] and with the apical surface of the RPE.[66] At the apical RPE border, tight junctions between adjacent cells constitute a complete diffusion barrier for virtually all matrix molecules in the interphotoreceptor space. Along the retinal aspect of the interphotoreceptor space, however, adhering junctions between the photoreceptor inner segments and Müller cells (the outer limiting membrane) present a less restrictive barrier to diffusion.[14] The RPE mediates the transfer of ions and molecules between the choroidal capillaries and the neural retina. Because there are no cellular junctions between the RPE and photoreceptor layers, and because the extracellular space between adjacent RPE cells is sealed by tight junctions near the apical border, this transfer must take place across the plasma membranes and cytoplasm of the RPE cells and through the interphotoreceptor matrix.

Retinal Detachment

Detachment of the retina from the RPE surface results not only in separation of the photoreceptor cell layer from the apical surface of the RPE but also in an expansion of the interphotoreceptor space (i.e., the subretinal space) and a concomitant change in the composition of the interphotoreceptor matrix.[2] In the detached state, extracellular material in the expanded subretinal space is usually referred to as subretinal fluid to distinguish it from normal interphotoreceptor matrix. Retinal detachment initiates a complex series of cellular and molecular changes in both retinal and RPE cells.[28,44] It may impede the normal transfer of ions and metabolites back and forth between the retina and the RPE-choroid.[69] It may also liberate or activate regulatory molecules sequestered in the interphotoreceptor matrix.[36,53] The severity of the resulting degenerative changes is clearly related to detachment duration and, under many circumstances, has serious adverse consequences for vision in the affected eye. Prompt reapposition of the retina and RPE layers can result in at least partial restoration of vision, implying that some of these abnormal changes can be arrested or even reversed by reattachment. This chapter reviews both the many changes that occur in retinal cells and the ensuing process of morphologic recovery, as revealed by studies of experimental retinal detachment and reattachment.

EXPERIMENTAL RETINAL DETACHMENTS

Animal Models of Detachment

Here we emphasize the results of the most recent studies using animal models of detachment and reattachment. These studies show that the responses of the RPE and neural retina to detachment fall into several general categories: (1) partial dedifferentiation of RPE cells with mounding of the apical surface and retraction of the apical processes, (2) proliferation and migration of RPE cells into the subretinal space, (3) degeneration of photoreceptor outer segments and synaptic terminals, (4) death of a variable population of photoreceptors, (5) neurite sprouting from second-order retinal neurons, (6) proliferation of all nonneuronal cell types within the retina,[29] and (7) Müller cell hypertrophy with eventual glial scar formation.

Our understanding of the cellular events that occur when the retina and pigment epithelium are detached or reattached derives principally from the study of experimental animal models. Because of the scarcity of human retinal tissue suitable for such studies, only recently has the histopathologic study of retinal detachments in humans been reported.[64,78] Several laboratories have developed different experimental models of retinal detachment. Although these models differ in the species used and in the exact method of separating the two layers, they have yielded similar results that constitute a relatively detailed profile of the ultrastructural changes that occur after detachment. Although fewer, experimental studies of reattachment have provided insight into the regenerative capacity of photoreceptor cells and may eventually lead to treatments that will enhance the recovery process.

The ultrastructural findings from experimental retinal detachments are currently being used as the basis for investigating the accompanying molecular changes. Such knowledge should eventually lead to a precise understanding of the degenerative processes that result in blindness or severely impaired vision, and they also will help in understanding serious complications of retinal detachment, such as proliferative vitreoretinopathy.

Ideally the characteristics of an experimental detachment should closely mimic those found in human patients while allowing for precise control over the extent of separation between the two layers (detachment height), the location of the detachment, its surface area, and the onset of detachment (or reattachment). The ideal is rarely attained, however, and some compromise has been required.

Because the retina and the RPE are tightly adherent, producing a detachment is not a simple process. Earlier work on experimental detachments was aimed at mimicking "rhegmatogenous detachment" (from the Greek *rhegma,* rupture or tear; and *genos,* origin). This technique uses a fairly large tear in the retina in combination with repeated aspiration of liquefied vitreous into the retinal hole.[48-50,58,62,63] Subsequent enlargement of the detachment away from the region of the tear tends to occur spontaneously. The disadvantage of this technique is that the onset of detachment at sites distant from the retinal tear cannot be defined precisely. Subsequent studies have used a somewhat different procedure, in which fluid is injected between the retina and the RPE through a metal needle or glass micropipette inserted into the subretinal space.* This produces a bullous retinal de-

*References 6, 9, 10, 28, 29, 35, 43, 44, 52.

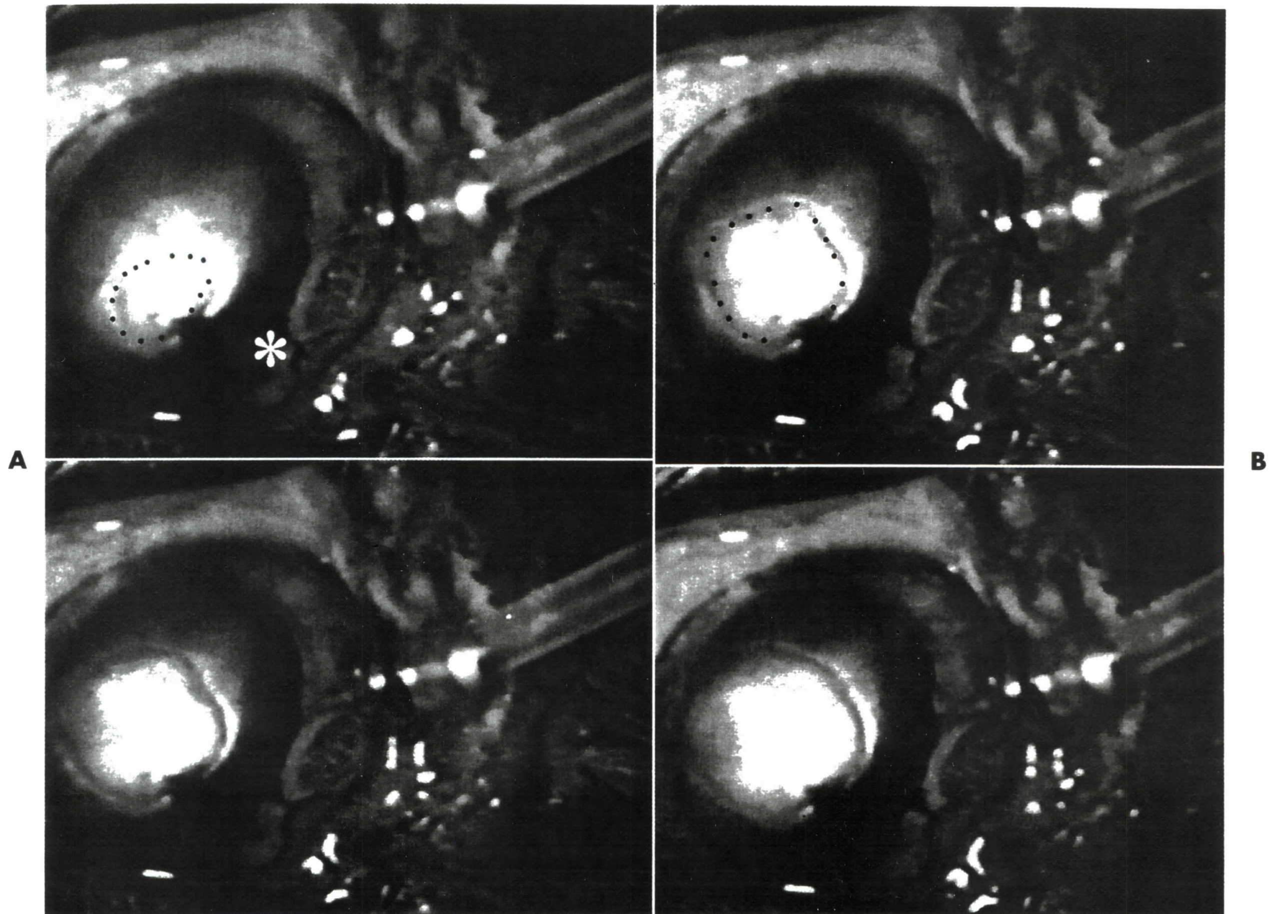

Fig. 119-1 Four frames taken from a videotape showing the production of an experimental retinal detachment in a cat. The detachment is produced by the injection of fluid into the subretinal space by a micropipette. The pipette holder is marked by the asterisk in **A.** The view is through the operating microscope, and the margins of the detachment are indicated by the dotted area in **A** and **B.**

tachment that can be either very small or expanded to fill a quadrant or more of the retina (Fig. 119-1). The major disadvantage of this technique is that, in most cases, the retina tends to flatten spontaneously, a graphic illustration of adhesive "force" and the retina's inherent capacity for self-repair. Because there is no large tear in these experimental detachments, it is not strictly equivalent to a clinical "rhegmatogenous" detachment. However, this technique does allow precise control over the onset of detachment and provides the investigator with the capability to control its size and location.

Photoreceptor-RPE Interface

The earliest effects of retinal detachment are seen at the interface of photoreceptors and the RPE. These changes include alterations in the RPE apical surface, proliferation of RPE cells, migration of cells into the subretinal space, degeneration of photoreceptor outer segments, and changes in photoreceptor outer segment renewal.

RPE Apical Surface. One of the earliest effects of detachment occurs at the RPE apical surface.[28] The long and elaborate sheetlike and villous processes that normally ensheathe the outer segments are lost within a few hours of detachment and replaced by a "fringe" of short microvilli (Fig. 119-2). Also occurring within the first 24 hours of detachment is a change in the overall surface morphology of these cells in which they begin to show a rounded contour, with cytoplasm protruding past the normal limits of the apical surface into the subretinal space[10] (Figs. 119-2 and 119-3). In addition, the nucleus is often displaced from its normal central location in the RPE cell to a more apical location. This response has been termed "mounding" of the apical surface, and experiments have shown that it begins within minutes of the production of a detachment in rabbit retinas.[44] On the basis of similar observations in cultured RPE cells, this effect may be 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 RPE cell bodies.[60]

Proliferation of RPE Cells. If tritiated 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 tritiated

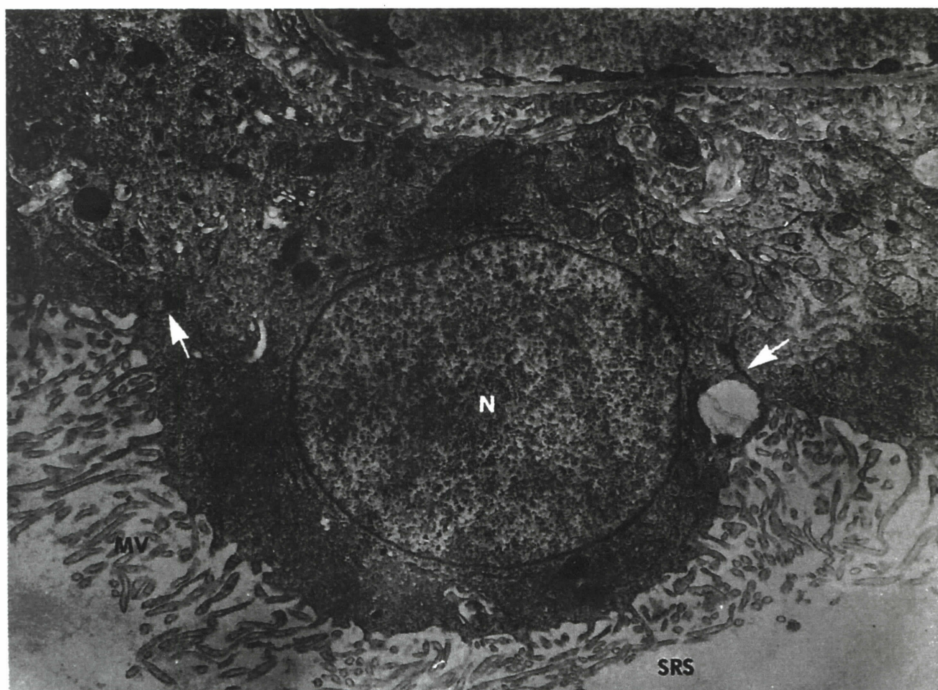

Fig. 119-2 An electron micrograph of the retinal pigment epithelium (RPE) 1 day after production of a retinal detachment. Compared with normal RPE cells, the apical surface is mounded. The sheetlike apical projections that normally ensheathe the outer segments have been replaced by a homogeneous fringe 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 (×6750). *SRS,* Subretinal space (From Anderson, DH, Stern, WH, Fisher, SK, Erickson, PA, and Borgula, GA: Invest Ophthalmol Vis Sci 24:909, 1983.)

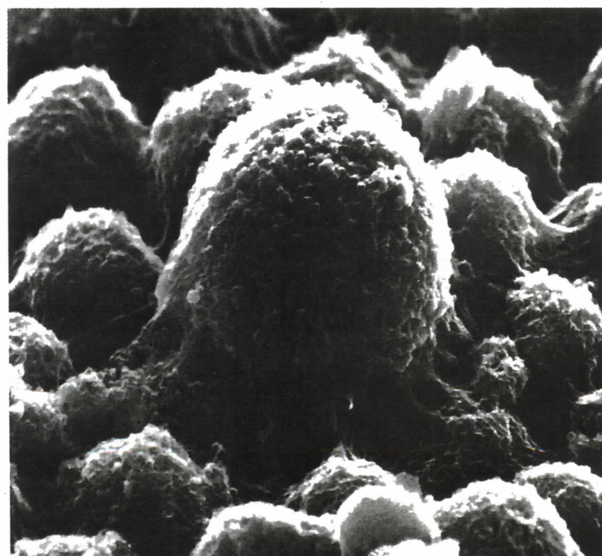

Fig. 119-3 Scanning electron micrograph of the apical surface of the retinal pigment epithelium 6 weeks after production of an experimental detachment, demonstrating the pronounced mounding response of the epithelial cells (×4800). (From Anderson, DH, Stern, WH, Fisher, SK, Erickson, PA, and Borgula, GA: Invest Ophthalmol Vis Sci 24:910, 1983.)

thymidine has been incorporated into DNA. When this technique is used, radiolabeled nuclei appear within the RPE cell layer within 24 hours after production of a detachment[9] (Fig. 119-4). This proliferative response can result in the transformation of the RPE'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 (7 days or more) the proliferative response may induce the formation of multiple layers of cells whose polarity does not necessarily match that of the original monolayer (Fig. 119-5). It is important to note that the molecular stimulus for DNA synthesis and RPE cell proliferation is un-

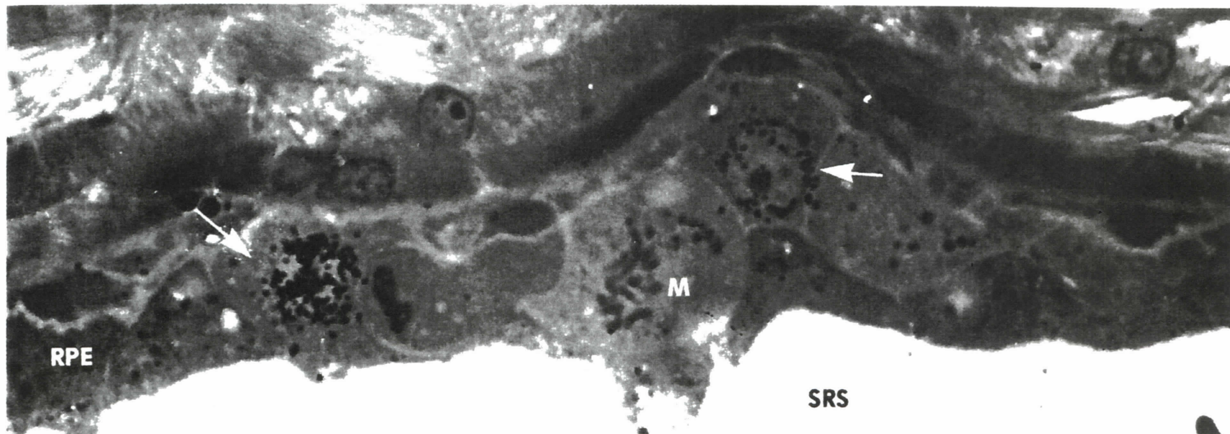

Fig. 119-4 Light microscopic autoradiogram of cat retinal pigment epithelium *(RPE)* 2 days after detachment. Tritiated thymidine was injected intravitreally 3 hours before fixation. Two labeled nuclei *(arrows)* and an adjacent mitotic figure *(M)* indicate that some RPE cells are proliferating at this stage (×850). *SRS,* Subretinal space. (From Anderson, DH, Stern, WH, Fisher, SK, Erickson, PA, and Borgula, GA: Invest Ophthalmol Vis Sci 24:911, 1983.)

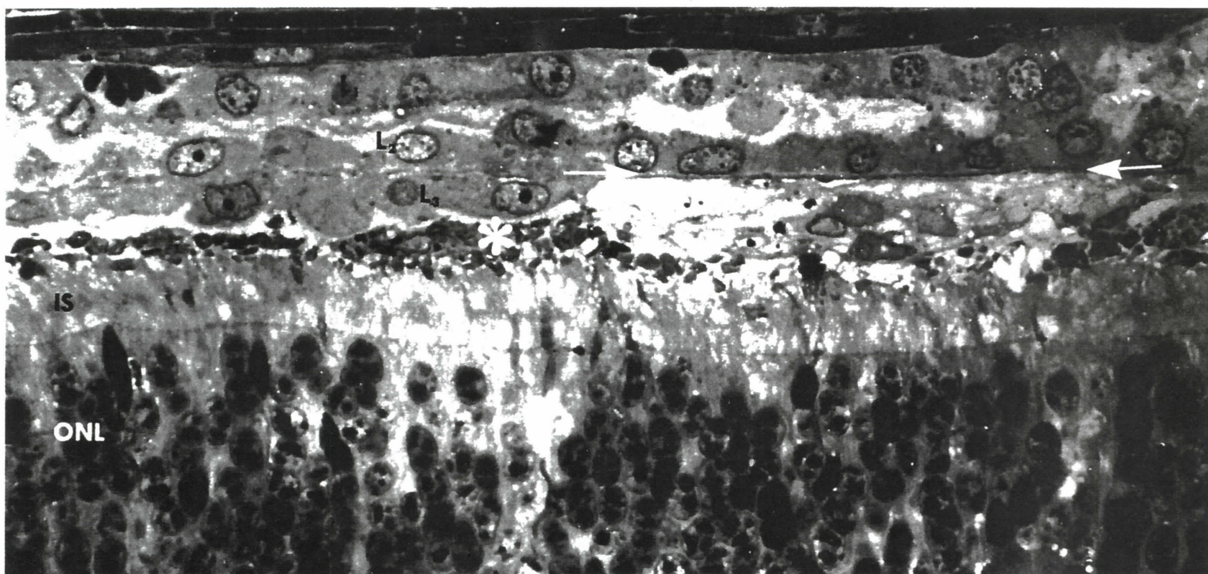

Fig. 119-5 A light micrograph of an area of RPE cell proliferation in a cat retina detached for 14 days and reattached for 30 days. Three monolayers of RPE cells are present (L_1, L_2, and L_3), each displaying different surface polarity. The apical surfaces of L_1 and L_2 face each other, as do the basal surfaces of L_2 and L_3. The basal lamina of L_2 is clearly evident *(arrowheads).* Only outer segment fragments *(asterisk)* appear near the inner segment *(IS)* tips (×800). *ONL,* Outer nuclear layer. (From Anderson, DH, Guerin, CJ, Erickson, PA, Stern, WH, and Fisher, SK: Invest Ophthalmol Vis Sci 27:174, 1986.)

known, but its effect appears to be limited to the region immediately underlying the detached retina; in attached regions the RPE remains mitotically quiescent. These data strongly suggest attachment of the RPE, and neural retina acts to keep the RPE mitotically inactive and its apical surface highly differentiated. The RPE cells in an area of detachment are similar to cultured RPE cells, and they display many of the morphologic and cytoskeletal characteristics found in confluent cell cultures.[60]

It does appear that the proliferative response of the RPE is self-limiting. In experimental detachments of both owl monkey and cat retinas, proliferation (measured by tritiated thymidine incorporation) is at very low levels after long detachment intervals (e.g., 12 to 14 months).[10,32,59] Proliferation of RPE during detachment is of clinical significance. The proliferation of the RPE cells in the subretinal space is probably the basis of pigmentary changes often observed during ophthalmoscopic examination of retinal detachments in hu-

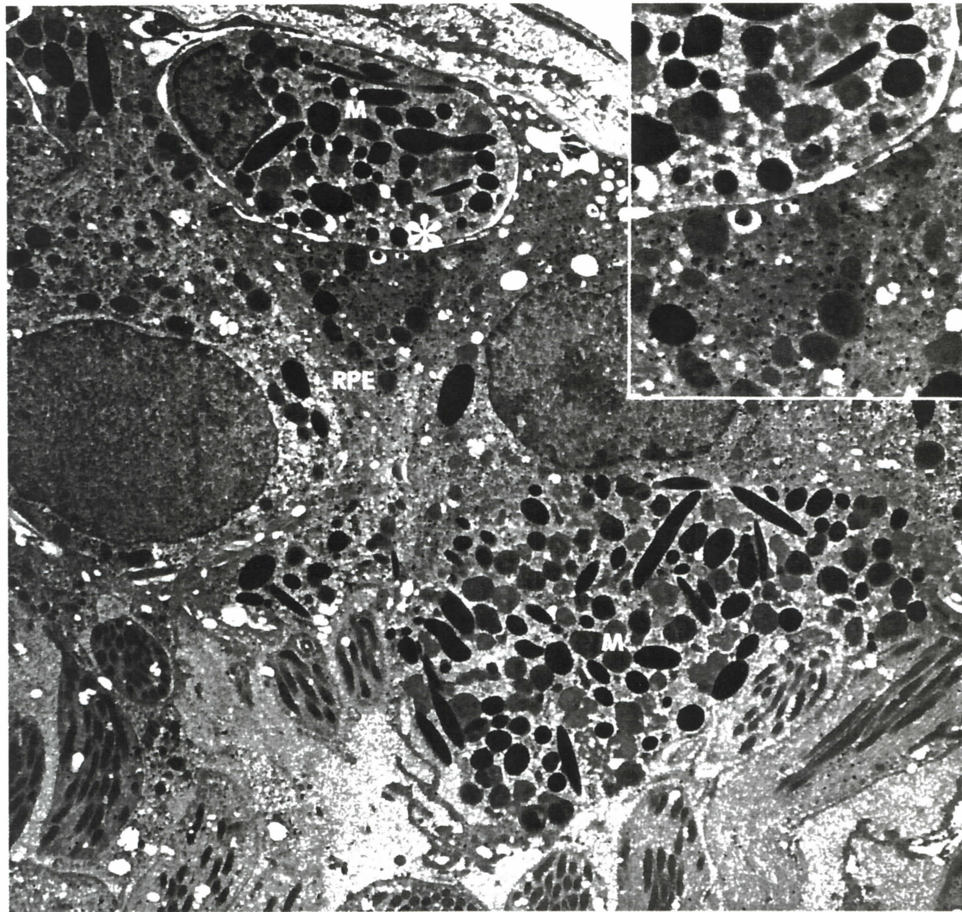

Fig. 119-6 An electron micrograph from a reattached monkey retina. The tissue sections were incubated with an antibody to cellular retinaldehyde-binding protein and then labeled with a secondary immunoglobulin-gold conjugate. The protein is expressed only in retinal pigment epithelial *(RPE)* and Müller cells. The small black spheres scattered throughout the cytoplasm of the 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 (×53,000; inset ×96,000).

mans. It is likely that the demarcation lines noted in human retinal detachments represent zones of proliferated RPE 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 regeneration of the photoreceptor outer segment after reattachment.[6,33]

Migration of Cells into the Subretinal Space. In mammalian retinas the interphotoreceptor space is normally free of cells. Within 24 hours of an experimental detachment, periodic acid–Schiff-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 RPE cells, apparently in the process of migration from the choriocapillaris into the subretinal space (Fig. 119-6). 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).[10,46]

RPE 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 RPE cells begin to lose their attachment to their neighbors and migrate into the subretinal space (Fig. 119-7). These cells also contain large packets of outer segment debris, indicating their probable role in scavenging material from the degenerating photoreceptors. Thus, at least two different sources for cells move into the subretinal space and phagocytose cellular debris. Some are of hematopoietic origin, whereas others are derived from the RPE 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, however, be used as immunochemical

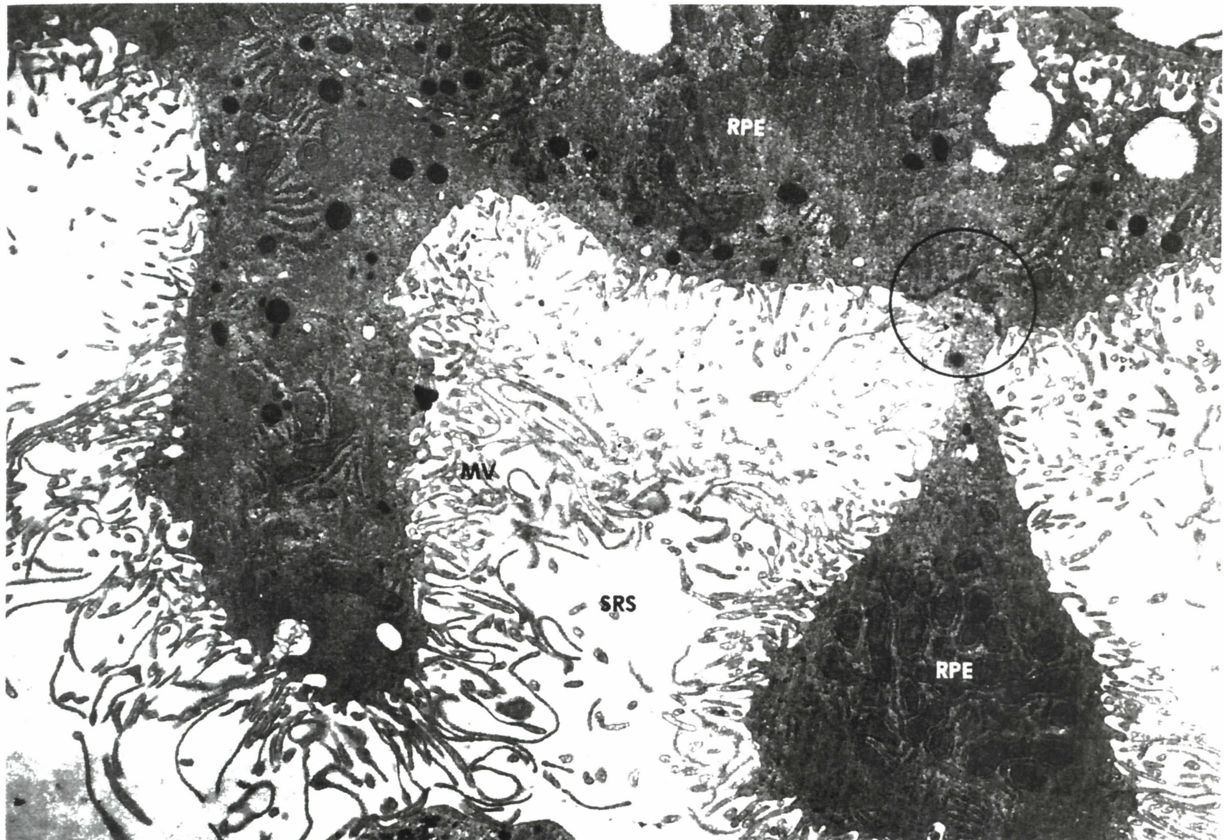

Fig. 119-7 An electron micrograph of the retinal pigment epithelium *(RPE)* 22 days after a detachment. The cell on the right has nearly separated from the monolayer. A narrow tail of cytoplasm retains junctions to neighboring cells *(circle)*. The apical processes *(MV)* of these highly mounded cells are longer than those found at earlier detachment times (×6250). *SRS,* Subretinal space. (From Anderson, DH, Stern, WH, Fisher, SK, Erickson, PA, and Borgula, GA: Invest Ophthalmol Vis Sci 24:920, 1983.)

probes to distinguish between different cell types. For example, cellular retinaldehyde-binding protein is found only in RPE and Müller cells.[13] Thus, an antibody to this protein can be used to identify cells that express it. 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-binding protein and, as such, can be positively identified as RPE cells (Fig. 119-8).

Degeneration of Photoreceptor Outer Segments. Like the RPE 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 detachment of any mammalian retina, whether it is experimental or clinical. The photoreceptor-RPE 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 calycle processes, their only connection to the inner segment is via a single ciliary stalk. As previously mentioned, the apices of the outer segments are enveloped by arrays of apical RPE processes (Fig. 119-9, *A*). The interphotoreceptor matrix has long been presumed to play a role in maintaining the structural integrity of these elements. There is very little information available on its disposition after detachment or reattachment. Interestingly, the aqueous insoluble portion of the interphotoreceptor matrix tends to remain adherent to the exposed photoreceptors when the retina is experimentally detached, and what appears to be residual matrix can still be identified for at least several days after the procedure. The measured adhesion between the two cell layers is reported to be strong,[82] 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

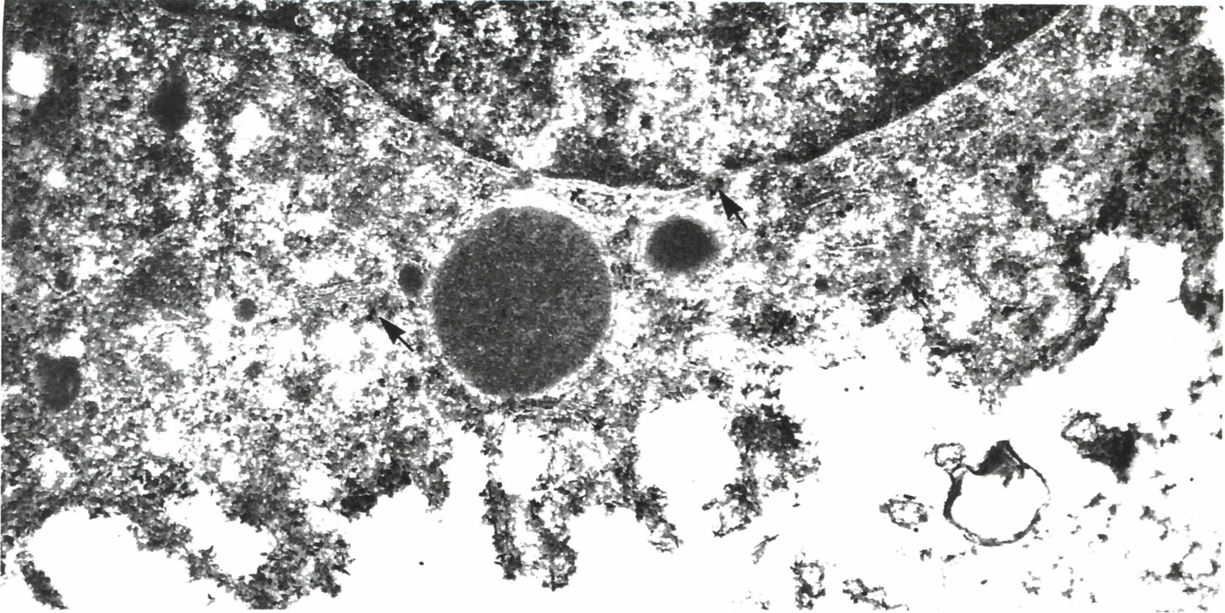

Fig. 119-8 An electron micrograph of a cell in the subretinal space in a cat retina detached for 2 months. The tissue sections were incubated as described in Fig. 119-6. The presence of gold spheres *(arrows)* scattered in the cytoplasm indicates that this subretinal cell is derived from the retinal pigment epithelium (×33,000).

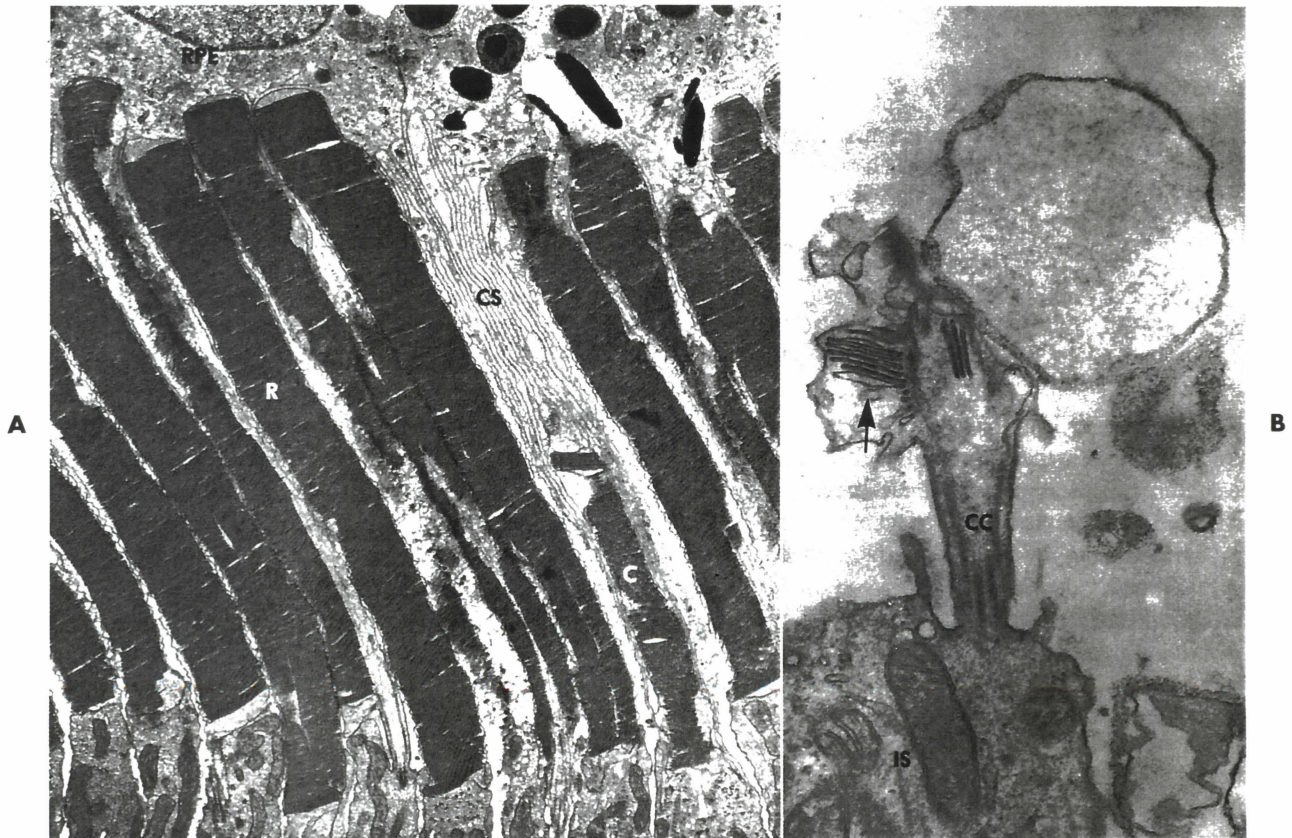

Fig. 119-9 **A,** An electron micrograph of the photoreceptors and retinal pigment epithelium in a normal cat retina. The outer segments are oriented along their longitudinal axes in a uniform, densely packed row. Cone outer segments *(C)* are shorter than those of rods *(R)* and are ensheathed by a 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 (×28,000). (**A** from Anderson, DH, Guerin, CJ, Erickson, PA, Stern, WH, and Fisher, SK: Invest Ophthalmol Vis Sci 27:173, 1986.)

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 may proceed until those in the zone of detachment appear only as empty sacs of membrane attached to the connecting cilium (Fig. 119-9, *B*). In other cases, severely truncated but recognizable outer segments can be present even after detachments of several weeks' duration.[51] 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 RPE cell.

Photoreceptor Outer Segment Renewal. Rod and cone outer segments are renewed by a continual process of disc addition at the base and loss at their tips.[4,40,72,79-81] Thus, any process that interrupts either the production of new discs, their shedding at the distal end, or their subsequent phagocytosis by the RPE will affect the integrity of the outer segment. There is experimental evidence that when photoreceptors are detached from the RPE, disc shedding cannot be induced by treatments that stimulate massive shedding in attached retinas.[77] It also appears from ultrastructural studies that the process of disc morphogenesis must slow down considerably while the retina is detached.[33,34] In long-term detachments, often the ciliary stalks with a few rudimentary discs or membrane evaginations are all that remain. In other cases, up to 25% of the outer segment may remain, although its structure is always disorganized. These disc membranes are found long after membranes synthesized before detachment would be expected to persist. Autoradiographic studies show that newly synthesized proteins continue to be transported and incorporated into these rudimentary outer segments.[51] Immunolabeling experiments indicate that these discs contain, among other outer segment specific proteins, opsin and peripherin/rds, two important structural components (Fig. 119-10). These results suggest that the process of disc membrane formation and opsin biosynthesis does not cease even after lengthy detachment episodes. It is not known, however, if the synthesis of visual pigment (i.e., opsin), or that of other molecular components of the outer segment, is altered during detachment.

Opsin, which is normally concentrated in the outer segment, begins to accumulate in the plasma membrane vitread to the connecting cilium within a day after an experimental retinal detachment. As outer segment degeneration proceeds, the number of cells showing this new pattern of rhodopsin distribution increases, as does the intensity of labeling in individual cells (see Fig. 119-10). After detachment, peripherin/rds, which, like rhodopsin, is an integral membrane protein but specific to the disc rims, begins to appear in cytoplasmic vesicles. As in the case with the shift in rhodopsin localization, the number of these vesicles increases with detachment time and correlates with outer segment degeneration. Peripherin/rds never appears in the plasma membrane, however.[26] Unpublished data from our own laboratories suggest that mRNA levels for rhodopsin remain at about the same levels as in normal retina in individual photoreceptors 28 days after an experimental detachment. These data, along with results showing that newly synthesized protein continues to be transported and incorporated into degenerating photoreceptor outer segments, suggest that rhodopsin synthesis and transport (and probably that of other outer segment components) persist after detachment but that, in the absence of an organized outer segment, membrane proteins such as opsin and peripherin/rds may be inserted at inappropriate cellular locations.[51]

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 highly likely that both the normal shedding process and disc formation are affected adversely by the separation of the two tissue 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.

Summary. The effects of detachment on the photoreceptor-RPE 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 of 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.

Inner Retina

Although the earliest and most obvious effects of detachment are seen at the neural retina–RPE 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 day. 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 within the retina. Rabbits, which have an "avascular" retina, show extensive and rapid degenerative effects in the layer of photoreceptor nuclei, often leading to degeneration of the entire layer of photoreceptors. Dogs, monkeys, humans, and cats have capillaries within the neural retina, and photoreceptor layer is apparently spared from total degeneration during detachment.

Photoreceptor Inner Segments. During the first day of a detachment, the inner segments appear essentially normal, but between the first and third days, they begin to show signs of degeneration: most commonly swelling, disruption, and loss

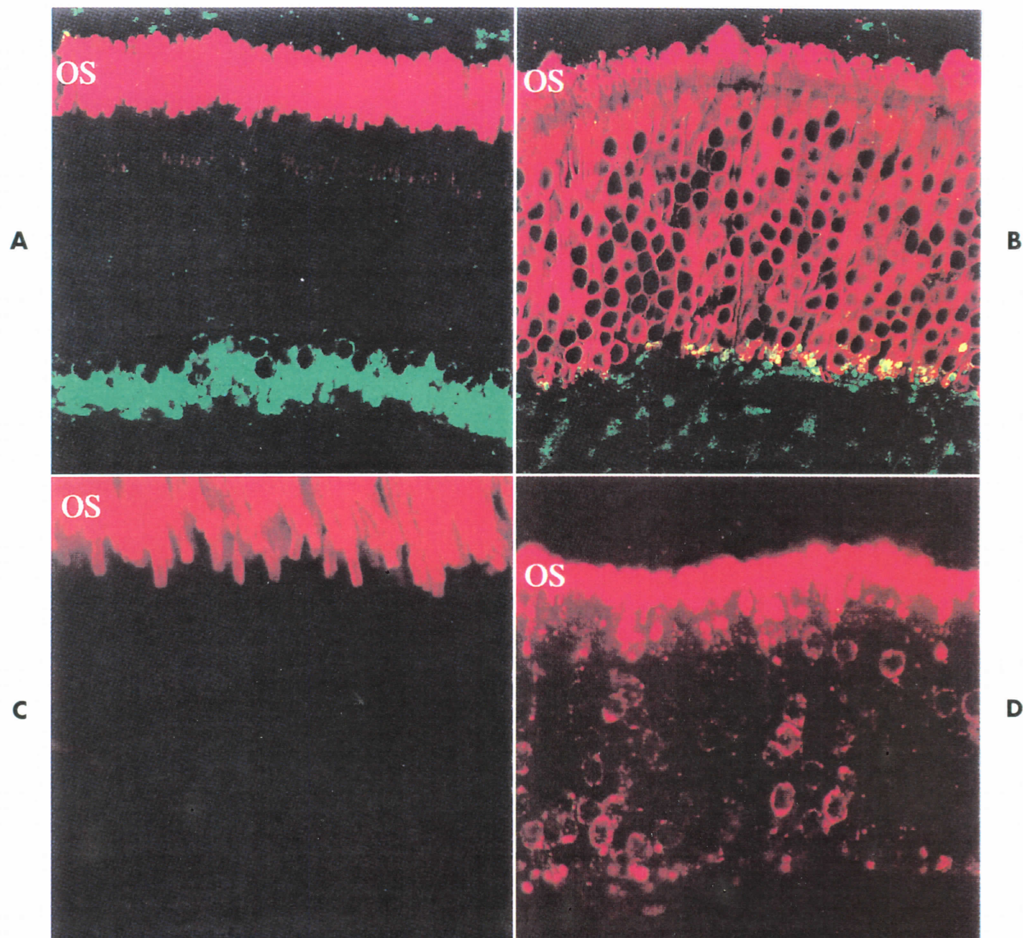

Fig. 119-10 Immunofluorescence images illustrating the changes in labeling with antibodies to rhodopsin, synaptophysin (**A** and **B**), and peripherin/rds (**C** and **D**) that occur after detachment. **A,** An antibody to rhodopsin intensely labels the rod outer segments in normal retina *(red)*, while the antibody to synaptophysin labels the terminals of rods and cones *(green)*. **B,** After 28 days of detachment, the antibody to rhodopsin now labels the entire rod photoreceptor plasma membrane *(red)*. The labeling with the antibody to synaptophysin shows the extent of disruption of the photoreceptor synaptic terminals in the 28-day detachment *(green)*. **C,** The antibody to peripherin/rds labels the edges of the outer segments in normal retina. **D,** After detachment this antibody labels intracellular structures (as well as the shortened outer segments) but not the plasma membrane.

of mitochondria in the ellipsoid region, an overall disruption of the organized rough endoplasmic reticulum and Golgi apparatus in the myoid region, and, within a few days, an overall size reduction of the inner segment. It is interesting to note that the *connecting cilium* is retained even in severely affected inner segments in long-term detachments. This is a crucial point because the connecting cilium is essential for production of 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).[67] The outer plexiform layer also contains the processes of second-order neurons, the cell bodies of which lie in the inner nuclear layer. These processes synapse with each other and with the photoreceptors. The photoreceptor cell bodies and synaptic terminals show a rapid response to detachment in feline retinas. Some of these cells will die (see below), and those that remain show extensive structural changes. By 3 days after detachment the cell bodies and terminals show extensive vacuolization and can also show distention 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 degenerative changes in these cells. Some of the most striking structural changes in the surviving photoreceptors occur in the cy-

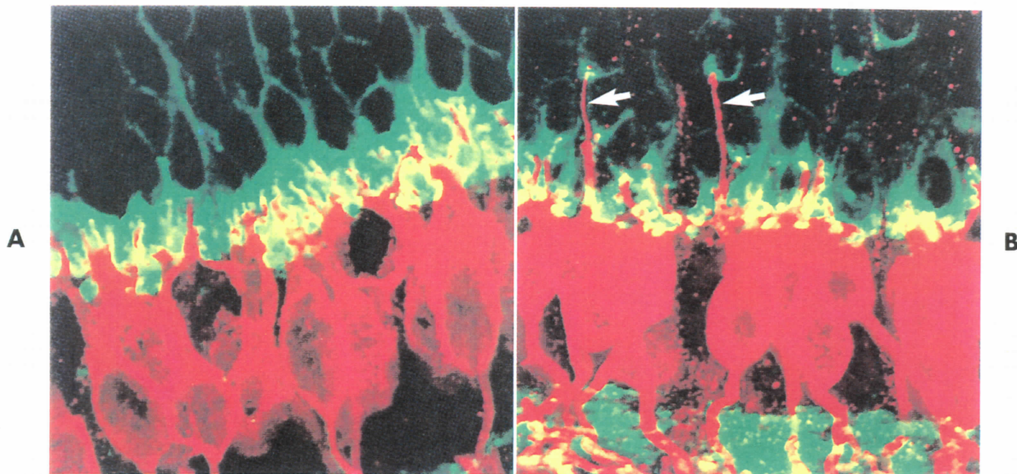

Fig. 119-11 Immunofluorescence images of normal **(A)** and 3-day detached **(B)** retinas labeled with antibodies to protein kinase C *(green)* and synaptophysin *(red)*. The antibody to protein kinase C labels only the rod bipolar cells and their dendrites, which connect to the terminals (*green*, synaptophysin labeled) of the rod photoreceptors. After detachment, the synaptic terminals degenerate, often withdrawing into the outer nuclear layer, and the rod bipolar cells sprout neurites that grow into this layer (**B**, *arrows*).

toskeleton of these cells. Both microtubules and filamentous-actin, which are highly organized in normal photoreceptors, show profound changes after detachment,[56] and since these structures are associated with the transport of molecules within cells and the maintenance of cell shape, their disruption would seem to have significant effects on these cells.

Not all photoreceptor cells degenerate (or die) 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 cones. In a region in which nearly all of the rod cell bodies show signs of degeneration and even cell death, 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. Many terminals appear as if they have "retracted" into the cell body, so synaptic structures generally associated with the outer plexiform layer now occur within the outer nuclear layer. As with the cone and rod 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 small synaptic vesicles.[22]

Recent studies using antibody probes have demonstrated the magnitude of the rod spherule response to detachment, and some unexpected changes in the second-order neurons that connect with them. Beginning by 1 day after detachment the outer plexiform layer exhibits disorganization of the photoreceptor synaptic terminals when they are labeled with an antibody to the protein synaptophysin (Fig. 119-11). Synaptophysin-positive terminals now begin to appear deep in the outer nuclear layer. At this same time, processes from the horizontal and rod bipolar cells (labeled with antibodies to calbindin D and protein kinase C) begin to grow beyond the normal layer of photoreceptor synaptic terminals and into the outer nuclear layer[55] (Fig. 119-11). The effect of these changes on visual recovery after reattachment is unknown. 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.[22]

Proliferation of Cells in the Inner Retina. In the first week after detachment, there is also a significant proliferative component to the cellular changes in the inner retina. Within 24 hours of the detachment all nonneuronal cell types within the retina, including astrocytes, Müller cells, pericytes, capillary endothelial cells, and microglia, display signs of proliferation as shown by tritiated thymidine autoradiography or when labeled with an antibody that recognizes proliferating cells.[29,32] By 2 days, some labeled Müller cell nuclei are translocated from their normal positions on the vitreal border of the inner nuclear layer into the outer plexiform and outer nuclear layers. This response apparently peaks within about 3 to 4 days after detachment and declines slowly to baseline levels several weeks later (Fig. 119-12).

Müller Cell Hypertrophy. Müller cells begin a process of glial scar formation almost immediately after detachment; changes in protein expression associated with this response are detectable within a day, and the actual growth of Müller cell processes within 3 days.[52,54] Presumably these cells fill the

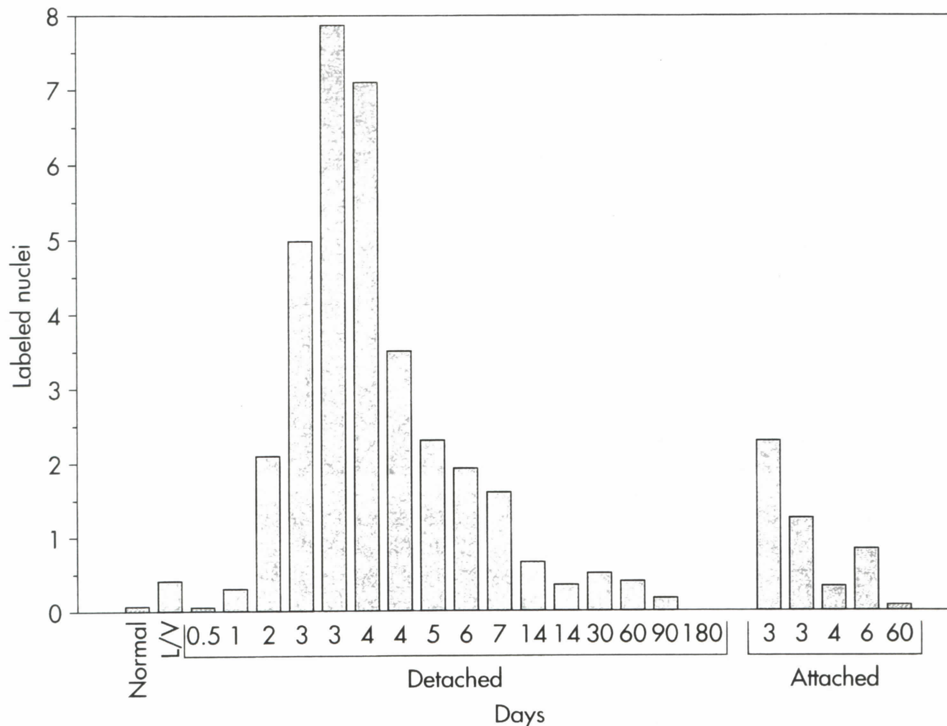

Fig. 119-12 Histogram showing the level of proliferation in nonneuronal cells within the retina as a function of time after retinal detachment. Cells with the morphology and distribution of astrocytes, Müller cells, vascular endothelial cells, microglia, and cells of monocyte/macrophage lineage were included in the counts. The total number of cell nuclei labeled with tritiated thymidine per millimeter of retinal length was counted in normal retinas *(Normal),* retinas that had undergone lensectomy/vitrectomy *(L/V),* retinas detached for intervals ranging from 0.5 to 180 days *(Detached),* and attached retinal regions adjacent to the zone of detachment *(Attached).* Proliferation levels peak 3 to 4 days after detachment and then decline rapidly to baseline levels after approximately 2 weeks. Retinal regions adjacent to the detached retina also show some evidence of proliferation at the 3-day, peak response time. (From Fisher, SK, Erickson, PA, Lewis, GP, and Anderson, DH: Invest Ophthalmol Vis Sci 32:1739-1748, 1991.)

spaces left by degenerating neurons, but they do more than that because they also grow rapidly into the subretinal space, and, more rarely in our model, onto the retinal vitreal surface. Müller cell bodies migrate, frequently being found in the outer nuclear and outer plexiform layers within 3 days. The cytoplasm of these cells is filled with 10 nm diameter (intermediate) filaments that, by immunochemical criteria, are composed of glial fibrillary acidic protein,[23] vimentin,[52] or both (Fig. 119-13). Beyond 3 days of detachment, Müller cell processes often extend into the subretinal space through localized disruptions in the outer limiting membrane. These processes become more commonplace and elaborate as detachment time lengthens. They often form a multilayered "glial scar" within the subretinal space (see Fig. 119-13), separating the neural retina from the RPE. 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, represent a common response to central nervous system injury—for example, in spinal cord axotomy or neurectomy.[47] In this case, the resulting glial scar is thought to block the regeneration of spinal cord axons. The intermediate filament 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 day of detachment these filaments begin to appear elsewhere in these cells, and eventually they occur throughout the retinal layers and into Müller cell processes that have invaded the subretinal space.[52]

The presence of Müller cell processes within the subretinal space appears to inhibit the regeneration of photoreceptors after retinal reattachment (see later discussion). What role, if any, their presence within the outer plexiform layer plays in the maintenance or loss of synaptic connections between photoreceptors and second-order neurons during a period of detachment is unknown.

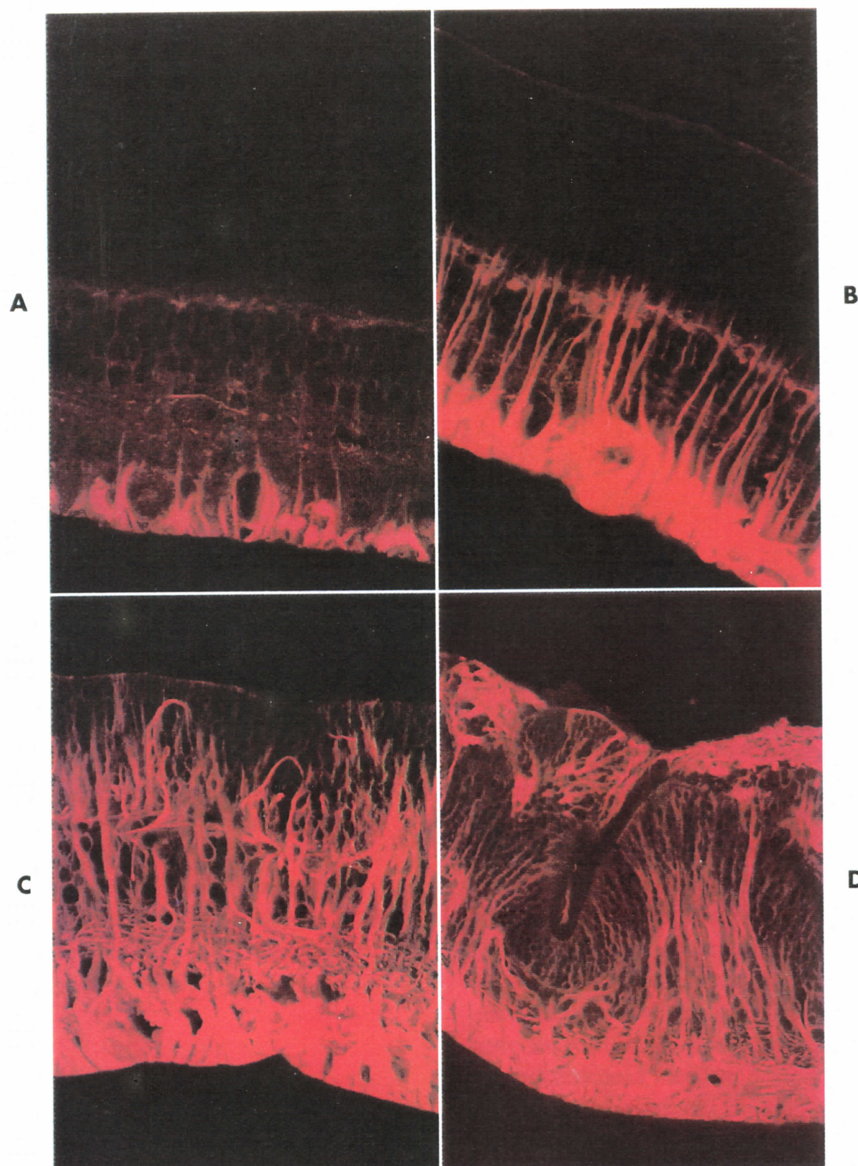

Fig. 119-13 A series of immunofluorescence images of normal **(A)**, 3-day **(B)**, 7-day **(C)**, and 28-day **(D)** detached retinas labeled with an antibody to glial fibrillary acidic protein (GFAP). In normal retina this protein is localized to the end-foot region of the Müller cells bordering the vitreous cavity (and astrocytes in this region, data not shown). After detachment the increase in GFAP within the Müller cells is an excellent marker for the reactivity and hypertrophy of these cells. In this 28-day example a large Müller cell scar occurs in the subretinal space, covering the photoreceptor layer. Labeled Müller cell processes can be seen extending into the subretinal space as early as 3 days after detachment.

Loss of Photoreceptor Cells. The loss of cells from the photoreceptor layer occurs by cell death and the extrusion of photoreceptor cell bodies past the outer limiting membrane into the subretinal space. Cell death among photoreceptor cells has been definitively shown to occur by apoptosis, and perhaps by necrosis.[19] The mechanism by which cells are extruded into the subretinal space 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 cat retinas, 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[22] (Fig. 119-14). 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, rabbits are the most severely affected,

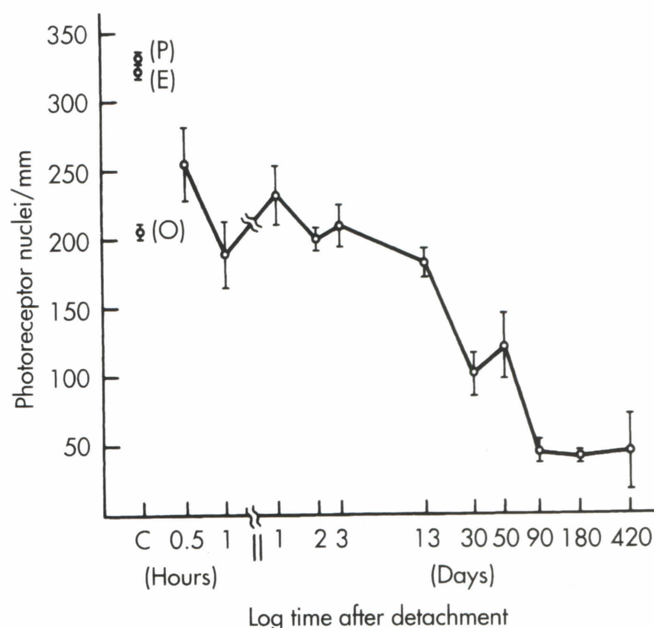

Fig. 119-14 A graph relating the number of photoreceptor nuclei in the outer nuclear layer to the time after retinal detachment (*error bars,* ±1 SD from the mean). The points indicated by *C* (control, attached retina) on the ordinate are from posterior retina *(P),* from equatorial retina *(E),* and from between 1 and 2 mm posterior to the ora serrata *(O).* All counts from retinal detachment are from equatorial or posterior retina. (From Erickson, PA, Fisher, SK, Anderson, DH, Stern, WH, and Borgula, GA: Invest Ophthalmol Vis Sci 24:938, 1983.)

with the retina being reduced to a single layer of cells in detachments of 4 months' duration.[62,63] 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.[48,50] On the basis of 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 human retinas. However, a recent histopathologic study by Wilson and Green[78] 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.[64]

Metabolic Changes During Detachment

Substantial metabolic changes must occur 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 earlier, Müller cells greatly increase 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 tritiated uridine, a specific precursor of RNA. Synthesis of RNA can be quantified in autoradiograms by counting exposed silver grains over individual cells or 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 tritiated 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. 119-15). 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. 119-15). 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 after detachment 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 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, the labeling intensity of the Müller cells significantly declines when stained with an antibody to the retinoid-binding protein known as cellular retinaldehyde-binding protein (Fig. 119-16). Similar immunolabeling experiments show a dramatic decrease in the levels of two Müller cell enzymes, carbonic anhydrase C and glutamine synthetase, during detachment (Fig. 119-16). Thus, detachment of the retina seems to produce changes in the metabo-

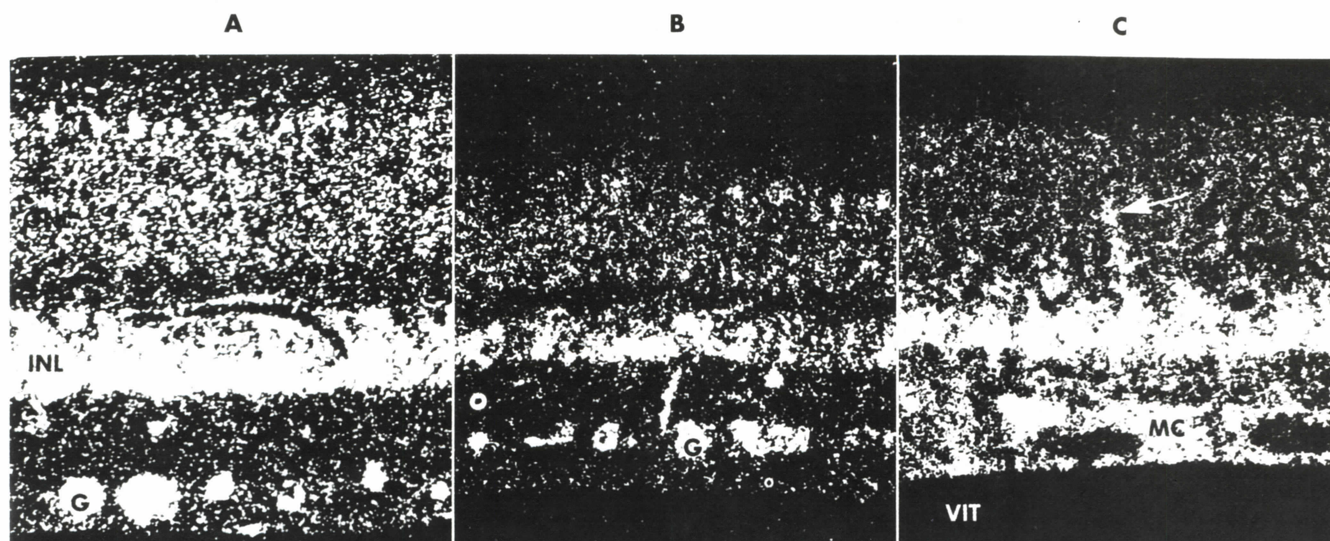

Fig. 119-15 Light microscope autoradiographs (dark-field exposures) of retinas detached for 1 day (**A** and **B**) and 3 days **(C)** and labeled for 24 hours with tritiated uridine. A is from an attached region adjacent to the area of detachment shown in **B**. Note the decreased intensity of labeling in the outer and inner nuclear layers in the detached region. **C,** 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)* (×325).

lism of retinal glia. The large increase in RNA synthesis seen in Müller cells likely represents a specific phase of altered transcription and translation that is a direct or secondary response to detachment.

Role of Regulatory Peptides in Events Induced by Retinal Detachment

Although our current level of understanding remains modest, the rapid proliferation of nonneuronal cell types after detachment, as well as the degeneration and eventual death of many photoreceptors, appear to be the events most likely to adversely affect the recovery of visual function following detachment. Any biologically active, nontoxic agent that acts to promote photoreceptor cell survival, maintain nonneuronal cells in a mitotically quiescent state, or sustain the differentiated phenotype of retinal neurons could be of potentially significant therapeutic value in the treatment of retinal detachment and other retinal diseases. This provides a compelling reason to examine the functional roles of regulatory peptides, particularly those factors that are normally present in the retina or adjacent tissue compartments, in the events triggered by retinal injury. A provisional list of such factors includes both acidic and basic fibroblast growth factors (FGF-1 and FGF-2), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), transforming growth factor-alpha, interleukin-1 beta, neurotrophin-3, neurotrophin-4, and insulin-like growth factor-2.[50a,50b,55a] In some cases, in situ hybridization and immunolocalization methods have been used successfully to identify the cellular and extracellular sites within the retina where these factors, and their receptors, are located. The underlying premise of such studies is that a more sophisticated understanding of their functions can be gained by knowing where they are sequestered precisely and which cell types serve as their targets.

One of these factors, FGF-2, has been shown to be associated with developing retinal vessels.[39] In the adult mammalian retina, it has been localized to a number of additional cellular and extracellular sites including the inner limiting membrane, Müller cells, and the insoluble portion of the interphotoreceptor matrix.[36,60a] When injected directly into the vitreous humor, FGF-2 can induce proliferative and fibrillogenic responses similar to those induced by experimental retinal detachment.[19,53] Similarly, exogenous injections of FGF-2 into the vitreous humor or subretinal space have been shown to arrest photoreceptor cell degeneration in various rodent models.[24,24a,50a] In theory, such "rescue" effects could be mediated through direct action of FGF-2 on the photoreceptor cells; alternatively, they could be secondary to a primary effect on another target cell type(s), such as retinal glia.[39a,53a]

Levels of FGF-2 immunoreactivity in the outer nuclear layer increase substantially following experimental optic nerve injury in rats,[47b] and increased FGF-2 immunoreactivity also is found in the rat interphotoreceptor matrix following experimental retinal detachment.[64a] These results imply that FGF-2 is released or activated in response to retinal injury or retinal detachment. In culture, the survival of rat photoreceptors is enhanced directly by exogenous FGF-2,[31a] and it has been shown recently that experimental retinal detach-

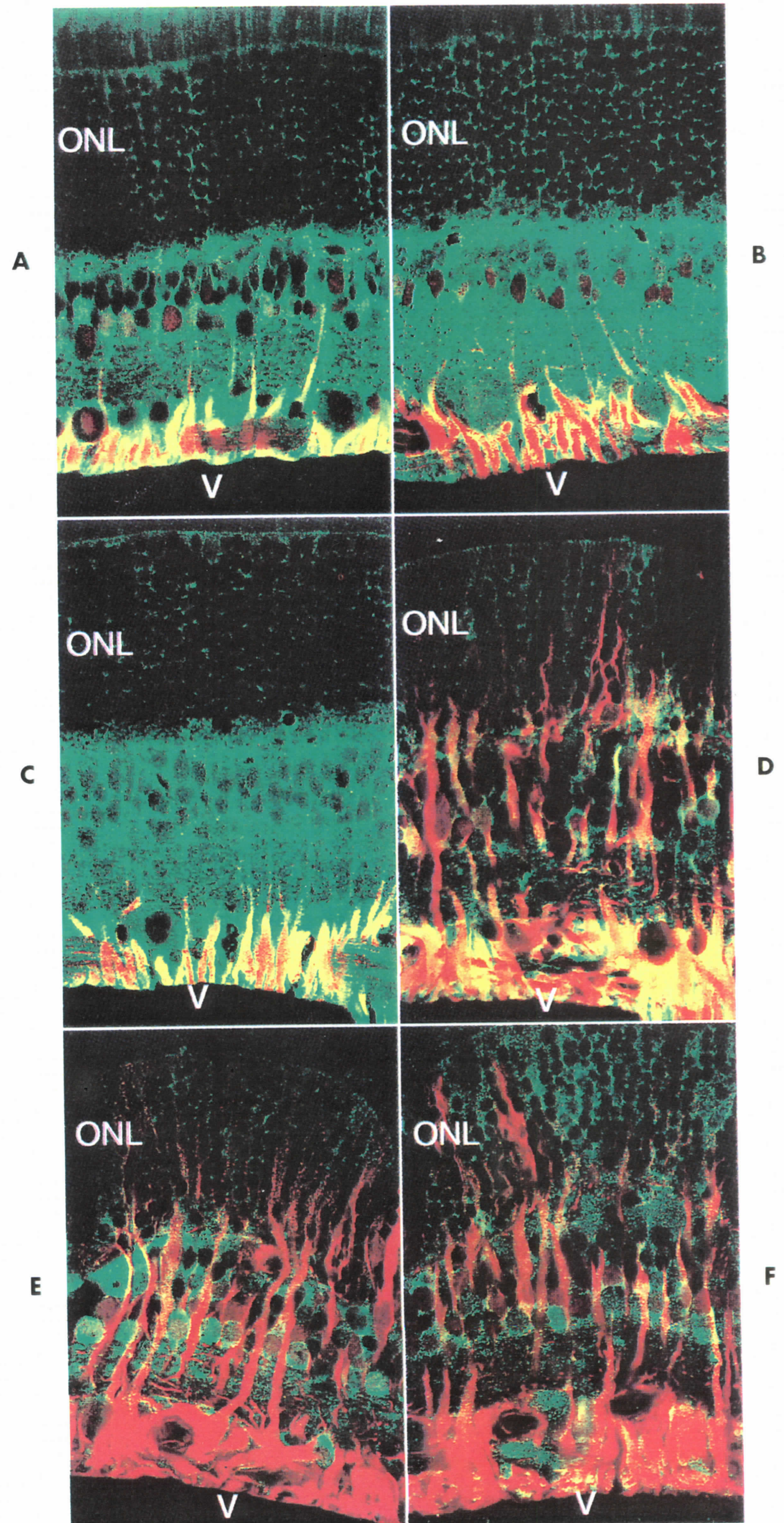

Fig. 119-16 Immunofluorescence images showing changes in the expression of Müller cell proteins after detachment. All sections were labeled with an antibody to vimentin, an intermediate filament protein (appears as red-orange), and then with an antibody to cellular retinaldehyde binding protein (**A** and **B**), carbonic anhydrase C (**C** and **D**), or glutamine synthetase (**E** and **F**) (green). In normal retina (**A**, **C**, and **E**) vimentin is found only in the Müller cell end-foot region (where it co-localizes with GFAP; see Fig. 119-13), whereas the other proteins are distributed throughout the cell. After 7 days of detachment (**B**, **D**, and **F**) vimentin expression increases in the Müller cells, whereas that of each of the other proteins decreases.

ment or focal injury is accompanied by a rapid increase in the expression of the high affinity fibroblast growth factor receptor (FGFR1) on the rat photoreceptor cell surface in vivo.[64a] These results strongly suggest that the "rescue" effect of FGF-2 on rat photoreceptors is mediated directly, rather than through some other cellular intermediary. Additional experimental studies will be required to elucidate the ensuing cascade of intracellular events and cellular interactions that accompany FGFR1 upregulation by photoreceptors and to clarify how other endogenous neurotrophins, including BDNF and CNTF, which apparently lack appropriate high affinity receptors on the photoreceptor cell surface,[47a,74a] exert their photoreceptor survival-promoting effects.[40a,50a,50b] Further investigation will be required to clarify the functional roles of regulatory peptides present in the normal retina, the RPE, or adjacent tissues, such as the vitreous and choroid, and to determine how these factors regulate the cellular events associated with detachment or other degenerative and proliferative retinal diseases.

EXPERIMENTAL 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 reversed successfully. The goal of experimental studies of reattachment is to understand more fully the cellular and molecular changes that accompany visual recovery. Eventually this should lead to improvements in the ability to manage, optimize, and ultimately manipulate the recovery process.[15,18,31,64] Discovery of the cellular mechanisms underlying this process and the variables that affect the extent of 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.[14,16,27] In the case of detachments involving the macula, there is no general consensus in identifying a critical period beyond which the prognosis for recovery dims. However, one study provides compelling evidence that visual recovery declines exponentially as a function of macular detachment duration.[15] This conclusion conforms with the morphologic results obtained from experimental models of reattachment.

Reestablishing the Photoreceptor-RPE Interface

It is evident that successful reestablishment of the photoreceptor-RPE interface is one, and perhaps the most important, aspect of morphologic recovery after reattachment. The cell-cell interactions that must occur between the photoreceptors and RPE during this recovery period are not known, but some relevant concepts have emerged from the experimental analysis. First, the photoreceptors are not reapposed to their original sites of interaction with the RPE when the retina is reattached. Therefore, site-specific interactions between these two cell types can be virtually ruled out as a factor in recovery. Second, rods and cones in many mammals, including humans, have different ultrastructural relationships with the RPE apical surface. It is still unclear to what extent these specific relationships are reestablished after reattachment and, moreover, whether this has implications for the successful regrowth of rod versus cone outer segments. Third, the cell-cell interaction that must occur after reapposition of the two layers is analogous to the interaction that takes place during RPE and photoreceptor development. Early in development, the photoreceptor inner segments are apposed to a mounded and undifferentiated apical RPE surface. Unknown metabolic and molecular processes that promote photoreceptor-RPE adhesion tend to maintain this apposition as the photoreceptor outer segments interdigitate with newly forming apical RPE 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 RPE apical surface, the reensheathment of the regenerating outer segments, and perhaps the resynthesis of interphotoreceptor matrix components as well. Finally, the photoreceptors and RPE must also reestablish a functional relationship. The transport of ions and molecules between the retina and RPE is affected to an unknown degree when the two cell layers are separated from each other. For example, retinoids (chemically distinct forms of vitamin A), coupled with their binding proteins,[13] must be transported back and forth between the neural retina and the RPE as part of the visual cycle. 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 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 often 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 later discussion). All these factors add to the difficulty in quantifying the rate of outer segment regrowth. However, recent morphometric and electron microscopic autoradiographic analyses of outer segment regeneration in monkeys whose retinas had been detached for 1 week and then reattached for up to 5 months have now begun to reveal some of the dynamics of this repair process.[34] The results suggest that the return of vision, at least in the first few weeks after reattachment, takes place against the background of an abnormally slow rate of photoreceptor outer seg-

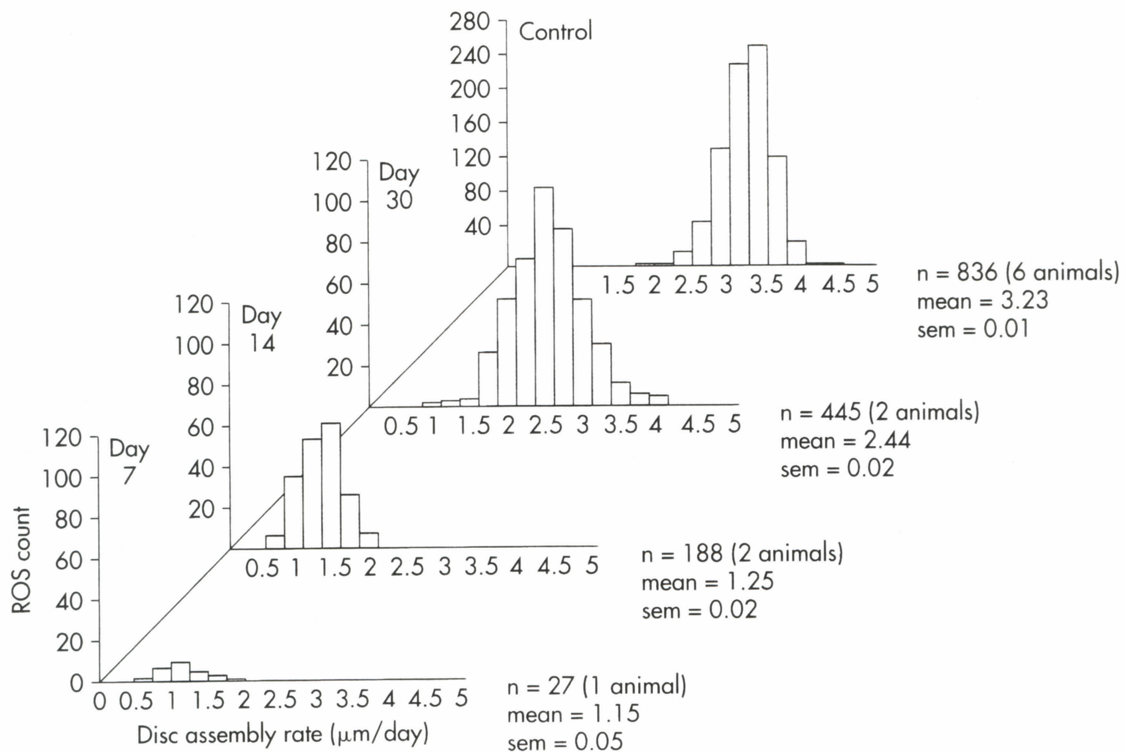

Fig. 119-17 Histograms illustrating the distribution of rod outer segment disc assembly rates after varying periods of retinal reattachment. The macular region in the retinas of rhesus monkeys were experimentally detached for 7 days. At 7, 14, and 30 days after reattachment, rod outer segment disc assembly rates (in micrometers per day) were calculated from electron microscopic autoradiograms in sample populations of macular rods located in the reattached regions. The number of rods was then plotted against assembly rate at several reattachment time points. The mean rate increased from a low of 1.15 μm/day at 7 days to 2.44 μm/day at 30 days. This compares with a mean normal rate of 3.23 μm/day.

ment disc membrane assembly (Fig. 119-17). In monkey retinas detached for 1 week, rod and cone outer segments regain approximately 30% of their normal mean length in the first 7 days after reattachment, 60% of their length after 30 days, and 100% by 150 days. In the first 30-day interval, the mean disc membrane assembly rate in rods is approximately one third slower than the normal rate (see Fig. 119-17); whether that diminution persists beyond that point remains an open question for the time being. Disc shedding, on the other hand, appears to engage after the first reattachment week.

In cat retinas detached for periods longer than 7 days, many outer segments remain shorter than normal several months after reattachment,[6] implying that defects in the assembly or shedding phases (or both) of the renewal process may persist well beyond 30 days in retinas detached for longer durations. In the future it would be highly desirable to have complementary electrophysiologic and psychophysical data in these experimental animal models to define more precisely the relationship between the recovery of visual function after reattachment and the time course of photoreceptor outer segment regeneration.

Patchwork Regeneration: the Result of Proliferative Changes. One of the most striking features of the reattached retina is the variability in outer segment length from region to region 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 RPE more rapidly and begin the regenerative process more quickly than do adjacent regions. In the experimentally reattached retina, photoreceptor regeneration occurs in a "patchwork" fashion, with areas of near-normal appearance adjacent to areas showing virtually no regeneration.[6] 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 RPE cells or Müller cells into the subretinal space is nearly always associated with poor or nonexistent photoreceptor recovery (Figs. 119-18 and 119-19). In the case of RPE proliferation, recovery seems to depend on

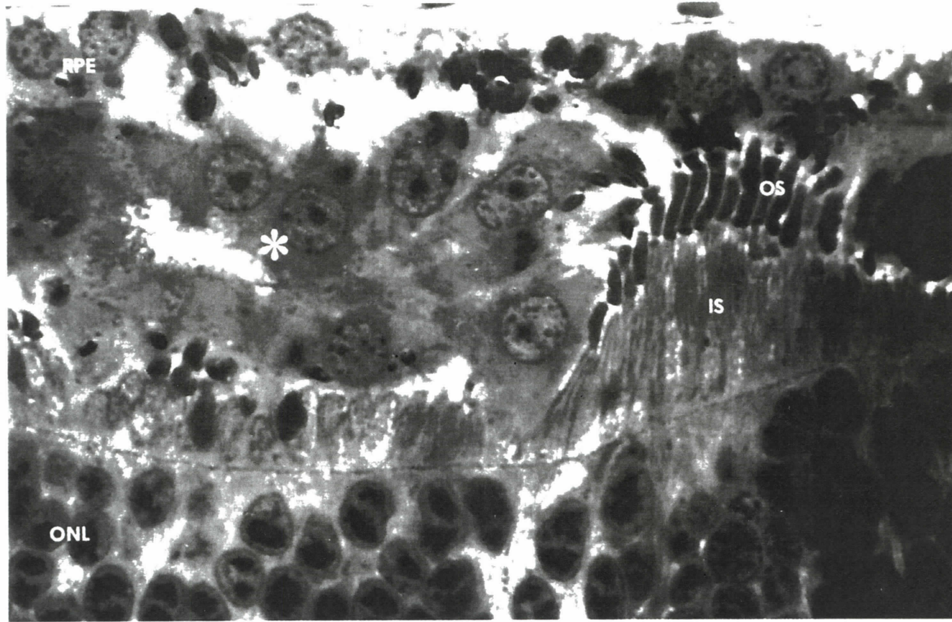

Fig. 119-18 A light micrograph showing an area of retinal pigment epithelium cell proliferation in a reattached retina. Only a few outer segment remnants remain under the cluster of proliferated cells *(asterisk)*. The dimensions of the outer segments *(OS)* adjacent to the zone of proliferation are nearly normal (×2200). *IS*, Photoreceptor inner segment; *ONL*, outer nuclear layer. (From Anderson, DH, Guerin, CJ, Erickson, PA, Stern, WH, and Fisher, SK: Invest Ophthalmol Vis Sci 27:173, 1986.)

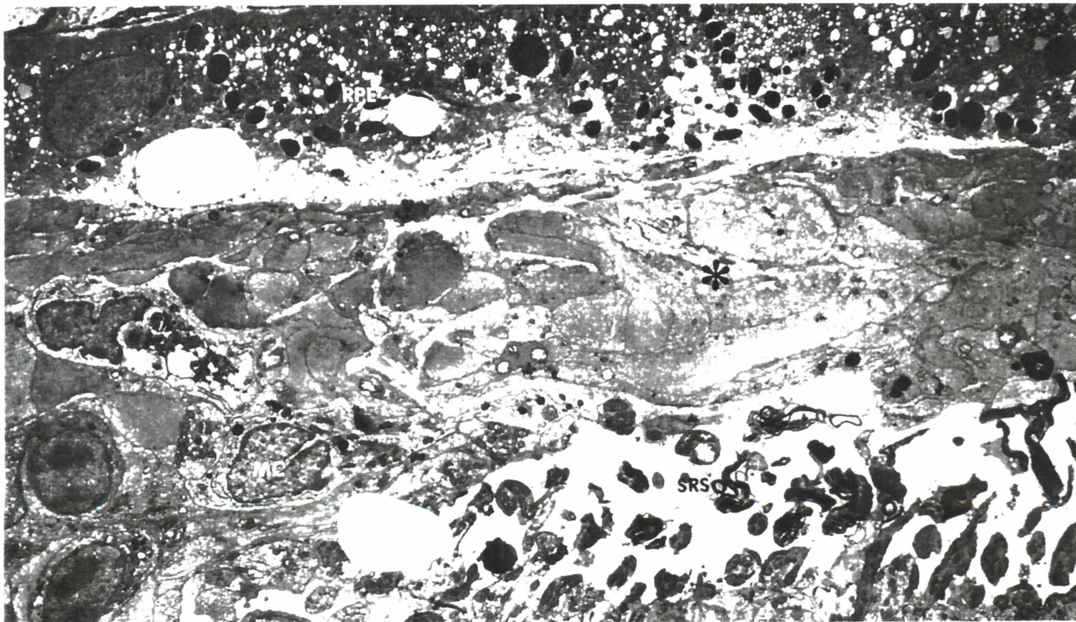

Fig. 119-19 An electron micrograph showing the presence of extensive Müller cell hypertrophy in the subretinal space in a retina that was detached for 6 weeks and reattached for 4 weeks. The Müller cell processes form a barrier between the retinal pigment epithelium and the photoreceptor cell layer (×2800). *ONL*, Outer nuclear layer; *asterisk*, Müller cell processes in the subretinal space *(SRS)*; *MC*, a Müller cell body in the subretinal space.

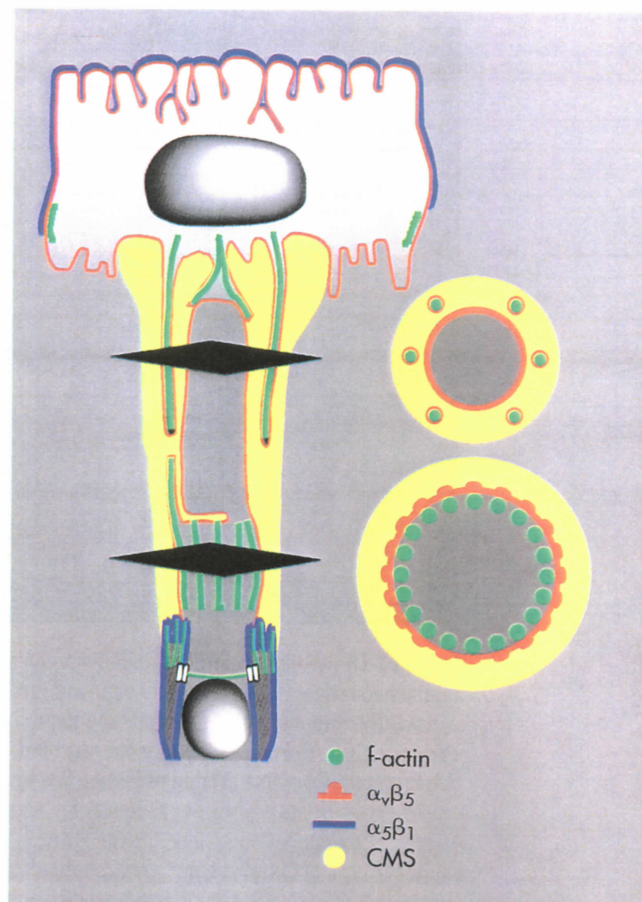

Fig. 119-20 Schematic representation showing the putative locations of $\alpha_v\beta_5$ and $\alpha_5\beta_1$ integrins at the photoreceptor-RPE interface, and their distributions in relation to the actin cytoskeleton, as found in this study. A cone photoreceptor drawn in a plane parallel to its longitudinal axis is shown on the left side of the illustration. Two cross-sectional views, one at the level of the cone outer segment and one at the level of the cone inner segment ellipsoid, appear on the right side of the illustration. The distribution of $\alpha_v\beta_5$ on the cell surface of the cone corresponds closely with the distribution of the CMS and with actin filaments located in the cone ellipsoids and myoids. A transcellular linkage between component(s) of the CMS, $\alpha_v\beta_5$ integrins on the surfaces of photoreceptor and RPE cells, and their actin cytoskeletons could function as a molecular mechanism for maintaining retinal adhesion.

the degree of differentiation and the polarity of the proliferated cells. In many instances the apical-basal surface morphology of proliferated RPE cells bears little resemblance to that of normal RPE. In others, additional layers of proliferated cells may have their polarity reversed, with the *basal* surface facing the photoreceptors (see Fig. 119-5). In either situation, regeneration is much poorer under such circumstances. 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 immediately or as soon as possible after detachment may well 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 comprehensive understanding of why the retina adheres to the pigment epithelium has yet to emerge. At this point, it appears most likely that multiple forces act in concert to maintain normal adhesion; Marmor reviews the morphologic, physiologic, and physicochemical factors that may contribute to retinal adhesion, as well as their therapeutic implications, in Chapter 116.

Perhaps the most intriguing addition to the list of potential contributing factors is the identification of aqueous insoluble domains of proteoglycan-rich interphotoreceptor matrix (IPM) that ensheathe rod and cone photoreceptor outer and inner segments.[45] The matrix sheath is a distinct anatomic entity that can be isolated as a "sheet" of interconnected elements, each of which envelops a single photoreceptor.[37] It is composed primarily of aqueous insoluble glycoconjugates rich in chondroitin 4,6-sulfate. It originates at the apical surface of the pigment epithelium, drapes the photoreceptor outer and inner segments, and terminates at or near the Müller cell microvilli at the level of the outer limiting lamina. Thus the biochemical composition and strategic location of the matrix sheath make it an ideal candidate for an endogenous retinal "glue" that spans the interphotoreceptor space (Fig. 119-20). Although the complete functional repertoire of the matrix sheath has not been elucidated, retinal peeling experiments in a number of species, including primate,[38] now demonstrate that cone matrix sheaths are apparently anchored to the surfaces of the cell types that border the IPM (i.e., RPE, photoreceptor cells, and, perhaps, Müller cells). As

such, identification of the specific molecular component(s) within the sheath that adhere to the plasma membranes associated with the photoreceptors and apical RPE surface could represent a significant step forward in our understanding of retinal adhesion.

In other systems, the mechanism by which cells adhere to their substrate is a process involving extracellular matrix, transmembrane, and cytoskeletal-related proteins. It is now recognized that a number of relatively well-characterized extracellular matrix molecules, including collagens, fibronectin, laminins, proteoglycans, and vitronectin, bind to heterodimeric membrane receptors (integrins), which, in turn, interact with subplasmalemmal proteins as well as cytoskeletal elements at sites of cell-substratum attachment.[42] By analogy, it would not be surprising to discover that molecules from the same or related families also mediate retinal adhesion, which can be conceptualized as an unusual in vivo example of cell-matrix–cell attachment.

New evidence for the expression of a variety of different adhesion-related receptors and extracellular matrix molecules at the photoreceptor-RPE interface has emerged within the past few years. The first reports suggested that a β1 integrin was expressed on the apical surface, as well as the basal surface, of mammalian RPE.[7,65] Subsequently, this was confirmed in lower vertebrates as well.[17] However, the most recent evidence strongly suggests that $\alpha_v\beta_5$ is expressed predominantly on the apical RPE surface,[8] whereas β1-containing integrins appear to be located primarily on the basolateral surface. Binding experiments in cultured RPE cells have confirmed that adherence of rod outer segments to the apical surface is mediated, at least in part, by $\alpha_V\beta_5$ in conjunction with vitronectin, its preferred ligand.[27,61]

In addition to $\alpha_V\beta_5$, several other molecules, including the multifunctional scavenger receptor (CD36),[68] a mannose receptor,[12] N-CAM,[35] N-acetylgalactosaminylphosphotransferase,[74] and S-laminin,[43,57] have also been advanced as candidate retinal adhesion molecules in recent years, most often in the context of outer segment binding prior to RPE phagocytosis. Nevertheless, a contribution of one or more of these molecules to retinal adhesion cannot be excluded. Eventually, identification of the full complement of adhesion molecules in the interphotoreceptor matrix, their plasma membrane receptors, and the cytoskeletal-related elements with which they interact, should provide a more complete understanding of the molecular basis of retinal adhesion and its potential relationship to the binding phase of outer segment phagocytosis.

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.[63] 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] On the other hand, Kroll and Machemer[49,50] concluded that owl or rhesus monkey retinas detached for up to 12 weeks can appear relatively normal after approximately 4 weeks of reattachment. More recent studies in cat retinas and in the macula of rhesus monkeys detached for 3 or 7 days and reattached for 3, 7, 14, or 30 days demonstrate a progressive increase in both rod and cone outer segment length and a tendency to return to their normal configurations[33,34] (Figs. 119-21 and 119-22). There is general agreement that after reattachment the photoreceptors do retain some capacity for outer segment regrowth, and regenerating outer segments do interdigitate with apical RPE 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] It also appears, however, 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 undergoing measurable or perceived deficits in function. At present it is simply 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 critical retinal location, such as the fovea, is involved.

Many of the changes described in experimental reattachments occur in small, localized regions, especially when the detachment interval is short. These may have no significant effect on the return of vision unless they occur within the macula, where even a small disruption of retinal structure could have a profound effect. The presence of a macular detachment is known to produce disturbances in acuity, metamorphopsia, and color vision that persist long after the retina is reattached. Damage to or loss of a percentage of the photoreceptor population within the macula, and particularly within the fovea, could account for chronic deficits in visual function. Although substantial individual variability in the packing density of photoreceptors occurs within normal primate maculas,[20] the adult visual system, because of its limited plasticity, may be unable to compensate for a reduction in the number of photoreceptors in an already established mosaic.

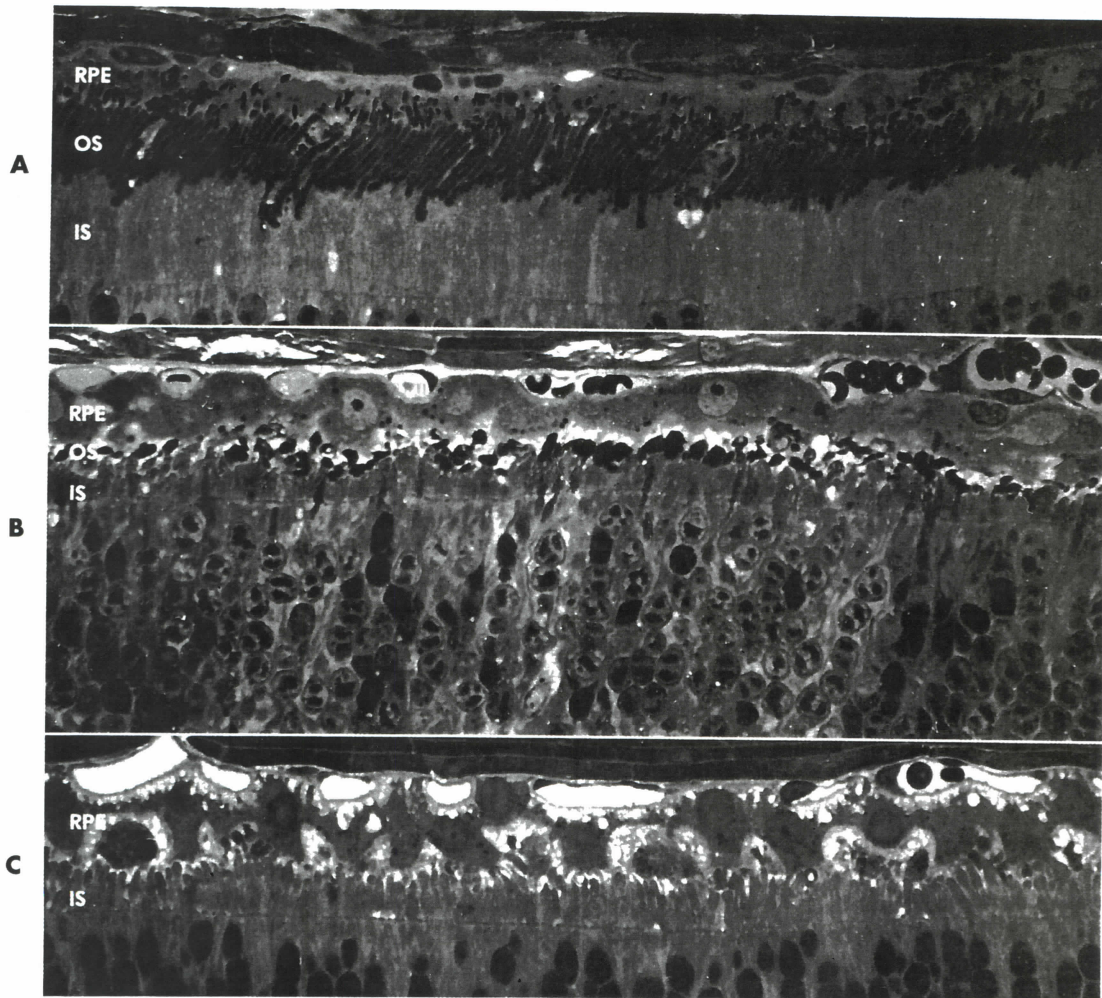

Fig. 119-21 Light micrographs of retinas detached and reattached for various intervals. **A,** The photoreceptor-RPE interface after a 3-day detachment and 1-month reattachment. After short detachment intervals the outer segments can reattain normal length in a relatively short time frame. Discrete areas of disruption, however, can still be identified. **B,** In contrast **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.

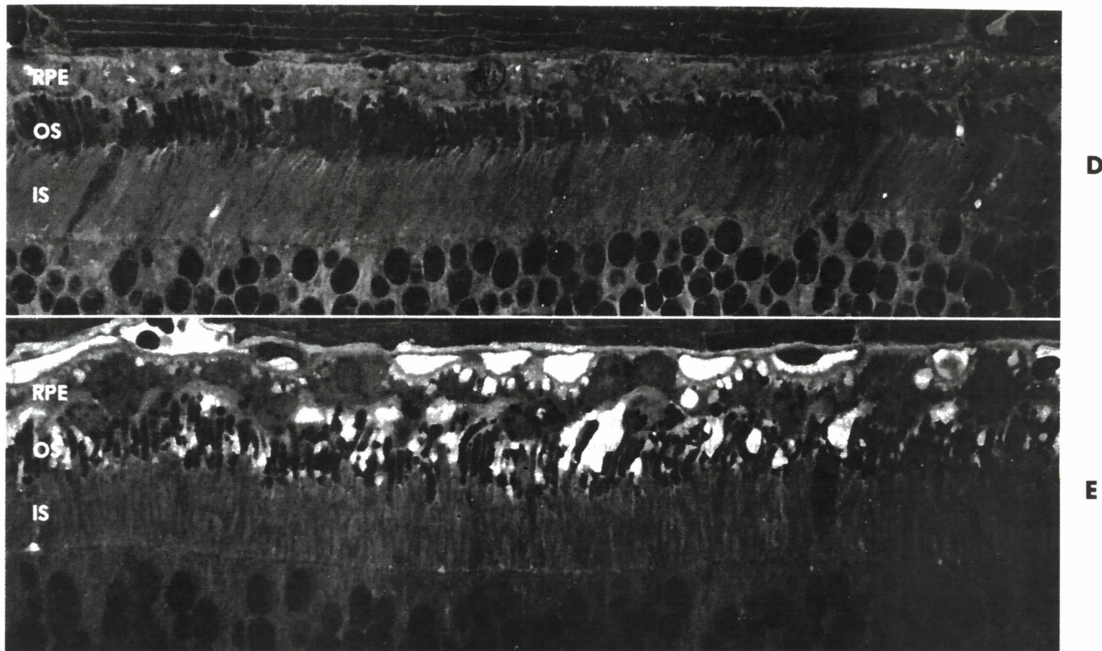

Fig. 119-21, cont'd **D,** An example from a second animal whose retina was detached for 5 days and reattached for 7 days. In this case the rod outer segments are approximately half of normal length. Cone outer segments are also abnormally short. **E,** In a retina detached for 8 days and reattached 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 (×800). *RPE,* Retinal pigment epithelium; *OS,* layer of photoreceptor outer segments; *IS,* layer of photoreceptor inner segments.

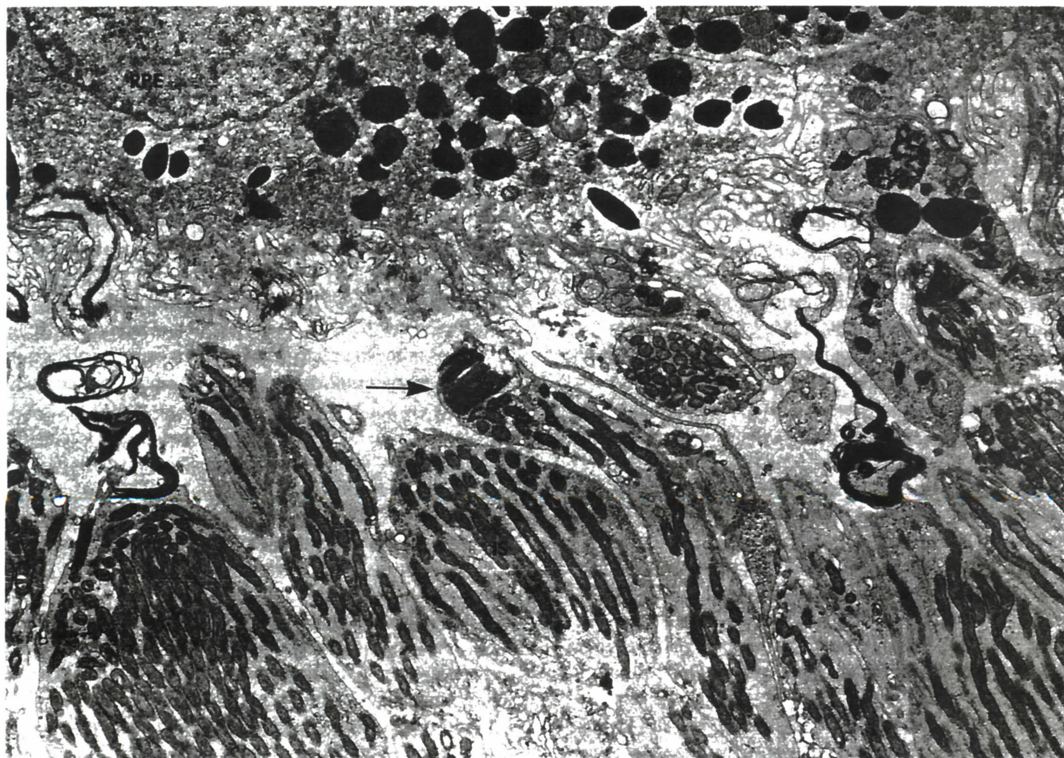

Fig. 119-22 An electron micrograph of the photoreceptor-RPE interface in the macula of a monkey retina detached 7 days and reattached for 3 days. Even after short reattachment intervals, some regenerating outer segments *(arrow)* can be identified (×7100). *IS,* Photoreceptor inner segment.

ACKNOWLEDGMENTS

Our research was supported by grants EY00888 and EY02082 from the National Eye Institute, National Institutes of Health. The opsin antibodies were gifts from Drs. Dean Bok and Robert Molday, and CRALBP antibody was a gift from Dr. Jack Saari.

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