
The onset of pigment epithelial proliferation after retinal detachment

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The adult mammalian retinal pigment epithelium (RPE) is mitotically inactive, yet retains the capacity to proliferate under certain conditions. To determine the onset of RPE proliferation after retinal detachment, we examined experimentally detached cat retinas of 12, 24, 48, and 72 hr duration. An additional animal served as a nondetached, sham-operated control. ³H-thymidine was injected into the vitreous chamber and the eyes were processed for light microscopic autoradiography. Autoradiograms from both the control and the 12 hr detachment showed no evidence of labeled RPE nuclei; however, labeled nuclei were present at both 24 and 48 hr after detachment. Labeled nuclei per millimeter of RPE at 24 hr were 55% of the 48 hr rate. Mitotic figures were noted only at 48 and 72 hr after detachment. No labeled RPE nuclei appeared in autoradiograms that bordered the detachment zone. Electron micrographs showed that proliferating RPE cells assume several configurations, some of which have been reported in other species. The proliferative response of the RPE occurs much sooner than had previously been thought. It appears to be a local effect that does not involve retinal regions beyond the detachment boundaries, and it may have potentially adverse effects when the retina and RPE are reapposed after retinal reattachment surgery.

Key words: retinal detachment, pigment epithelium, proliferation, retina, autoradiography

Early in development, the retinal pigment epithelium (RPE) cells undergo a rapid phase of mitosis that is completed prior to the differentiation of the photoreceptors.¹ After maturation, the RPE cells are generally con-

sidered to be mitotically inactive, as are neurons and a few other cell types in adult mammals. One exception, however, may occur in the adult rat retina, where the RPE cells apparently turnover at a very low rate.^{2, 3} Several studies have shown that the adult mammalian RPE retains the capacity to proliferate under certain conditions, i.e., in cases of tumors of the RPE and choroid,^{4, 5} in response to injury from intense light exposure,⁶⁻⁸ after cryotherapy,⁹ or after retinal detachment.¹⁰

In this report we show that the onset of RPE proliferation in the cat begins about 24 hr after the retina is surgically detached from the RPE and that the response is confined to the zone of detachment. The proliferation may continue even after retinal reattachment

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surgery.¹¹ Apparently, the separation of the retina from the RPE is sufficient to release the RPE cells from their postmitotic state and to initiate the sequence of events leading to cell division.

Methods

To determine the onset of proliferation, monocular retinal detachments were made in adult cats. Four eyes were enucleated and fixed at 12, 24, 48, and 72 hr after detachment. An eye from a fifth animal, which underwent all of the surgical procedures except for the detachment surgery, was used as a sham-operated control. All the animals were maintained on the same lighting schedule (12L:12D).

Surgery. Animals were anesthetized and extracapsular cataract extraction was performed through a 180-degree corneal incision. The posterior capsule was excised, and that was followed by a partial open-sky vitrectomy. The cornea was sutured closed and the eye was allowed to heal for 4 weeks. In the second stage of the surgery, the posterior vitreous cortex was removed with a vitreous suction cutter, which was then used to place a small retinal hole in the superior nasal retina. The suction cutter was replaced with a curved needle through which isosmotic Ringer's solution was injected into the subretinal space with a mechanical drive syringe. This resulted in a bullous retinal detachment that radiated outward from the superior nasal quadrant to below the optic disc. Only detached regions distant from the retinal hole were studied histologically.

Fixation. All the eyecups were fixed by immersion in 1% glutaraldehyde, 1% paraformaldehyde in phosphate buffer (pH 7.1) for approximately 12 hr. After aldehyde fixation, the eye cups were washed in phosphate buffer (plus sucrose at 45 mg/ml), post-fixed in 2% barbital (Veronal) acetate-buffered osmium tetroxide, dehydrated in a graded ethanol-H₂O series, and embedded in Araldite 6005.

Autoradiography. A solution of 200 μ Ci of ³H-thymidine (50 mCi/mmol specific activity) in 0.2 ml of distilled H₂O was injected into the vitreous chamber of the control eye and into the eyes that had had detachment surgery 12, 24, or 48 hr previously. All injections were made through the pars plana with a 26-gauge needle. A preplaced suture surrounding the injection site was closed immediately after withdrawal of the needle in order to ensure that all the injected fluid remained within the eye. All the eyes were injected during the

middle part of the light cycle in order to control for diurnal fluctuations in the cell cycle. After an incubation interval of 3 hr, the eyes were enucleated, the anterior one third of the globe was dissected away, and the eyecup was immersed in fixative. Light microscopic autoradiograms were prepared from 1 μ m tissue sections that were dipped in a 1:1 solution of Kodak NTB-2 and distilled H₂O maintained at 43° C. The slides were then placed in light-tight boxes and exposed from 5 to 10 days at 4° C. Finally, the slides were developed for 2 min in full-strength D-19 (at 20° C), washed, fixed, and stained with methylene blue-azure II or basic fuchsin.

Samples for autoradiography were taken from the following regions: (1) normal retina from the sham-operated control eye; (2) an area of detachment (5 mm in length) from each of the 12, 24, and 48 hr eyes; (3) ora serrata close to the zone of detachment from the 12 and 48 hr eyes; (4) ciliary epithelium close to the zone of detachment from the 24 and 48 hr eyes; and (5) an area of attached retina adjacent to the detachment zone (a transition zone) from the 48 hr eye.

The number of labeled RPE nuclei per millimeter of RPE was determined for the control, 12, 24, and 48 hr animals (1 and 2 above) in the following manner. A minimum of 13 autoradiograms, each containing ten 1 μ m tissue sections, were prepared for each animal. One section was randomly selected from each of the 13 slides for counting purposes. Because each of the tissue blocks was 5 mm long, a minimum of 65 mm of RPE was examined and counted in each of the eyes.

Results

Light microscopic examination of 1 μ m sections from the control eye showed that the surgical procedures preceding the detachment had no effect on retinal-RPE morphology. The outer segments of both rods and cones showed their usual cylindrical organization, and the relationship between the outer segments and the apical RPE appeared normal (Fig. 1, *a*). Phagosomes could be identified within the RPE cell bodies and within the apical RPE processes, indicating that the phagocytic role of the RPE cells remained unaffected. No labeled RPE cell nuclei were ever observed in autoradiograms from the control eye, and only a few scattered silver grains were present over the inner

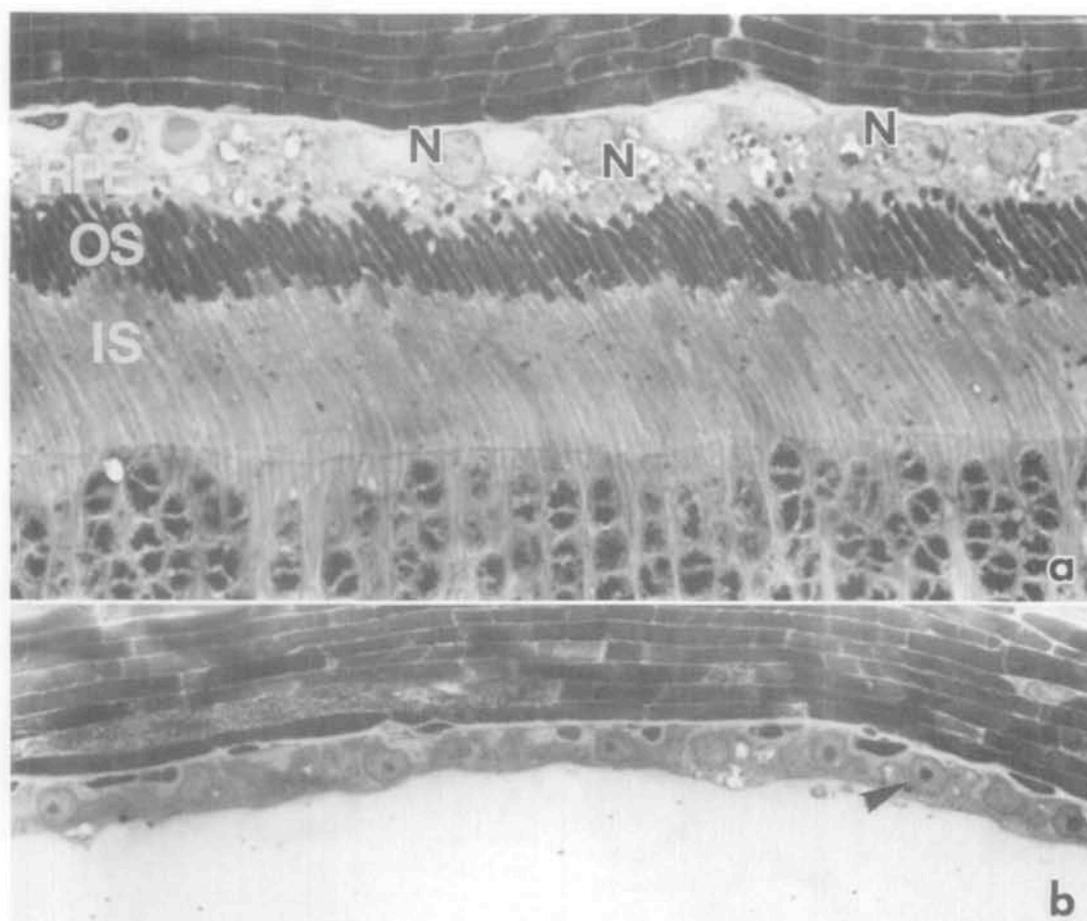


Fig. 1, a and b. a, Light microscopic autoradiogram from the control retina. The relationship between the photoreceptor outer segments (OS) and the retinal pigment epithelium (RPE) is normal. The RPE nuclei (N) are not labeled. A few scattered silver grains appear over the photoreceptor inner segments (IS). ($\times 840$.) b, Autoradiogram from the 12 hr detachment. There is no evidence of labeled RPE cell nuclei (arrow) in this animal. ($\times 450$.)

segments of the photoreceptors (Fig. 1, a). As expected, there was no labeling of photoreceptor nuclei or of tapetal cell nuclei.

In the 12 hr detachment some important morphological changes were detected in the RPE cells; however, no evidence of labeled RPE nuclei or mitosis was found (Fig. 1, b). Phagosomes that were clearly identifiable within the RPE cell bodies of the control eye were absent in the RPE cells of the 12 hr detachment. Furthermore, the apical processes that normally ensheath the outer segments were gone and only a fringelike apical border could be detected in the light microscope. A few labeled cells, presumably invading white blood cells, were found in the ret-

ina close to the outer margin of the inner nuclear layer, thereby indicating that the labeled compound had indeed been available for incorporation.

In contrast to both the control and the 12 hr detachments, heavily labeled RPE nuclei were evident at both 24 and 48 hr after detachment, and mitotic figures were observed at both 48 and 72 hr after detachment (Fig. 1, d). At 24 hr, labeled RPE nuclei were found occasionally, and at 48 hr it was not unusual to find two or more adjacent RPE nuclei labeled (Fig. 1, c). Labeled nuclei per millimeter of RPE 24 hr after detachment were about 55% of the 48 hr rate (Fig. 2). Neither the photoreceptor nuclei nor the tapetal cell nu-

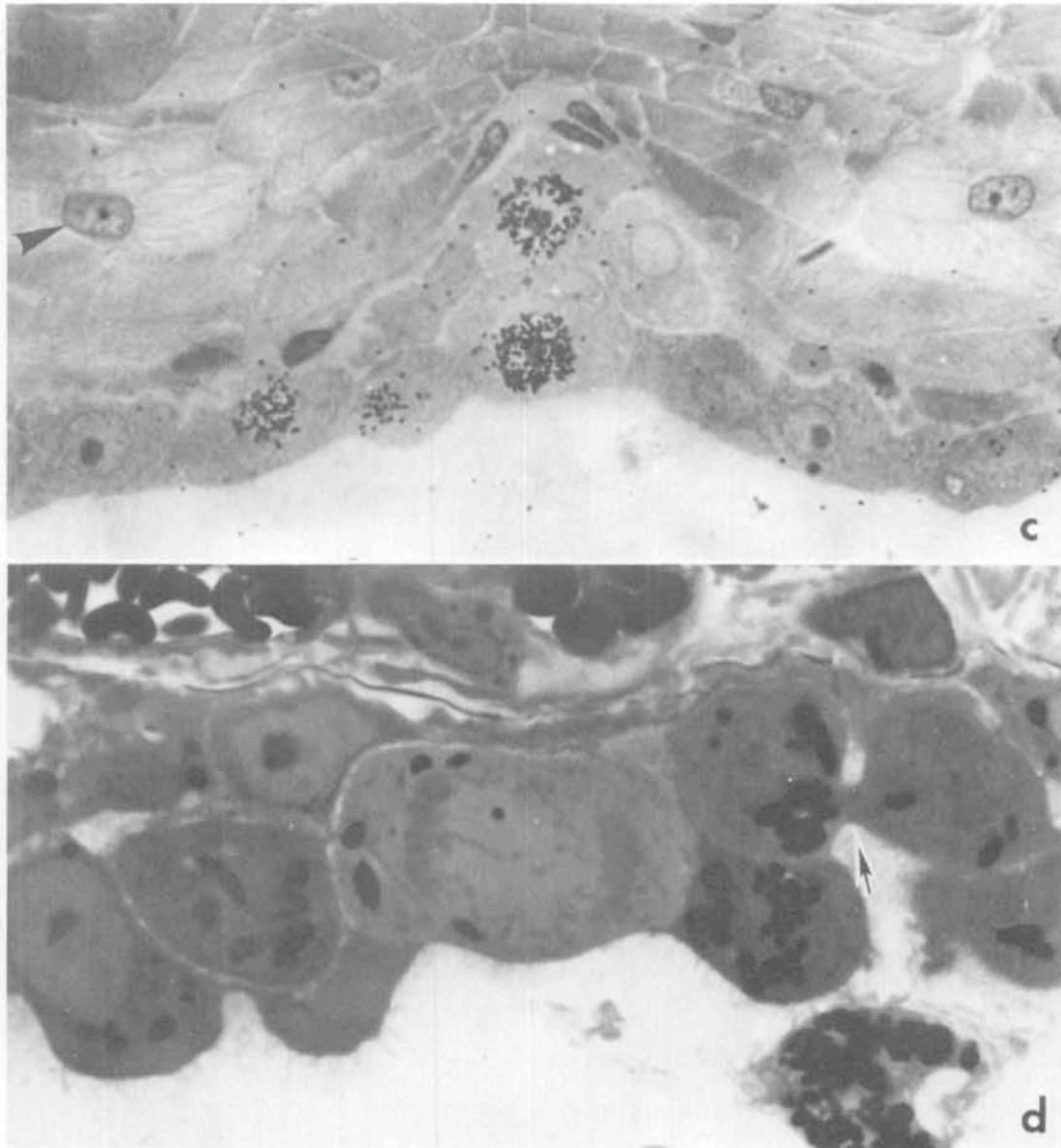


Fig. 1, c and d. c, Autoradiogram from the 48 hr detachment. Four adjacent RPE cell nuclei are intensely labeled, indicating that they were in the S-phase of the cell cycle for at least a portion of the ^3H -thymidine incubation period. The tapetal cell nuclei (arrow), which lie immediately above the RPE monolayer, remain unlabeled. ($\times 1200$.) d, Light micrograph from the 72 hr detachment. In the center of the micrograph, a mitotic figure in anaphase appears within the RPE cell monolayer. The chromatids have migrated to opposite poles of the cell. The cell on the right side of the micrograph appears to be in the final stages of cleavage prior to division (arrow). ($\times 1650$.)

clei showed any evidence of ^3H -thymidine incorporation at these times.

We examined autoradiograms of the RPE that bordered the area of detachment in order to determine whether the proliferation

was strictly localized to the detachment zone or was a more generalized response. We found no mitotic figures or labeled RPE cells in these areas at 12, 24, or 48 hr after detachment. Furthermore, we found no evi-

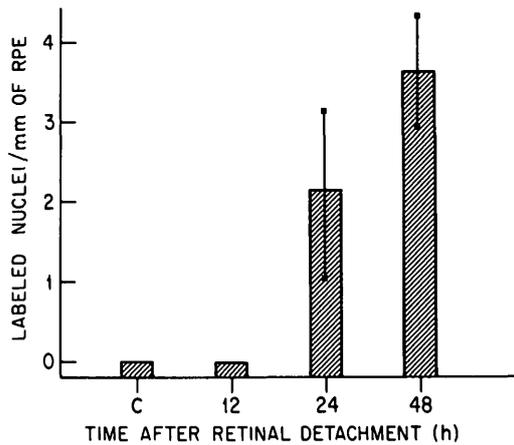


Fig. 2. Number of labeled RPE nuclei/mm as a function of the time (hr) since retinal detachment surgery. Each vertical bar represents the mean number of RPE nuclei labeled during one 3 hr interval after a single injection of ^3H -thymidine. C, Control animal; error bars = ± 1 S.D.

dence of mitosis in the ciliary epithelium adjacent to the detachment zone and, with one exception, no ciliary epithelial cells were labeled at the times sampled.

After 72 hr, small areas of RPE hyperplasia began to appear in the RPE of the detached retina. In Fig. 3, the proliferating RPE cells appear histologically as a second layer of cells parallel to the original monolayer. Their nuclear membranes are more scalloped than those of normal RPE cell nuclei, and the usual apical-basal surface polarity is not evident. The cell surfaces are lined with many short, undifferentiated processes that sometimes interdigitate with similar processes from adjacent cells. The cells are joined by adhering junctions to each other and to cells in the original monolayer (Fig. 3, inset). There is no sign of any fibrous extracellular matrix or basement membrane associated with the proliferating cells at this stage.

Discussion

RPE proliferation has been shown to occur in several species, including humans, in response to a variety of different stimuli such as xenon arc⁶ and laser⁷ photocoagulation, and exposure to ophthalmoscopic light⁸ and as a result of overlying choroidal tumors,⁵ cryotherapy,⁹ or retinal detachment.¹⁰ The mor-

phological features of the proliferative response are similar in all these studies despite the different conditions that elicit it. The areas of proliferation assume one of several specific configurations. With time, these areas may appear as plaques of spindle-shaped cells. The normal polarity of the cells is usually modified, and they are interconnected by adhering junctions. Their pigmentation is altered to some degree, and they are often associated with a fibrous intercellular matrix presumed to be secreted collagen. The RPE cells are capable of separating from Bruch's membrane, and there is suggestive evidence that they become migrating phagocytes in the subretinal space.

This morphological profile is also found in cat retinas detached for longer than a few days.^{11, 12} Proliferating RPE cells appear histologically as localized mounds of cells joined by adhering junctions (Fig. 3, inset) to each other and to the original layer, as multilayered sheets of cells parallel to the original monolayer (Figs. 3), or as folds of cells that protrude into the subretinal space. In addition, electron microscopic observations show that some RPE cells in the detached retina separate from Bruch's membrane and float freely in the subretinal space. These cells tend to be pigmented in areas of pigmented retina and nonpigmented in tapetal regions, thereby indicating their probable RPE origin. The cells appear to migrate toward the tips of the degenerating outer segments, where they phagocytize outer segment debris.

Only rough estimates of when the proliferative response first begins have been made in previous investigations. Proliferating cells were first observed as early as 2 weeks in light-damaged retinas⁶ and between 1 and 8 weeks in experimentally detached retinas.¹⁰ The issue is a significant one because of the involvement of proliferating RPE cells in massive periretinal proliferation^{13, 14} and because of the potentially adverse effects of RPE proliferation with respect to retinal reattachment surgery. If the experimentally detached cat retina accurately reflects the time course of the response in other mammalian retinas, then the onset of the proliferative re-

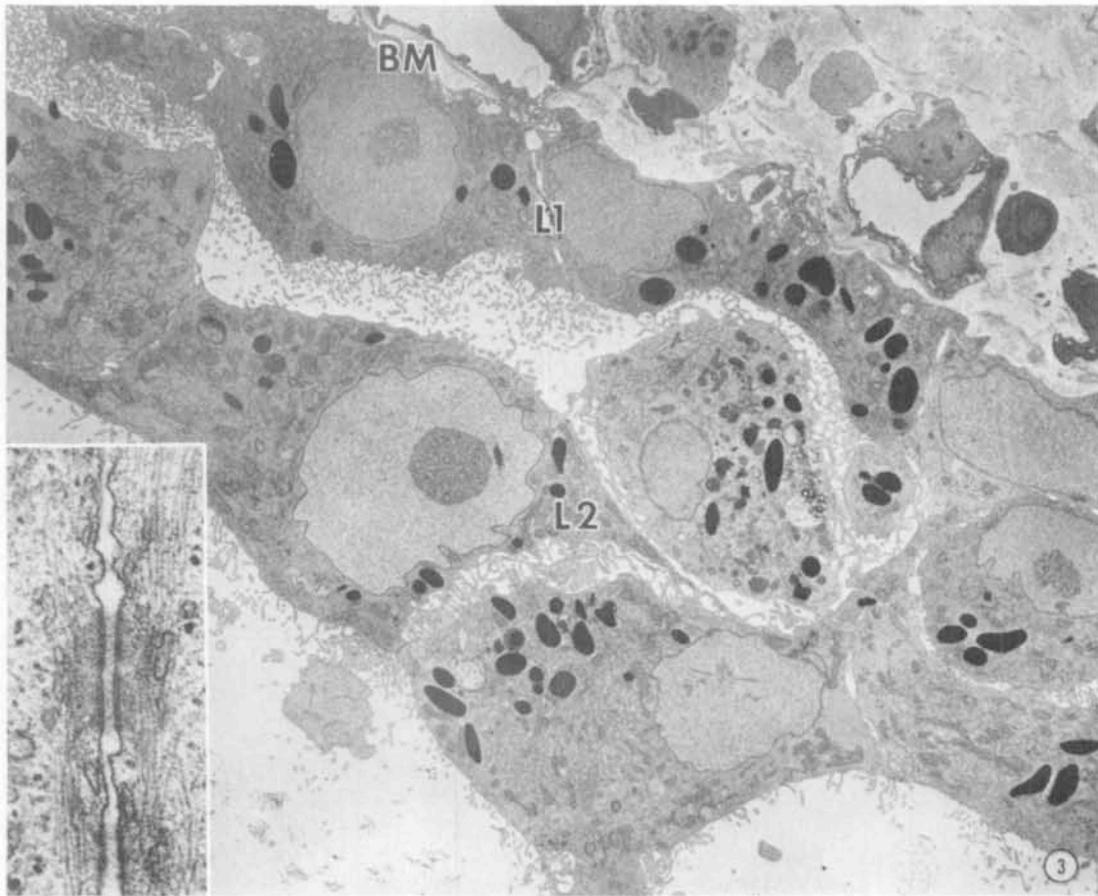


Fig. 3. Area of proliferated RPE cells 72 hr after experimental detachment in the cat. A second layer (*L2*) of RPE cells parallels the original monolayer (*L1*) in the upper portion of the figure. The new layer of RPE cells has not retained the apical-basal polarity characteristic of normal RPE cells. *BM*, Bruch's membrane. **Inset**, New cells are linked by adhering junctions. ($\times 9600$; inset $\times 150,000$.)

response occurs much sooner than had previously been reported.

In the cat retina and in all other vertebrate retinas examined thus far, processes from the apical RPE surface envelop or ensheath the outer portion of both rod and cone outer segments.¹⁵ This close intercellular association between the ensheathing processes and the outer segments provides the structural framework for the exchange of ions and molecules between the outer segments and the RPE cells.^{16, 17} The loss or disruption of this normal metabolic exchange through detachment may act as the stimulus for the dedifferentiation of RPE cells and the resulting proliferative response. This type of proliferation is not without precedent in other tissues.

Other *in vivo* examples of stimulus-dependent DNA synthesis and cell division in mitotically quiescent cell populations occur in mammalian liver, kidney, uterine and lens epithelium, mammary gland, and several other tissues.¹⁸ In all these examples, the proliferative response occurs after a single stimulus application and becomes evident only after an interval of 12 to 72 hr. In some of these systems, the response involves a significant fraction of the cell population. Whether or not this is true for the RPE remains an intriguing but unanswered question.

There are several potential pathological consequences of reapposing the retina to proliferating RPE cells. Our results point toward two related conclusions that could ad-

versely affect the re-establishment of the normal photoreceptor-RPE interface and therefore the return of vision after retinal reattachment surgery. First, in the earlier stages after reattachment surgery, the RPE may continue to proliferate. We have identified RPE mitotic figures in cat retinas that have been detached for 2 weeks and reattached for 1 month.¹¹ What is not yet certain is whether this represents the residual response to detachment or whether the proliferation continues uninterrupted despite reapposition of the photoreceptors to the apical RPE. Second, the presence of multiple layers of RPE cells may interfere with the re-ensheathment of regenerating outer segments by apical RPE processes and therefore with the phagocytosis of shed-disc packets and with the transfer of metabolites from the choroidal vasculature to the retina. It remains to be seen whether the daughter cells adjacent to the original RPE monolayer are able to fulfill their specialized role in the outer segment renewal process after retinal reattachment.

Many of the major characteristics of RPE proliferation in the detached cat retina have also been identified in cases of human retinal detachment.¹⁹ Consequently, the experimentally detached retina can be used as a model system in providing quantitative estimates of the rate and time course of RPE proliferation, in determining the effectiveness of various pharmacological agents in inhibiting proliferation, and in assessing the viability of the restructured RPE-photoreceptor relationship after retinal reattachment surgery.

REFERENCES

1. Mann I: The Development of the Human Eye. New York, 1969, Grune & Stratton, Inc.
2. T'so MOM and Friedman E: The retinal pigment epithelium: I. Comparative histology. *Arch Ophthalmol* 78:641, 1967.
3. LaVail MM: The retinal pigment epithelium in mice and rats with inherited retinal degeneration. *In The Retinal Pigment Epithelium*, Zinn KN and Marmor MF, editors. Cambridge, Mass., 1979, Harvard University Press, pp. 357-381.
4. T'so MOM and Albert DM: Pathological conditions of the retinal pigment epithelium: neoplasm and nodular non-neoplastic lesions. *Arch Ophthalmol* 88:27, 1972.
5. Wallow IHL and T'so MOM: Proliferation of the retinal pigment epithelium over malignant choroidal tumors. A light and electron microscopic study. *Am J Ophthalmol* 73:914, 1972.
6. Wallow IHL and T'so MOM: Repair after xenon arc photocoagulation. 2. A clinical and light microscopic study of the evolution of retinal lesions in the rhesus monkey. *Am J Ophthalmol* 75:610, 1973.
7. Marshall J and Mellerio J: Disappearance of retino-epithelial scar tissue from ruby laser photocoagulations. *Exp Eye Res* 12:173, 1971.
8. T'so MOM: Photic maculopathy in the rhesus monkey: a light and electron microscopic study. *INVEST OPHTHALMOL* 12:17, 1973.
9. Laqua H and Macheimer R: Repair and adhesion mechanisms of the cryotherapy lesion in experimental retinal detachment. *Am J Ophthalmol* 81:833, 1976.
10. Macheimer R and Laqua H: Pigment epithelial proliferation in retinal detachment (massive periretinal proliferation). *Am J Ophthalmol* 80:1, 1975.
11. Anderson DH, Stern WH, Fisher SK, Erickson PA, and Borgula GA: The onset of pigment epithelial proliferation following retinal detachment. *INVEST OPHTHALMOL VIS SCI* 19(ARVO Suppl.):251, 1980.
12. Anderson DH, Stern WH, Fisher SK: Transmission and scanning electron microscopy of the detached cat retina. *INVEST OPHTHALMOL VIS SCI* 18(ARVO Suppl.):86, 1979.
13. Clarkson JG, Green WR, and Massof D: A histopathologic review of 168 cases of preretinal membrane. *Am J Ophthalmol* 84:1, 1977.
14. Macheimer R, van Horn D, and Aaberg TM: Pigment epithelial proliferation in human retinal detachment with massive periretinal proliferation. *Am J Ophthalmol* 85:181, 1978.
15. Anderson DH, Fisher SK, and Steinberg RH: Mammalian cones: disc shedding, phagocytosis, and renewal. *INVEST OPHTHALMOL VIS SCI* 17:117, 1978.
16. Steinberg RH and Miller SS: Transport and membrane properties of the retinal pigment epithelium. *In The Retinal Pigment Epithelium*, Zinn KN and Marmor MF, editors. Cambridge, Mass., 1979, Harvard University Press, pp. 205-225.
17. Young RW and Bok D: The metabolism of the retinal pigment epithelium. *In The Retinal Pigment Epithelium*, Zinn KN and Marmor MF, editors. Cambridge, Mass., 1979, Harvard University Press, pp. 103-123.
18. Baserga R: Biochemistry of the cell cycle: a review. *Cell Tissue Kinet* 1:167, 1968.
19. Morris DA and Henkind P: Pathological responses of the human retinal pigment epithelium. *In The Retinal Pigment Epithelium*, Zinn KN and Marmor MF editors. Cambridge, Mass., 1979, Harvard University Press, pp. 247-266.