Acute Changes in RPE Apical Morphology After Retinal Detachment in Rabbit

A SEM Study

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The morphology of the apical surface of rabbit retinal pigment epithelium was studied by scanning electron microscopy from the first minute to several hours after making small non-rhegmatogenous retinal detachments (blebs). From 0 to 5 min, there were only slight changes in the homogenous, dense mat of filamentous microvilli. From 5 to 30 min, filamentous microvilli retracted exposing larger processes. From 30 to 60 min blunt processes became completely exposed and sheet-like processes disappeared. At about 60 min, cone sheaths were no longer identifiable in most specimens. Between 60 min and the time of retinal reapposition (several hours), the apical surface became highly rounded. Colchicine and cytochalasin-D had no effect on the time required for fluid resorption, but colchicine greatly accelerated and enhanced cell rounding, while cytochalasin-D produced prominent apical tufts. Invest Ophthalmol Vis Sci 27:1770–1776, 1986

Changes in RPE apical morphology have been observed 24 hours to several weeks after experimental rhegmatogenous detachment of the retina,1,2,3,4,5 or within minutes after making the vitreous severely hyperosmotic.6 The aim of this study is to examine the apical surface immediately after relativelyatraumatic mechanical detachment in the rabbit eye, and to determine the time of onset and the sequence of changes in RPE surface morphology.

Materials and Methods. Our technique for making small non-rhegmatogenous retinal detachments (blebs) has been described in detail elsewhere.7 In brief, Dutch rabbits weighing approximately 1.5 kg were anesthetized with pentobarbital, and a glass micropipette filled with Hank’s balanced salt solution (Gibco Chagrin Falls, OH), with a tip diameter of 40 to 50 μm, was advanced through a limbal incision to the retinal surface. Air pressure slowly expelled the fluid from the micropipette so that upon penetrating the retina and

* On leave from the Department of Ophthalmology, Osaka University Medical School, Osaka, Japan. Supported by NIH Grant EY03277 and the James S. Adams Award (Dr. Pederson) from Research to Prevent Blindness, Inc, New York, New York. Submitted for publication: November 14, 1985. Reprint requests: Jonathan E. Pederson, MD, University of Minnesota, Box 493 Mayo, Minneapolis, Minnesota 55455.

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entering the subretinal space, fluid elevated the retina and formed a bleb within 3 to 4 seconds (occasionally as long as 10 sec). For some blebs, 1 mM colchicine (Sigma St. Louis, MO) or 10 µg/ml (plus 1% DMSO) cytochalasin-D (Sigma) was added to the injection saline. Blebs were 2 to 3 mm in diameter and were made in the central retina, approximately 3 mm inferior to the optic nerve head. The limbal incision was sutured closed for observations greater than 20 min. All procedures conformed to the ARVO Resolution on the Use of Animals in Research.

After enucleation, the anterior segment and vitreous were removed. A small piece of tissue containing the blebs was excised and immersed in a dish of fixative (1% formaldehyde, 1% glutaraldehyde, 0.086 M phosphate without calcium) where the retina was gently peeled away from the blebs. Time from enucleation to fixation was 30 to 60 sec. Specimens were fixed overnight (refrigerated) and processed in the usual manner for light or scanning electron microscopy. One µm light microscopic sections were stained with toluidine blue. Scanning electron microscopic (SEM) material was coated with 50 Å of gold and viewed on an ISI-40 scanning electron microscope.

**Results.** Time course of RPE apical surface changes.

0 to 5 min: When observed by scanning electron microscopy within 5 min after bleb formation, the RPE apical surface was an even mat of filamentous microvilli 0.1 to 0.2 µm in diameter, through which the tips of cone sheaths and a few blunt processes projected (Figs. 1A-1B). Cone sheath microplicae during this period were well-organized and tightly-wrapped (Fig. 1B). Cone outer segments were not observed associated with cone sheaths, although outer segment fragments within the sheath might not be visible. We estimate that approximately one half to two thirds of the cone sheath was obscured by microvilli. A fully exposed cone sheath from a later timepoint (25 min) is shown for comparison in Figure 1C. A few flattened and blunt processes similar to those observed later were sometimes seen between the filamentous microvilli.

5 to 30 min: During the next 25 min, sheet-like microplicae became more exposed (Figs. 2A-2B), but were overshadowed by thick blunt processes that grouped together into elevated tufts (Fig. 2C). RPE cells in some areas showed an overall rounding of their apical surface.

60 min: About 1 hour after bleb formation, cone sheaths had disappeared in most blebs (Fig. 2D) and in some were shortened with disorganized lamellapodia. Sheet-like processes were few and less extensive, many looking like remnants of cone sheaths, and rounding of the RPE cell apical surface was very apparent.

3 to 4 hr: By 3 to 4 hours, 50 to 100% of the retina was reapposed to the RPE (see ref. 7). Filamentous microvilli were still present but sheet-like processes and cone sheaths were not found. The apical surface was populated predominantly by thick, blunt processes and displayed very pronounced rounding (Figs. 3A-3B). Intact and burst spherical objects 3 to 5 µm in diameter were occasionally found on the surface (Fig. 3). Pits and spherical objects were often found in the halo re-
region and were most numerous 24 hours after bleb formation.

24 hr: After about 20 hours of retinal reapposition (24 hr after bleb formation), blebs could be identified ophthalmoscopically by RPE pigment abnormalities. The morphology of the apical RPE surface observed by SEM, was very different compared to earlier times. The margin of the bleb appeared irregular and scalloped; not a clearly defined circle as it normally appeared before retinal reapposition. Some areas of the margin were covered with outer segment fragments presumably torn off by removal of the retina under fixative. A thick fibrous material was found between the apical processes which were almost all of the blunt type (Fig. 3C). Pits, 2 to 5 μm in diameter, were most numerous at this time.

RPE apical surface in the halo region: Most blebs were surrounded by a thin annulus or halo in which the attached retina appeared faintly whitened. This halo was evident ophthalmoscopically within the first few minutes after bleb formation and could persist for up to 1 hr. Apical process morphology in the halo region
differed markedly from those in the central region (Fig. 4). At the innermost edge of the halo many filamentous microvilli and microvilli were disarrayed or stretched (Fig. 4A). Within the halo itself, there were many elongated processes, often with diameters less than 0.1 μm, lying horizontal to the apical surface (Figs. 4B-4C). These processes were shorter at the inner margin, longer at the outer margin, and always radially arranged, pointing towards the center of the bleb. Pits, lined with flat, sheet-like processes occurred near the inner margin and many flattened processes were found near the outer margin of the halo.

Effects of cytoskeletal disrupters. Colchicine: The addition of 1 mM colchicine to the injection saline did not alter resorption time or the ophthalmoscopic appearance of the retina. At 5 and 16 min, the apical surface of the RPE had a normal appearance and lamellapodia of cone sheaths were still intact. By 30 min, however, the cells had become intensely rounded (Fig. 5A). At this time cone sheaths and a few filamentous microvilli remained but most apical processes were blunt and thick. After 3 hr (the time for resorption in this sample), the RPE cells were extremely rounded and tufts were barely discernible (Fig. 5B). (A trial in-
Fig. 4. A, Innermost margin of the halo region 25 min after bleb formation, h = halo area; b = bleb area. Magnification bar = 5 μm. B, Halo region 2 min after formation. Sheet-like (open arrow) and filamentous processes are oriented toward the center of the bleb. o = outer portion of halo; i = inner portion of halo. Note the flattened pit (arrow). Magnification bar = 5 μm. C, Higher power view of the outer margin of the halo. Magnification bar = 1 μm.

Injection of 4 mM colchicine caused retinal opacity; therefore, this concentration was not studied further.

*Cytochalasin-D:* 40 to 50 min after bleb formation with saline containing 10 μg/ml cytochalasin-D the apical surface had a normal, flat appearance. Rounding of the entire cell as seen with the addition of colchicine was not observed. However, tufts of blunt processes appeared to become more prominent and projected farther than normal from the apical surface (Fig. 5C). Observed ophthalmoscopically, the RPE and choroid became very red in color. The retina over these blebs was whitish and slightly opaque, and, upon dissection, was very friable and tore easily. Bleb resorption time was unaffected.

**Discussion.** The rabbit RPE showed progressive morphological changes beginning within the first few minutes after gentle detachment. The end result of this progression was a rounded apical surface populated by numerous short, thick processes, in agreement with published accounts of RPE 24 hr or more following retinal detachment. The appearance of different types of processes after detachment may simply rep-
Fig. 5. A, 30 min after exposure to 1 mM colchicine. Arrows show cone sheaths. B, 3 hr after exposure to colchicine. Cells have become intensely rounded. C, 50 min after injection of saline containing 10 μg/ml cytochalasin-D. The apical surface appears normal except for prominent apical tufts. Magnification bars = 4 μm.

Recent uncovering of extant processes after the retraction of the fine filamentous microvilli. Alternatively, new processes might have been formed either from modification of microvilli or newly added membrane material. The mechanism of cell rounding is unknown, but probably is not an expansion of the cell contour from membrane material donated by retracted processes, since colchicine caused intense rounding without the disappearance of cone sheaths, blunt processes or filamentous microvilli. Although this may implicate cytoskeletal modification the contribution of fluid influx cannot be ruled out.

Two novel features of the rabbit RPE only become visible after detachment, viz spherical objects and pits (Figs. 3A-3C, 4B). We suspect that the spherical objects are RPE oil droplets which are found in rabbits, and not in other mammals. The pits may correspond to locations where oil droplets were expelled by the RPE cells although it is not clear why this occurs.

An important physiological consideration is whether the alterations in the apical morphology we observe are a direct response to the loss of outer segment apposition to the RPE or a response to secondary changes in the extracellular milieu after bleb formation. An
earlier study demonstrated that injections of a highly hyperosmotic solution into the vitreous rapidly caused retinal detachment and disruption of RPE apical morphology in a sequence of changes similar to (but more rapid than) the ones described herein. The solutions injected into blebs during the present experiments were essentially isoosmotic with tissue fluids, but other abnormalities of the extracellular milieu might be present.

Our method for producing non-rhegmatogenous retinal detachments in rabbit is relatively gentle insofar as morphological criteria are concerned. Comparison of our SEMs of the apical surface to published transmission electron micrographs of attached RPE-retina shows that delicate apical processes and cone sheaths survive our procedure intact, and are visible immediately following detachment. Nonetheless, the halo region was obviously highly disturbed and we think it represents the point at which the RPE was still adherent to the inflating retina. Since the retina over the bleb was necessarily stretched, there must have been considerable force acting to stretch adherent RPE processes in the halo region, thus accounting for their orientation and appearance.

Key words: RPE, apical morphology, retinal detachment

Acknowledgments. The authors are indebted to Susan Lauber and Suzanne Tharpe for valuable assistance in preparations for electron microscopy.

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References


Comparative Aqueous Outflow Facility Measurements by Pneumatonography and Schiotz Tonography

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Tonography was performed on 36 eyes of 15 normal and 3 primary open angle glaucoma patients using pneumatonography and classical Schiotz tonography. The average values of the coefficient of outflow facility (C) for the whole sample were virtually identical with both methods. However, both intersubject and interobserver variability were significantly higher with pneumatonography. Although both methods provide comparable aggregate estimates of aqueous outflow facility, we think that Schiotz tonography is more reliable than pneumatonography because of the greater mechanical stability of the Schiotz instrument on the eye. On the other hand, pneumatonography offers the advantage of a shorter test period (2 min instead of 4). Invest Ophthalmol Vis Sci 27:1776–1780, 1986

Measuring aqueous outflow facility (C) tonographically has wide applications in clinical glaucoma research. However, because tonography involves some inherent sources of error,1,2 some technical limitations3 and subjective approximations in estimating the C value,2 and because the test is usually not necessary for the management of individual glaucoma patients,4,5 it has been largely abandoned in clinical practice.