The integrity of the RPE barrier function in retinal detachment was studied in vitro. The retinal pigment epithelium (RPE)-choroid tissue was isolated from cynomolgus monkey eyes with acute (<1 hr), subacute (1–2 weeks), and chronic (8–20 months) retinal detachments, and clamped between Ussing-type chambers. Electrical characteristics and choroid-to-retina permeability to carboxyfluorescein were determined. In the HEPES-buffered bathing solution, transepithelial potential difference and resistance in eyes with acute retinal detachments (0.2 mV and 134 ohm-cm², respectively) were significantly lower than subacute (7.9 and 350) and chronic (10.4 and 348) retinal detachments. Furthermore, the permeability was increased five-fold in acute retinal detachments with respect to subacute and chronic retinal detachments, indicating a breakdown of the RPE barrier in acute retinal detachment. No statistical difference was found between subacute and chronic retinal detachments. In this animal model, RPE barrier function is destroyed at the onset of retinal detachment, but recovers in a week or two, and is maintained in the chronic stage. Histological examination revealed that RPE recovery was accomplished by RPE proliferation and hyperplasia.

Materials and Methods

Rhegmatogenous RDs were created in one eye of each of 11 cynomolgus monkeys of both sexes weighing 2.5 to 3.5 kg. Animal usage conformed to the ARVO Resolution on the Use of Animals in Research. The details of the surgical procedure are described elsewhere. Briefly, a total vitrectomy was followed by insertion of a tapered polyethylene cannula in the subretinal space. One milliliter of the Ringer’s solution was injected as fluid was removed from the vitreous cavity. A large retinal hole was then it is modified by acetazolamide and furosemide. Thus, RPE barrier function involving fluid and electrolyte transport is retained or even accelerated in chronic RD. The question arises as to how this can occur in eyes with RD in spite of damage to the RPE. The present study was undertaken to investigate this question.

It has been shown that the transepithelial potential difference in the RPE-choroid preparation is an indicator of the electrogenic RPE electrolyte transport system. Therefore, RPE-choroid was isolated from monkey eyes with experimentally created RDs of various durations to evaluate the alterations of RPE barrier function. The choroid-to-retina (inward) carboxyfluorescein permeability was also measured, which represents the diffusional component of tracer movement and thus is a good indicator for the breakdown of the RPE barrier.
created with the vitrectomy instrument. Five of the 11 monkeys were sacrificed 1 to 2 weeks later (subacute RDs), when a total funnel-shaped RD was observed ophthalmoscopically. The remaining six monkeys were sacrificed 8 to 20 months after the creation of RD (chronic RDs). Moreover, in five of the 11 monkeys, RDs were created in the fellow eyes less than 1 hr prior to sacrifice (acute RDs). One of the intact fellow eyes served as a histological control.

All monkeys were killed with an overdose of sodium pentobarbital and the eyes with RDs were enucleated and bisected at the pars plana. The posterior segment was placed in a Petri dish containing the bathing solution and divided into three pieces. Two of these were subjected to histological examination. The remaining piece, consisting of the macular and temporal area, was further processed for the in vitro study.

Under an operating microscope, the RPE-choroid was carefully removed from the sclera using Vannas spitz forceps. The isolated RPE-choroid was placed on a stainless mesh, retinal side upward, and clamped between Ussing-type half-chambers made of Lucite (12.5 ml each). The area of the tissue exposed to both solutions was 0.67 cm². A full description of the chamber is made elsewhere.° HEPES-buffered solution (pH = 7.4 at 37°C, 300 mosmol/kg) was used.°

The transepithelial potential difference and short-circuit current across the RPE-choroid were recorded through two pairs of 3% agar-3 M KCl bridges and calomel electrodes, using an automatic voltage clamp device.° The transepithelial resistance was calculated from Ohm's law. After potential difference became stabilized, a 10 µl aliquot of 6-carboxyfluorescein was added to the solution facing the choroidal side of the tissue (final concentration, 10⁻⁴ g/ml). Each of the bathing solutions was continuously mixed by 95% O₂ and 5% CO₂ gas bubbling. A 100 µl sample was then taken from the solution facing the retinal side every 15 min for 60-90 min. The concentration of carboxyfluorescein in the samples was measured by a flash fluorophotometer.° We then calculated the choroid-to-retina (inward) premeability from the rate of appearance determined by linear regression.°

For histological examination, one piece of RPE-choroid-sclera from each eye was fixed in 10% neutral buffered formalin, processed for routine light microscopy and stained with hematoxylin and eosin. Another piece from each eye was fixed in 1.25% glutaraldehyde and 1% paraformaldehyde in phosphate buffer at a pH of 7.2. All such tissues were stored in phosphate buffer and prepared for scanning electron microscopy simultaneously. Each tissue was dehydrated in graded ethanol, critical point dried, sputter coated with gold palladium and examined with a scanning electron microscope.

Results

Ophthalmoscopically, the RPE observed through the retinal hole was characterized by an abnormal light brown color (accounting for 30-60% of the entire RPE) and the normal dark-brown color (Fig. 1). The light-colored area, characteristic of the underlying RPE from which the sensory retina had been originally separated during surgery, was always included in the isolated RPE-choroid exposed to the solution. However, the area of mechanical retinal pigment epithelial damage from the cannula was not included in the isolated RPE of the acute RD. In the subacute and chronic stage, the cannula track could not be identified even under the operating microscope.

The electrical characteristics of the isolated monkey RPE-choroid shown in Table 1 were stable for more than 3 hr. The potential difference of 7.9 to 10.4 mV, retinal side positive, in eyes with subacute and chronic RDs was comparable to the isolated frog, chicken, and dog RPE-choroid.° Moreover, the resistance of these eyes, 348-350 ohm-cm², was among the highest studied so far,° affirming the in

![Fig. 1. Fundus photograph of the RPE observed through the retinal hole in chronic retinal detachment. Note light and dark areas of the RPE (see text).](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933131/)
vitro clamping technique in the present study. However, the electrical parameters of the RPE-choroid in the acute RD were markedly lower than the subacute and chronic RD.

The appearance of carboxyfluorescein in the chamber facing the retinal side was constant during the experiment, indicating a stable permeability value. The inward permeability in the subacute and chronic RD was comparable to monkey RPE in vivo and dog RPE-choroid in vitro (4.8 and 5.4 × 10⁻⁷ cm/sec, respectively). However, the inward permeability was significantly increased in the acute RD (Table 1). There was no statistical difference between electrical parameters and permeability of the subacute and chronic RDs.

Light microscopy of the light-colored area in the acute RD showed either absence or depigmentation of the retinal pigment epithelial cells (Fig. 2A). Although the light-colored area was still recognizable ophthalmoscopically in the subacute and chronic RD (Fig. 1), retinal pigment epithelial cell loss was rarely found in any specimen with RD more than 1 week old. In the subacute and chronic RD, the RPE had a scalloped border and was occasionally multilayered, indicating retinal pigment epithelial proliferation. Most cells were heavily pigmented and some cells contained giant or multiple nuclei yielding a thickened layer of RPE (Fig. 2B, C). The RPE from the control eye was a single cell layer with a clear basal region containing a single nucleus and a densely pigmented, evenly thick apical region (Fig. 2D).

Scanning electron microscopy of the apical surface of the RPE following acute RD showed areas where the cell layer was flat with indistinct boundaries. Other areas showed spaces in the epithelium where cells were damaged. Enlarged cells with retracted microvilli were also present (Fig. 3). The RPE from an eye with a subacute RD showed a continuous cell layer with no missing cells. In some areas the cell layer contained flat cells with indistinct cell borders, but in most areas clumps of large round retinal pigment epithelial cells were noted (Fig. 4). The RPE from chronic RDs was similar to that seen in subacute RDs. Occasionally, individual enlarged, multinucleated cells were found suggesting hyperplasia and clumps of retinal pigment epithelial cells were noted indicating proliferation (Fig. 5).

Discussion

Although the RPE is thought to be mitotically inactive in adult mammals, it may proliferate in re-
Fig. 3. Scanning electron micrograph of the retinal pigment epithelium following acute retinal detachment. Some cells have been damaged, leaving spaces. A few large, round cells with retracted microvilli are also present. Most of the retinal pigment epithelial cells exhibit a flattened appearance (×220).

Fig. 4. Scanning electron micrograph of the RPE following subacute retinal detachment of 2 weeks' duration. The cell layer is intact and covered with mossy-appearing elongated microvilli. The cell borders are distinct due to their scalloped shape and lack of apical connections between adjacent cells. The overall appearance is one of a sculptured carpet (×220).
The RPE proliferation in RD was noted by Machemer and Laqua in 1975 as a pathogenesis of proliferative vitreoretinopathy. However, the present study reveals that RPE proliferation and hyperplasia lead to reconstruction of the RPE barrier, both anatomically and functionally, thus highlighting an advantageous aspect of RPE proliferation.

Previous in vitro studies have shown that the ouabain-sensitive electrogenic pump (Na-K ATPase) is located on the apical (retinal side) membrane of the RPE. Inhibition of this pump almost abolishes the potential difference in chicken and dog RPE. Therefore, the marked reduction in potential difference in the acute RD (only 2.5% of the subacute RD) is not surprising, since histological examination shows extensive destruction of the apical membrane. During separation of the sensory retina, the RPE apical membrane may remain partially attached to the sensory retina, thus causing a defect or depigmentation of the RPE.

The acute RPE barrier damage results in increased diffusional permeability of carboxyfluorescein. The size of the RPE defects shown in Figure 3 would also allow other substances present in the serum, some of which are harmful to the sensory retina, to traverse the RPE more readily. Rapid recovery of the RPE barrier may help avoid "intoxication" of the sensory retina, and thus help attain a favorable visual prognosis after reattachment. It is reported that in the cat, RPE proliferation begins about 24 hr after the onset of RD. In the present study, functional recovery was complete in 1–2 weeks.

It is now commonly held that the RPE transports fluid from the retinal to the choroidal surface (absorption). This fluid transport is a mechanism for retinal reattachment and apposition. Since the fluid transport is coupled with the apical electrogenic pump, a high potential difference in subacute and chronic RD implies reactivation of the metabolic fluid absorption. A preliminary in vitro volumetric study has shown that the water transport rate in the monkey RPE-choroid of chronic RD is higher than in the dog and frog (unpublished data). Therefore, RPE proliferation in the course of RD may be a self-healing mechanism for the RD itself.

The present study confirms the efficacy of the monkey experimental RD model, where RPE transport and permeability have been studied in vivo. Transmission electron microscopy with horseradish peroxidase has previously revealed "tight" RPE junc-
tions in the monkey chronic RD. The present physiological and scanning electron microscopic studies indicate that these "tight" junctions are partly newly-formed, and are representative of the whole, not only local, RPE.

It is unknown whether the newly-formed RPE is "normal." Although its histological difference from the normal RPE is striking (Fig. 2), it does act as a diffusion barrier, and has electrical characteristics comparable to other species studied in vitro. Therefore, in terms of barrier function, newly-formed RPE may be virtually normal. However, it should be noted that barrier function is only one part of the activity of the RPE. Furthermore, in the cat RD, morphological recovery of the photoreceptor-RPE complex after reattachment is poor, especially in areas of proliferation and hyperplasia.

Acute RPE damage from separation of the sensory retina has been negligible in the frog, chicken, rabbit, and dog, where removal of the sensory retina in vitro does not cause reduction of the electrical parameters of the RPEchoroid. Histologic studies of rabbit RPE after acute separation of the sensory retina shows only slight changes in the microvilli. However, in the in vitro monkey eye removal of the sensory retina is extremely difficult and the electrical parameters are greatly reduced, suggesting a more complicated photoreceptor-RPE relationship in primates than other animals. Thus, the RPE barrier recovery following RD is important in primates and requires further study.

As a result of recovery, movement of large molecules becomes restricted, while transport of water is regained across the RPE in RD more than 1 week old. This is consistent with the hypothesis that vitreous humor flows into the subretinal space via the retinal hole, resulting in the accumulation of protein in the subretinal space over time. A portion of the protein of serum origin would, however, directly come across the RPE before its recovery.

The damage to the RPE from this primate model of RD is undoubtedly more severe than occurs in spontaneous RD in humans. The RD in monkeys is created in less than 1 minute whereas the RD may evolve over days or weeks in humans. Thus, direct comparison to the clinical setting must be made keeping this difference in mind. Nevertheless, the RPE of primates does show remarkable capacity to regain its physiological function.

Key words: retinal pigment epithelium, retinal detachment, permeability, barrier, cynomolagus monkey

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References


