Photic maculopathy in rhesus monkey
A light and electron microscopic study

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Photic maculopathy was produced in rhesus monkeys after exposure to the light of an indirect ophthalmoscope for one hour. This experimental model provided an opportunity for light and electron microscopic study of a relatively mild photic insult to the macula. During the first week, there was retinal edema with damage to the photoreceptor cells and to the retinal pigment epithelium. Beginning in the second week, an influx of macrophages to the subretinal region was observed. Later the macrophages left the site of injury by way of the choroidal circulation. Depigmented retinal pigment epithelium remained. After the first month, active repair and regeneration took place. A placoid proliferation of the retinal pigment epithelium developed in the center of the lesions. Histologically such proliferation resembled "fibrous metaplasia" of retinal pigment epithelium in man, but electron microscopy showed that the cells retained their epithelial characteristics. Macrophages were still present between the proliferated cells of the retinal pigment epithelium five months after exposure. The photoreceptor cells overlying the depigmented and proliferated retinal pigment epithelium regenerated their outer segments. This study demonstrated that the photoreceptor cells can regenerate their outer segments and provided an explanation for the observation that patients with solar retinitis can show gradual but remarkable recovery.

Key words: photic maculopathy, light, indirect ophthalmoscope, photoreceptor cells, retinal pigment epithelium.

Penner and McNair reported that patients experiencing a solar retinitis after viewing an eclipse showed remarkable recovery, with 27 of 52 eyes regaining visual acuity of 20/20 in six months. Tissue from these patients, however, was not available for pathologic study to document the extent of the initial retinal injury or to explain the gradual but astonishing recovery. Exposure to the light of an indirect ophthalmoscope has been shown capable of producing a photic maculopathy in rhesus monkeys under certain conditions. This experimental model provided pathologic material for the study of the retinal response to photic damage somewhat comparable to that sustained by sun gazers or eclipse watchers, as well as for an investi-
Fig. 1. A rhesus monkey under general and retrobulbar anesthesia was exposed to the light of an indirect ophthalmoscope through a 20 diopter condensing lens. The filament of the bulb of the ophthalmoscope was focused in the fovea (Armed Forces Institute of Pathology [AFIP] Neg. 72-3188-1.)

Aetermination of the capability of the retina for recovery. The purpose of this communication is to present the pathologic and electron microscopic features of this maculopathy. The clinical and clinicopathologic aspects of this entity have been reported elsewhere.

Materials and methods

The maculas of 19 young adult rhesus monkeys were exposed to the continuous light of an indirect ophthalmoscope (American Optical Co.), focused on the retina with a 20 diopter lens for one hour (Fig. 1). The details of the experimental procedure have been described elsewhere. The macular lesions were followed by serial fundus photography. The animals were put to death at five hours (five monkeys), 24 hours (two monkeys), 48 hours (one monkey), one week (one monkey), three weeks (one monkey), four weeks (one monkey), three months (three monkeys), and five months (three monkeys). One animal was still alive and under observation at the time of this report. The enucleated eyes were fixed in either Dalton's chrome oximem for one hour or in four per cent buffered glutaraldehyde and postfixed in Dalton's chrome oximem. The specimens were then dehydrated by ascending concentrations of alcohol and embedded in epoxy resin and sectioned with a glass knife. Sections for light microscopic study were stained with toluidine blue or with paraphenylenediamine. Sections for electron microscopy were treated with uranyl acetate and lead citrate.

Results

The evolution of the pathologic processes in the retina in this photic maculopathy could be conveniently described in three stages: (1) the initial period of injury during the first week; (2) the macrophagic response during the remainder of the first month; and (3) the prolonged stage of repair and recovery of the retina during the next four months. There was considerable overlapping of these stages.

Stage 1. Within 24 hours after exposure, the retina became mildly edematous histologically (Fig. 2), and the retinal pigment epithelium showed mild derangement of its postfixed in Dalton's chrome oximem. The specimens were then dehydrated by ascending concentrations of alcohol and embedded in epoxy resin and sectioned with a glass knife. Sections for light microscopic study were stained with toluidine blue or with paraphenylenediamine. Sections for electron microscopy were treated with uranyl acetate and lead citrate.

lost their regularity (Fig. 3). Electron microscopically, the photoreceptor lamellas...
Fig. 2. Within 24 hours after one hour exposure to the light of an indirect ophthalmoscope, the retina is edematous. There is focal pyknosis of the nuclei of the photoreceptor cells. (Paraphenylenediamine. ×145. AFIP Neg. 72-3188-5.)

Fig. 3. Within 24 hours after the exposure to the light, the photoreceptive elements are irregular and the retinal pigment epithelium shows mild derangement of the pigment granules. Two pigment epithelial cells (arrow) and fragments of degenerated photoreceptive elements are noted in the subretinal space. (Toluidine blue. ×350. AFIP Neg. 72-3188-5.)
of the outer segments were broken down into vesicles and tubules (Fig. 4).

As early as five hours after exposure, occasional cells laden with pigment granules were observed in the subretinal space (Fig. 3). These cells showed marked vacuolation of their cytoplasm, and their nuclei were shrunken and appeared degenerated, but cell junctions between adjacent cells were observed (Fig. 5). Serial sections through such areas showed no evidence that these cells were artificatially detached from Bruch's membrane.

Two to 3 days later, the retina appeared more edematous. Pyknosis of some nuclei in the retinal pigment epithelium and in the outer nuclear layer was more evident.

Stage 2. After the first week, the pathologic picture was dominated mainly by the macrophagic response and the process of depigmentation. Ophthalmoscopically, focal areas of hyperpigmentation of the macula were observed (Fig. 6). Histologically, the hyperpigmentation was attributable to phagocytic cells laden with pigment granules accumulating beneath the degenerated photoreceptor cells (Fig. 7). Electron microscopically, these phagocytic cells had numerous slender cell processes, and they surrounded damaged inner and outer segments of photoreceptor (cells (Fig. 8). Much cellular debris in various stages of degradation was observed within the cytoplasm of these macrophages. Some of the phagocytosed cellular debris appeared to have a lamellated appearance similar to that of the degenerated outer segments of the photoreceptor cells (Fig. 9). Other particles appeared as granular dense bodies in the cytoplasm of the macrophages. The melanin granules within the phagocytic cells appeared in two forms (Fig. 9). Some melanin granules were observed within phagocytic vacuoles together with other cellular debris; other melanin granules appeared to be contained in the cytoplasm with or without a membranous envelope.

The macrophages at times were so numerous that they arranged themselves as a sheet in the subretinal space between the
Fig. 5.  **Top,** Pigment epithelial cells in the subretinal space within 24 hours after exposure. The nuclei of the cells are shrunken, and the cytoplasm is markedly vacuolated. The cells contain melanin granules and phagosomes in the cytoplasm. (Original magnification ×2,700.) **Bottom,** Dense cell junctions (heavy arrow) are noted between these degenerated cells. Noteworthy are melanin granules within phagosomes (double arrow) and melanin granules that apparently lie free in the cytoplasm (single arrow). (Original magnification ×3,500. AFIP Neg. 72-3188-4.)

photoreceptor cells and the retinal pigment epithelium. No cell junctions or basement membranes, however, were observed along these sheets between adjacent macrophages (Fig. 9).

While most of the phagocytic cells were observed in the subretinal space, similar cells laden with pigment granules and cell debris were observed between the retinal pigment epithelium and Bruch’s membrane (Figs. 10 and 11) or in the choroid lying between the uveal melanocytes (Fig. 12). On the other hand, mononuclear cells without pigment granules were seen between the choriocapillaris and Bruch’s membrane as well as between Bruch’s membrane and the retinal pigment epithelium (Fig. 13).

The retinal pigment epithelium became progressively more depigmented (Figs. 14 and 15). The depigmented cells were joined to one another by zonula adherens and zonula occludens as well as by an occasional desmosome. These cells contained mitochondria, a moderate amount of smooth- and rough-surfaced endoplasmic reticulum, and lamellated membranous
Fig. 6. Two weeks after exposure, the macula shows irregular pigmentation. (AFIP Neg. 72-3188-2.)

bodies. Most had lost the basal infoldings of their plasma membranes, and the plasma membrane and the basement membrane were in approximation (Fig. 14). Conversely, the basal plasma membrane of other depigmented cells of the retinal pigment epithelium was slightly raised from Bruch’s membrane and had produced a new basement membrane superimposed on the persistent original basement membrane along the inner surface of Bruch’s membrane (Fig. 15).

Stage 3. After the first month, active repair and recovery became apparent. Histologically, proliferation of the retinal pigment epithelium was observed as early as two weeks after the injury (Fig. 16). In the early phase a plaque of irregularly pigmented cells with no definite layering arrangement was observed between Bruch’s membrane and the photoreceptor cells. The latter showed extensive loss of inner and outer segments. No interruption of Bruch’s membrane was observed, nor was there any proliferation of blood vessels into the subretinal area.

Three to five months after the injury a plaque consisting of spindle-shaped cells arranged in layers was noted (Fig. 17). By electron microscopy, two types of cells were identified in this plaque. Some of the spindle-shaped cells were arranged in sheets and were identified as modified cells of the retinal pigment epithelium (Fig. 18). The adjacent cells were joined together by cell junctions consisting mostly of desmosomes (Fig. 19). Some of the cells contained melanin, but others were devoid of pigment granules. The normal arrangement of the cells of the retinal pigment epithelium into an apical and a basal portion was not present. Instead, basement membrane material lined the inner and outer surface of each sheet of cells. The cells in the center of the plaque tended to produce basement membrane that was thicker and more multilaminar. The cells that were in approximation with Bruch’s membrane produced an additional thin basement membrane on top of the persistent continuous basement membrane in the inner side of Bruch’s membrane.

On the surface of the plaque, a layer of cuboidal epithelial cells had formed (Figs. 17 and 18). These cells, in contrast to those in the center of the plaque, became more completely differentiated, forming an apical and a basal region. In the apex, numerous villi were in contact with the outer segments of the photoreceptor cells. Zonulae adherens and occludens joined adjacent cells. Pigment granules were randomly distributed in the cells. The basal region of the cells showed numerous infoldings of the basement membrane. The basement region of the cells showed numerous infoldings of the plasma membrane. The basement membrane was present only at the base of the cell and showed irregular infolding following the contour of the basal plasma membrane.

The second cell type in the plaque consisted of macrophages (Fig. 18). They were so identified because they lacked basement membranes and cell junctions but had slender cell processes and phagosomes with engulfed cellular debris.

In between the cells, filamentous material was observed embedded in a matrix of ground substance (Fig. 19). Some had a periodicity of 500 to 600 Å.
Fig. 7. Pigment-laden macrophages (arrows) clustering around outer segments of the photoreceptor cells. The retinal pigment epithelium is partially depigmented. (Paraphenylendiamine. ×440. AFIP Neg. 72-3188-2.)

Fig. 8. A macrophage (M) extends fine cell processes surrounding inner segments (IS) and outer segments (OS) of photoreceptor cells. PE, pigment epithelium. (Original magnification ×7,200. AFIP Neg. 72-3188-7.)
basement membranes, namely the basement membrane of the original but now missing retinal pigment epithelium and a new basement membrane produced by the regenerated retinal pigment epithelium. On the side of the choriocapillaris two basement membranes could also be observed in places. One belonged to the old choriocapillaris, which had disappeared, and one belonged to the new choriocapillaris, which had produced a new basement membrane.

The photoreceptor elements overlying the plaque appeared somewhat irregularly aligned (Fig. 17). Some of the inner segments of the photoreceptor cells developed enormous calyceal processes around the outer segments (Figs. 21 and 22). Within these calyceal processes mitochondria and microtubules were observed. At the junction of the outer and inner segments, the cilia appeared unremarkable (Fig. 22), but the proximal end of the outer segments consisted predominantly of tubules and vesicles (Fig. 21) and were sometimes irregularly arranged (Fig. 22). Fragments of lamellated material compatible with the outer segments of photoreceptor cells were also observed within the inner segments of photoreceptor cells.

Discussion

Exposure of the maculas of rhesus monkeys to the light of an indirect opthalmoscope for one hour produced retinal damage that led to the development of a distinct photic maculopathy. With this experimental model a pathologic study of photic maculopathy in its several stages of development was undertaken by light and
Fig. 10. Portion of a macrophage (M) containing cellular debris is located external to the cell junction (heavy arrow) of the retinal pigment epithelium but internal to Bruch's membrane. Other macrophages (Mₙ) are present in the subretinal space. The retinal pigment epithelium (PE) is largely depigmented and has produced a new basement membrane (double arrow) internal to the continuous original basement membrane (single arrow) lining the inner side of Bruch's membrane. The macrophages do not have a basement membrane. (Original magnification x9,000. AFIP Neg. 72-3188-12.)

Fig. 11. A macrophage (M) containing phagocytosed pigmented granules and cellular debris is located in the subretinal pigment epithelial region. PE, retinal pigment epithelium; BM, Bruch's membrane. (Original magnification x8,300. AFIP Neg. 72-3188-12.)

Fig. 12. A macrophage (M) containing cellular debris is present in the choroid between uveal melanocytes (U). (Original magnification x2,800. AFIP Neg. 72-3188-12.)

Fig. 13. Two mononuclear cells (M), one on each side of Bruch's membrane (BM) are noted. Noteworthy is the fact that both cells do not have pigment granules in their cytoplasm. PE, pigment epithelium; C, choriocapillaris. (Original magnification x5,700. AFIP Neg. 72-3188-12.)
Fig. 14. A depigmented cell of the retinal pigment epithelium (PE). The basal plasma membrane of this cell has few infoldings and is resting on Bruch's membrane (BM). In the cytoplasm there are abundant mitochondria, ribosomes, and endoplasmic reticulum. (Original magnification ×6,300. AFIP Neg. 72-3188-6.)

Fig. 15. A depigmented cell of the retinal pigment epithelium (PE) that has produced a new basement membrane (double arrows) in addition to the persistent continuous basement membrane (single arrow) on the inner surface of Bruch's membrane (BM). Dense lamellated bodies (L) in the cytoplasm of the cell resemble the degenerated outer segments of the photoreceptor cells (OS). A desmosome (heavy arrow) joins adjacent pigment epithelial cells. (Original magnification ×4,200. AFIP Neg. 72-3188-6.)
electron microscopy. An opportunity was provided to study the response of the retina to this mild photic insult and its capability for recovery.

In the acute stage, the inner and outer segments of the photoreceptor cells were disrupted. The photoreceptive lamellae were broken down into vesicles and tubules. The breakdown of the photoreceptive lamellae in this manner is not unique. A similar process has been observed in a variety of conditions ranging from experimental detachment of the retina in rhesus monkeys to the physiologic breakdown of the outer segments of photoreceptor cells in the pineal organ of frogs. This breakdown of photoreceptive lamellae, however, is in sharp contrast to that observed when the outer segments are damaged by the high intensities of the ruby laser or by the Zeiss photocoagulator. The focal densities observed along the photoreceptive lamellae in the latter conditions were not seen in this experimental model.

In the second stage of the photic maculopathy, macrophages dominated the pathologic picture. The cellular debris from the necrotic retinal pigment epithelium and damaged photoreceptor cells was removed by macrophages. Therefore, degenerated materials suggestive of outer segments of photoreceptor cells and melanin granules were found in various stages of degradation within the cytoplasm of the macrophages. This was in contrast to the observation of others made in the study of mild photic damage to the retina of rats, in which case the damaged cells had been removed from the area by "some quick and mysterious process."

The route of exit of these macrophages from this area has been a subject of controversy. In our experiment, however, macrophages laden with pigment granules were observed in the subretinal space beneath retinal pigment epithelium and in the choroid. These findings were interpreted as evidence that the macrophages left the area of injury by way of the choroid.

The origin of these macrophages has also...
been a subject of dispute. Mononuclear cells without pigment were observed between Bruch's membrane and the retinal pigment epithelium, as well as external to Bruch's membrane. These cells were interpreted as derived from the bloodstream in the choriocapillaris, migrating toward the site of injury. They were so interpreted be-
cause they had no cytoplasmic melanin granules. The possibility that the retinal pigment epithelium may be one of the sources of these macrophages could not be completely ruled out. Some of the melanin granules within these macrophages appear to be contained within phagoctytic vacuoles. Others appeared to be native to the cell, lying freely or enclosed in an envelope in the cytoplasm. Evidence of epithelial characteristics in the macrophages, however, could not be found. In contrast, epithelial characteristics such as cell junctions were observed in degenerative pigment epithelial cells in the subretinal space during the acute stage.

With increasing macrophagic response, the retinal pigment epithelium in the region of injury became progressively depigmented. Despite the loss of melanin granules, the cells of the retinal pigment epithelium appeared to be active cells. Some of them had produced an additional new basement membrane on top of the persistent continuous basement membrane along the inner side of Bruch’s membrane. It is possible that these cells of the retinal pigment epithelium were new cells that had proliferated to replace those that had become necrotic. Other depigmented cells of the retinal pigment epithelium, however, seemed to rest on the original basement membrane along the inner surface of Bruch’s membrane, but they did not have the normal infoldings of the basal plasma membrane. There is, however, no other evidence to show that these were newly proliferated cells. It is possible that some of the retinal pigment epithelium survives the insult but responds to the mild injury by losing some of its melanin granules. Pigmentless filamentous organelles (referred to as pigmentless melanin granules of Stage I or II by Moyers\textsuperscript{11} and as premelanosomes by Fitzpatrick and associates\textsuperscript{12} and by Mund and associates\textsuperscript{13}) were not observed in the retinal pigment epithelium throughout this study.

The placoid proliferation of the retinal pigment epithelium observed in the center of the lesion appeared to be like that described as “fibrous metaplasia” in man.\textsuperscript{14, 15} The spindle-shaped cells in the plaque were identified as modified retinal pigment epithelium because these irregularly pigmented cells were surrounded by basement membrane and adjacent cells were joined by cell junctions. Thus it is believed that even though these cells had lost their cuboidal form, they retained other epithelial characteristics and had not been transformed completely into fibroblastic cells, as implied by the term “fibrous metaplasia.” On the other hand, filamentous material identical to collagen was seen between these cells. Since there was total absence of invasion of blood vessels in the subretinal region, we believe that this collagen was produced by the retinal pigment epithelium, probably in conjunction with the production of basement membrane. This is not a unique example of production of collagen by cells of neuroepithelial origin. Collagen filaments in the vitreous body are also believed to be produced by the nonpigmented ciliary epithelium, which is also of neuroepithelial origin. In a recent study of proliferation of retinal pigment epithelium over malignant choroidal tumors in man,\textsuperscript{16} plaques of proliferated cells were considered as exhibiting “fibrous metaplasia” histologically; electron microscopic study, however, showed that these cells retained some of their epithelial characteristics but with the deposition of collagen in between the cells in the plaque.

The morphologic differences between the retinal pigment epithelium on the top of the plaque from those in the center of the plaque are striking. The former were cuboidal cells with a basement membrane at the base and numerous villi at the apex. These cells also develop zonulae adherens and occludens joining adjacent cells in a single plane. The basement membrane of these cells, however, showed marked infoldings following the contour of the basal plasma membrane. The normal retinal pigment epithelium has a straight basement membrane in contact with the collagenous
Fig. 18, for legend see opposite page.
Fig. 19. Adjacent cells in one sheet of proliferated retinal pigment epithelium are joined by cell junctions (heavy arrows). Filamentous (thin arrow) materials are present between the sheets of cells. (×16,000. AFIP Neg. 72-3188-10.) *Inset,* Filamentous material (arrow) with cross-striations of 500 to 600 Å periodicity is embedded in a ground substance between the sheets of proliferated retinal pigment epithelium. (×28,000. AFIP Neg. 72-3188-10.)

portion of Bruch’s membrane, and the infolding involves only the basal plasma membrane. We speculate that the differentiation of the cells lining the top of the plaque must have been influenced by the proximity of the overlying retina.

Within the plaque, scattered macrophages were observed five months after the injury. It seemed that these macrophages had been trapped within the plaque and the subretinal space for a long time without being able to reach the choroid. These

Fig. 18. A, Cells (PE) on top of a placoid proliferation of retinal pigment epithelium are cuboidal and are joined to adjacent cells with zonulae adherens and occludens (heavy arrow). They are polarized into apex and base. In the apex, villi are in contact with outer segments of photoreceptor cells. The basal plasma membranes of the cells are infolded. The basement membrane (thin arrow) shows numerous infoldings following the contour of the basal plasma membrane. The cells within the placoid proliferation consist of modified retinal pigment epithelial cells (PE), which are surrounded by basement membrane and macrophages (M), which do not have a basement membrane. (×9,000. AFIP Neg. 72-3188-9). B, Portion of a plaque of proliferated pigment epithelium on Bruch’s membrane (BM). The modified cells of the retinal pigment epithelium (PE) are irregularly pigmented and are surrounded by basement membrane. In the center of the plaque the cells produce thick and multilaminated basement membrane (heavy arrows), while the basement membrane (thin arrows) produced by the cells in approximation to Bruch’s membrane is thin and single layered and is located internal to the original continuous basement membrane on the inner side of Bruch’s membrane. In contrast, the macrophages (M) are irregularly pigmented and do not have a basement membrane. They have slender cell processes and phagosomes in their cytoplasm. (×6,600. AFIP Neg. 72-3188-9.)
Fig. 20. Unusual arrangement of basement membranes of Bruch's membrane (BM). Two basement membranes are found on each side of Bruch's membrane. The retinal pigment epithelium (PE) has produced a new basement membrane (single heavy arrow) on top of the original basement membrane (double heavy arrows) on the inner surface of Bruch's membrane. Endothelial cells of the choriocapillaris (C) have also produced a new basement membrane (single thin arrow) in addition to the original basement membrane (double thin arrows) on the outer surface of Bruch's membrane. Macrophages (M) do not produce a basement membrane. (×3,000. AFIP Neg. 72-3188-20.)

Macrophages partially explain the persistent irregular pigmentation of the fundus for long periods after injury.

Three to 5 months after the initial insult, the photoreceptor elements have reappeared over the depigmented retinal pigment epithelial cells as well as over the plaque of proliferated retinal pigment epithelium. The outer segments were somewhat irregularly aligned. These photoreceptor cells, however, had large calyceal processes from the inner segments within which many mitochondria and microtubules were found, suggesting that these cells were unusually active in metabolism. The proximal end of
the outer segments showed numerous tubules and vesicles. The reappearance of the photoreceptor elements after initial damage and removal of photoreceptor cells seems to provide evidence that the photoreceptor cells can regenerate their photoreceptor elements. This seems to give an explanation for the gradual recovery of vision by patients who have had a solar retinitis.

REFERENCES