Degenerative Changes in a Retina Affected with Autosomal Dominant Retinitis Pigmentosa

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The eyes of a 17-year-old male donor who was affected with autosomal dominant retinitis pigmentosa with variable expressivity have recently become available for study. Initial macroscopic examination of the fundus revealed bone spicules located in 180° of the postequatorial fundus centered on the inferonasal quadrant. Light microscopic examination of the retina showed degeneration within each quadrant characterized by an absence of rods and cones in the equatorial areas, and the presence of photoreceptors in the more peripheral and central retina. Ultrastructural examination disclosed photoreceptors that were abnormal in all regions when compared to a control eye from a 26-year-old donor. Intact rods were restricted to the peripheral quadrants, and intact cones were identified in the fovea and far periphery. In areas of intermediate degeneration, many outer segments were either shortened and disorganized or absent. Regions of severe degeneration were characterized by the complete loss of the photoreceptors and apposition of the external limiting membrane to the retinal pigment epithelium. The density of rods and cones was found to be substantially lower than normal in all regions. In areas of relatively intact photoreceptor outer segments, we found ultrastructural evidence of recent phagocytic activity, and fluorescence microscopy revealed no unusual accumulation of lipofuscin within the pigment epithelium or subepithelial debris. The choroid and inner retina were normal throughout the eye. The normal condition of the choroid, retinal pigment epithelium, and inner retina implies that the primary disorder resides within the photoreceptor cell.

Retinitis Pigmentosa (RP) is the name given to a group of inherited disorders which give rise to degeneration of the outer layers of the retina. RP is characterized by night blindness, and progressive loss of the peripheral visual field. The typical clinical findings are pigmentary retinopathy, visual field loss beginning at 20° of eccentricity, elevated dark-adapted threshold and reduction of the electroretinogram in the early stages of the disease. Little is known of the pathogenesis of these retinal dystrophies. The amount of ultrastructural information regarding the progression of the degeneration in the photoreceptor layer is limited since the majority of the retinas acquired to date have been from donors with advanced disease, and were often not preserved within the brief period necessary to avoid major changes due to autoysis. These limitations are illustrated by the current literature, which contains only 14 ultrastructural studies of retinitis pigmentosa donor eyes, of which just four have been cases of autosomal dominant RP. These examples of dominant disease were obtained from donors who were over 50 years of age, and had end-stage disease in which the majority of the photoreceptors were either absent or severely degenerate.

In a previous publication, we reported the development of microtechniques for the morphological and biochemical analysis of loci within human retina. Those techniques were used to establish baseline maps of the distribution of rod and cone photoreceptors as well as the levels of cyclic GMP, cyclic AMP and protein in the normal retina. Maps of the distribution of rods and cones and the location of surviving photoreceptors in one of the two retinas of RP donor eyes can be used to make morphological and biochemical correlations with the other retina, since...
the pattern and progression of the disease is usually remarkably symmetrical between the two eyes of affected individuals.8

In this study, we compare the histopathology and distribution maps of photoreceptors in the retina of a 17-year-old donor affected with autosomal dominant retinitis pigmentosa with those of normal donors in the same age range. This is necessary due to the reduction in the foveolar cone number with age, as recently reported by Gartner and Henkind,9 and Marshall and Laties.10 The fellow eye of the RP donor was frozen and analyzed biochemically; these results and immunocytochemical data from the fixed eye will be presented in subsequent reports.

Materials and Methods

The donor was a member of a family in which retinitis pigmentosa was inherited in an autosomal dominant fashion with variable expressivity (also termed reduced penetrance); no consanguinity was recorded in the family (Fig. 1). The donor's paternal great-great-grandfather, (I/I), was known to have had "moon blindness" and was blind in late life. Six additional relatives were known to have been affected by retinitis pigmentosa (III/1, 5, 7, 8; IV/3, 6). All were night blind in early life, some were severely handicapped in late life and with the exception of (I/I), all patients had been diagnosed as having retinitis pigmentosa by an ophthalmologist. Ophthalmic examination has been undertaken on four relatives. One uncle (IV/6) was examined at the age of 27 years, at which time visual acuity with each eye was normal, the fundi showed the typical changes of retinitis pigmentosa, and dark adaptation testing showed a 2 log unit elevation of the final threshold. The electro-oculogram (EOG) light-induced rise in ocular potential was reduced (OD 140%, OS 120%), and the electroretinogram (ERG) was not recordable. The father (IV/8), another uncle (IV/7), and the patient's sister (V/8) had normal final thresholds on dark adaptation, normal ERGs, and in none was any fundus abnormality identified.

The donor was first examined at the age of 15 years at which time there was symptomatic loss of night vision. Visual acuity at that time was normal, but the fundi had the typical appearance of retinitis pigmentosa. Dark adaptometry was tested with a Goldmann-Weekers adaptometer using a large target, and no fixation strategy. A normal threshold was reached in the central field after 30 min with a normal cone/rod break. The scotopic and photopic responses were
just recordable on ERG testing. Visual fields were not available on this patient. These results were considered to confirm the diagnosis of retinitis pigmentosa.

The RP donor died as a result of injuries incurred in a motorcycle accident, and was on a respirator for a short period before death. The donor had no chemotherapy or other factors known to compromise the ultrastructure of the retina. Normal eyes from a 12-year-old female and 26-year-old male donor were used as controls. All donor eyes were enucleated within 1 hr of death, each right eye (OD) was opened through the pars-plana and immediately fixed by immersion in a solution of 2.5% glutaraldehyde and 2% formaldeyde in 0.1 M Na phosphate buffer at pH 7.2. The left eye (OS) was immediately frozen and kept at −80°C for biochemical analysis.

**Tissue Processing**

The anterior segments of the aldehyde-fixed eyes were removed and the eyecups were cut into temporal and nasal calottes through the optic nerve, and subdivided into four quadrants (Fig. 2). Quadrants were divided into five wedges which were then cut into central, equatorial and peripheral regions resulting in a total of 61 blocks. In order to keep the macula complete in one quadrant, the temporal calotte was divided asymmetrically, including the macula within the superior quadrant. Tissue fragments were transferred to a storage solution of 2% formaldehyde in 0.1% Na phosphate buffer, pH 7.4 for 5 days at 40°C, to increase antigenicity. Blocks were divided into three groups before further processing. The group for ultrastructural examination was post-fixed in 4% OsO₄, dehydrated in ethanol, infiltrated in propylene oxide, and embedded in Araldite 502 epoxy resin (Ciba Products Co., Summit, NJ). The two remaining groups were processed for immunocytochemistry by dehydration in a graded series of dimethyl formamide or ethanol without post-fixation. The dimethyl formamide-treated group was embedded in Lowicryl K4M (Chemische Werke Lowi, Waldkraberg, West Germany) methacrylate resin. The ethanol-dehydrated group was embedded in LR White methacrylate resin (Polysciences, Inc., Warrington, PA).

**Nomenclature**

We used the nomenclature of Duke-Elder for the primary regional division of the retina. The central retina was subdivided into three regions according to Polyak, and Hogan et al. The region of retina devoid of ganglion cells is termed the foveola and is ~0.35 mm in diameter. The fovea is ~1.85 mm in diameter. The outer margin of the fovea is the point of greatest retinal thickness. Parafovea is the central retina with greater than five rows of cells in the ganglion cell layer; it extends to ~1.4 mm from the center of the foveola. Perifovea is the most peripheral part of the central region, in which there are two to four rows of ganglion cells; it extends to ~2.9 mm from the center of the foveola.

The equatorial region was defined as a belt ~8 mm wide. This was subdivided into post-equatorial, just peripheral to the central region, equatorial, and pre-equatorial—which separates the equatorial and peripheral regions. The peripheral region is the area anterior to the pre-equatorial region, extending to the ora serrata. The near periphery is ~1.5 mm across and most posterior, the mid-periphery is ~3 mm across, and the far-periphery is asymmetrical, being 9 to 10 mm across on the temporal side and 16 mm on the nasal side in the horizontal meridian.

**Light and Electron Microscopy**

One-micron sections from regions embedded in Araldite were stained with Toluidine Blue for light
microscopy. Photomicrographs were taken at a magnification of ×252. Thin sections (400 Å) for EM were stained with uranyl acetate and lead citrate and examined with a JEOL 100-C electron microscope. Fluorescence microscopy was performed on 1 μm sections of LR White-embedded retina with a Zeiss Photomicroscope III (Carl Zeiss GMBH, Oberkochen, West Germany) equipped epifluorescence condenser III RS, excitation filter UG 51 and BG 3, and barrier filter LP 478.

Morphometric Data

**Photoreceptors:** Counts of rods and cones were made from 1 μm sections using a calibrated measuring reticule. Receptor counts were independently confirmed by a second investigator (ACB). The number of rods and cones were counted in 18 different regions, representing each quadrant from the foveola to the ora serrata in both the 17-year-old RP eye and a normal 26-year-old eye. Rods and cones were identified on the basis of inner segment morphology. The criterion for inclusion in the tabulation was the presence of a recognizable inner segment. Photoreceptors with varying degrees of outer segment disorganization ranging from well-organized to nonexistent were tabulated equally in the RP retina. Numerical data were processed with a VAX 11/780 computer (Digital Equipment Corp., Maynard, MA) and Statistical Analysis System (SAS) software. Retinal locations are represented by a Cartesian coordinate system. The X and Y axes represent distances from the geometric center of the retina in the horizontal and vertical diameters respectively. The height along the Z axis describes the photoreceptor density at a specific retinal location. A smooth representation of the retinal surface was produced by interpolating between the data obtained from the tabulation of photoreceptors.15,16

**Optic nerve:** The number of myelinated axons per unit area in the orbital portion of the optic nerve was estimated. Axons were identified on light micrographs, and the areas measured using a digital planimeter (Laboratory Computer Systems, Cambridge, MA). The density of axons in the posterior part of the lamina cribrosa was derived from these measurements.

Results

**Gross Morphology**

The cornea, anterior chamber, ciliary body, and sclera of the RP donor eye all appeared normal. There were no indications of cataract. Gross examination revealed that the macula was intact, and there was no sign of retinal detachment. The equatorial fundus was the most affected region, in which there was loss of pigment at the level of the RPE. Long-standing disease, indicated by the presence of bone spicule pigmentation in the inner retina, was observed in 180° of the equatorial fundus, predominantly in the inferonasal quadrant. No retinal pigmentation was observed in the superior temporal quadrant (Fig. 3a, b). The retinal blood vessels supplying the equatorial retina appeared narrow but those supplying the posterior pole were of normal caliber. No evidence of closure of the choriocapillaris was identified, and the optic disc appeared normal.

**Light and Electron Microscopy**

Examination of the retina by light microscopy revealed the degeneration to be greatest in the equatorial region, and least in the foveola and far periphery. In the fovea, numerous photoreceptors with intact, organized but shortened outer segments were found (Fig. 4A). Ultrastructural examination revealed that
The foveola of the RP donor. Foveolar cones are well-organized structurally, but have a slightly reduced packing density (A) (x630). The outer segments of foveal cones are well-organized. Vesiculation observed at the base of the outer segments (arrow) is most probably due to postmortem autolytic changes, not as a result of the inherited degeneration (B) (x2300). High power view of vesiculation at the base of foveal cones (C) (x9100).
outer segments of the photoreceptors retained an orderly stacking of the discs (Fig. 4B), but they were shortened, and less densely packed than in the control. The nuclei of the photoreceptors were normal in appearance. Very few inclusion bodies or autophagic vacuoles were noticed within the photoreceptors. An ultrastructural abnormality noted was the presence of vesicles in place of the most basal discs, and swollen inner segment mitochondria.

Outside the fovea, an increasing proportion of the photoreceptor cells were severely altered, with morphologic changes in both the inner and outer segments. The parafoveal region (Fig. 5) was characterized by disordered, shortened outer segments throughout. The number of photoreceptors possessing recognizable outer segments rapidly decreased in the more peripheral region of the perifovea, culminating in an architecture nearly devoid of both rod and cone outer segments in the post-equatorial area (Fig. 6).

In the transition from the post-equatorial to the equatorial retina (Figs. 7-10A), all photoreceptor outer segments were lost and the inner segments became increasingly abnormal in appearance. The subretinal space also became progressively narrowed in this region. This degenerative pattern culminated in a complete absence of photoreceptors within the equatorial region (Fig. 10B). Within the lateral expanse of several hundred microns, the subretinal space was constricted to the point of direct apposition of the outer limiting membrane to the apical membrane of the retinal pigment epithelium (Fig. 10B). The most extensive domain of complete photoreceptor loss was observed in the equatorial region of the nasal hemisphere.

The pre-equatorial retina was characterized by the reappearance of photoreceptor outer segments and the reestablishment of the normal separation of inner retina from the apical membrane of the RPE. The far periphery throughout the RP donor retina sustained less degenerative changes in retinal morphology than other regions (with the exception of the foveola) and retained substantial numbers of photoreceptors which had well-organized outer segments (Fig. 11).

The pattern of degeneration was not found to be radially symmetric throughout the retina. The superior nasal and inferior temporal quadrants were comparable in terms of the magnitude of degenerative change to the photoreceptors in both the central and equatorial regions. Photoreceptors in these regions were less severely disorganized than cells located in corresponding regions of the inferonasal quadrant. In much of the superior nasal and inferior temporal quadrants, the subretinal space persisted, and numerous truncated outer segments were retained. Small loci within the equatorial regions of these two quadrants also contained structurally intact photoreceptor inner segments. The equatorial region of the inferior temporal retina contained the greatest morphologic variability of any region, with small areas (<1 mm in diameter) of severe degeneration devoid of outer segments interspersed with regions of intermediate outer segment disorganization. This quadrant sustained the greatest reduction in density of photoreceptor cells in the far periphery.

The superior temporal retina was demonstrably the least affected quadrant, possessing the narrowest lateral expanse of pre-equatorial and equatorial retina devoid of intact photoreceptors of any quadrant, and retaining many well-organized outer segments in both the post-equatorial and peripheral retina. The inferior nasal quadrant contained the most widespread severe degeneration of any quadrant, with a lateral expanse of over one centimeter with complete photoreceptor loss, which extended from the perifovea to the near periphery.

A single layer of RPE cells was observed overlying the neurosensory retina, and no region was devoid of RPE. In the most affected post-equatorial tract of the inferior nasal quadrant, there were several loci in which multiple layers of RPE were observed, probably as a result of RPE migration or proliferation. Discrete, isolated, pigment-containing cells were also found within the outer plexiform layer of the equatorial region of the nasal quadrants. These pigmented cells have the appearance of either macrophages or RPE cells which had disengaged from Bruch's membrane. In addition, several equatorial loci within all of the quadrants bore RPE cells with a "heaped" appearance, distinguished by the appearance of dual layers of RPE cells supplanting the usual monolayer epithelial organization (Fig. 12).

Light microscopic examination of the choriocapillaris disclosed it to be normal in all areas. Ultrastructural examination revealed a normal complement of endothelial fenestrations throughout the choriocapillaris. The inner neurosensory retina appeared normal throughout the eye, with the exception of the sporadic presence of pigment-containing cells.

Photoreceptor Morphometry

In the normal human retina, the packing density of rods increases gradually across each quadrant when measured in the posterior direction from the ora serrata to the perifovea. Exemplified by the 26-year-old donor (Fig. 13A), the normal density profile of rods is asymmetrical, reaching a maximum in the perifovea of the inferior temporal quadrant. Additionally, representation of the rod density as a surface yields a
Fig. 5. In the parafoveal region of the RP donor retina, the outer segments of both cone and rod photoreceptors are markedly shorter and less organized than in the foveal region. Henle's fiber layer can be observed in the lower third of the figure (x1350).
Fig. 6. The photoreceptor layer within the perifoveal retina is characterized by the absence of well-ordered outer segments. The remaining outer segments (OS) observed are severely attenuated. The retinal pigment epithelium in this region has assumed a scalloped appearance along its apical surface. A small number of autophagic vacuoles (arrow) are observed in the inner segments of the photoreceptors (×3150).
Fig. 7. The post-equatorial retina includes regions in which the subretinal space is reduced, leaving the remaining photoreceptor inner segments in direct apposition to the apical membrane of the RPE. The last vestiges of two photoreceptor outer segments are observed (arrows) (x3330).
Fig. 8. Müller cells in the post-equatorial retina have retained their structural integrity to a greater extent than the neighboring photoreceptor cells. The undamaged microvilli of a Müller cell extend beyond the external limiting membrane, immediately adjacent to a photoreceptor inner segment (IS) in the terminal stages of degeneration. Intact zonula adherentes are seen (arrows) joining the Müller cells and the photoreceptor inner segments (×9350).
Fig. 9. Amoeboid cells (m) are occasionally observed between the photoreceptor inner segments and the apical surface of the RPE in the pre-equatorial retina. These cells have the appearance of macrophages, with a prominent nucleus and a large number of vesicles within their cytoplasm (X6950).

sharp concavity slightly inferior and temporal to the geometric center, illustrating the abrupt decrease in rod density (to zero) which occurs in the foveola. The tabulation of rod and cone densities in the RP donor is based upon rod or cone photoreceptor inner segments; photoreceptor cells with either intact, disrupted or absent outer segments possess equal weight in the plots. Therefore, the plots represent only the absolute loss of entire photoreceptor cells, and not necessarily the magnitude of degenerative change to the retina in an area.

The rod distribution pattern observed in the RP donor, (Fig. 13B) contrasts sharply with that seen in the normal retina. No area in this RP donor retina retained a full, normal complement of photoreceptors, in terms of either number or ultrastructure. The magnitude of degenerative change in the RP retina is borne out by the minimal differences in rod density observed between the foveola, (normally devoid of rods) and the central and equatorial areas. This is due to the nearly total loss of rod photoreceptors in the equatorial region. The proportion of rod and cone cell loss is attested to by the differences in absolute numbers on the Z axis between the RP and normal donor retinas (Fig. 13A, B).

The far periphery of the RP retina was comparable to the fovea in terms of preservation of receptor structure, however, despite significant loss of rod cell density in both regions. If the regions are contrasted, rod photoreceptor loss in the peripheral retina was less prominent in the superior hemisphere (~40,000/mm² in ST, ~27,000/mm² in SN), than in the inferior hemisphere (~20,000/mm² in IN, <1500/mm² in IT). The rod densities that we estimated represent decrements in the outermost periphery of 99% in IT, 89% in IN, 81% in SN, and 62% in
The post-equatorial retina contains transition zones in which the photoreceptor inner segments are lost and the subretinal space is occluded (A) (×3000). In the equatorial region of the RP donor, the apical pigment epithelium is directly in contact with the external limiting membrane; the entire photoreceptor layer is degenerate (B). In some areas the RPE cells have a "heaped" appearance, possibly due to RPE cell migration or proliferation. The neural retina is characterized by gliosis. Pigment-containing cells (arrows) are observed in the neural retina. These pigmented cells are distinguished from the "bone spicules" observed by gross or clinical examination by their location in the retina, bone spicules existing exclusively near the retinal veins (×3350).
Fig. 11. Many intact rods and cones with long outer segments are observed in the far peripheral retina, within several millimeters of the ora serrata (×9600).
Fig. 12. Changes in the RPE suggesting migration or proliferation were observed in a few limited areas of the equatorial retina extending into the neural retina (×2085).

ST compared to the values measured in the 26-year-old normal (Table 1). Overall, the superior temporal retina was spared the most grave reduction in rod photoreceptor density.

The density of foveal cones in the RP retina (Fig. 14B) was reduced from the normal (Fig. 14A) by approximately 25%. Cones in the far periphery sustained density changes which were generally less than that of rods, with drastic reductions (>99%) occurring only in the superior temporal quadrant. In the equatorial regions, however, the complete ablation of photoreceptors also applied to cones.

Optic Nerve Morphometry

We examined several cross sections representing an area greater than 5 mm² through the external part of the lamina cribrosa in the orbital portion of the optic nerve of the RP donor and normal donor eyes. Analysis of this region is the most sensitive morphologic index of the loss of ganglion cells from the retina. The axons become myelinated in this region, and both oligodendrocytes and astrocytes were observed in the electron microscope. We were unable to identify any degenerating ganglion cell axons in these sections. The optic nerve of the RP donor eye contained a mean density of 130 axons/mm², SD = 44 in this region which was lower than that of the normal donor (mean = 249 axons/mm², SD = 55), but the difference did not reach α = 0.025 level of significance.

Fluorescence Microscopy

Fluorescence microscopy revealed only small amounts of lipofuscin in the pigment epithelium within various regions of the retina. Figure 15A is from the equatorial region of the superior nasal quadrant, a region in which the photoreceptor layer has completely disappeared. Fluorescence microscopy of a 12-year-old normal donor eye (Fig. 15B) demon-
Fig. 13. Rods per mm² shown on a Cartesian coordinate system in the 17-year-old RP donor eye (B) and in the normal donor retina (A). There is a marked reduction in the number of rods throughout the central and equatorial region in all four quadrants of the RP fundus. Regional differences are evident in the peripheral retina, with the most severe decrement occurring in the inferior temporal quadrant (lower left). The largest number of intact photoreceptors remains in the superior temporal quadrant (upper left).

...strated an equivalent amount of lipofuscin in the RPE. Fluorescence in the RP donor retina was observed emanating from two sources, melanolysosomes of the apical retinal pigment epithelium, and pigment granules within the macrophages and bone spicules of the inner retina.

Discussion

The donor's family pedigree (Fig. 1) leaves little doubt as to the autosomal dominant inheritance, with diseased individuals in three successive generations, and transmission from father to son. 17 Autosoma-
Table 1. Photoreceptor distribution in 26-year-old normal and 17-year-old RP retina. Estimated number of rods and cones per mm², ± 1 standard error of the mean (areas devoid of a photoreceptor layer do not have standard error)

<table>
<thead>
<tr>
<th>Retinal location</th>
<th>Normal rods</th>
<th>RP rods</th>
<th>Normal cones</th>
<th>RP cones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macula</td>
<td>0</td>
<td>0</td>
<td>117,551 ± 10,849</td>
<td>88,293 ± 8149</td>
</tr>
<tr>
<td>st1</td>
<td>92,125 ± 5776</td>
<td>0</td>
<td>3265 ± 291</td>
<td>0</td>
</tr>
<tr>
<td>st2</td>
<td>92,125 ± 16,382</td>
<td>0</td>
<td>4702 ± 419</td>
<td>0</td>
</tr>
<tr>
<td>st3</td>
<td>108,844 ± 7510</td>
<td>2644 ± 182</td>
<td>3265 ± 133</td>
<td>188 ± 8</td>
</tr>
<tr>
<td>st4</td>
<td>67,929 ± 22,273</td>
<td>37,746 ± 12,276</td>
<td>4702 ± 1128</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>sn1</td>
<td>162,003 ± 18,322</td>
<td>0</td>
<td>9437 ± 598</td>
<td>3951 ± 250</td>
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<tr>
<td>sn2</td>
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<td>0</td>
<td>10,580 ± 1828</td>
<td>0</td>
</tr>
<tr>
<td>sn3</td>
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<td>0</td>
<td>7347 ± 859</td>
<td>39 ± 5</td>
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<tr>
<td>sn4</td>
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<td>27,461 ± 6480</td>
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<td>3800 ± 995</td>
</tr>
<tr>
<td>it1</td>
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<td>315 ± 17</td>
<td>9437 ± 941</td>
<td>3265 ± 325</td>
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<tr>
<td>it2</td>
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<td>11,788 ± 553</td>
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<tr>
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<td>158 ± 62</td>
<td>5518 ± 652</td>
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<tr>
<td>it4</td>
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<td>3265 ± 285</td>
<td>384 ± 34</td>
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<td>9437 ± 566</td>
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<td>6400 ± 1331</td>
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<tr>
<td>in4</td>
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<td>20,408 ± 3871</td>
<td>6400 ± 911</td>
<td>0</td>
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</table>

st = superior temporal, sn = superior nasal, it = inferior temporal, in = inferior temporal, 1 = most central, 2, 3 = equatorial, 4 = peripheral.

The form of retinal disease observed clinically in the fundus and the severe depression of the ERG are quite characteristic of retinitis pigmentosa (Fig. 2). In addition, the clinical observation of normal retinal sensitivity centrally in the presence of severe equatorial atrophy is unusual but not rare in RP patients. In view of the severe morphologic changes observed postmortem, it is possible that electrophysiologic abnormalities would have become evident with more sensitive testing methods. The reporting of “normal” dark adaptation is probably due to the testing method used, in which the patient is allowed to search for the target, and the few rods that might have existed in the posterior pole may have given rise to relatively good final thresholds.

We observed many structurally intact photoreceptors in the foveal region of this donor. The cones were shorter than normal, due to a loss of outer segment discs, and slightly reduced in number. The changes observed in the fovea were restricted to attenuation and disorganization of the outer segment disc stack. Although morphological artifacts were minimized by fixation within 1 hr postmortem, the disorganization of the outer segments in foveal cones is probably attributable to postmortem autolytic changes rather than the disease process itself. In addition, there may have been ultrastructural changes due to anoxia during the short time in which the donor was maintained on a respirator.

The extent of pathologic changes observed in the retina became more severe anteriorly, from the foveola to the equatorial region. The retinal architecture progressed from disorganization and loss of numerous photoreceptor outer segments in the parafovea, to disruption of both inner and outer segments in the perifovea, culminating with complete loss of the photoreceptor layer and apposition of the outer limiting membrane to the apical RPE in the equatorial retina.

Anterior to the equatorial region, in the pre-equatorial retina, the structure of the retina became progressively more intact. The transition in retinal morphology, however, was not as gradual as that observed in the post-equatorial region. Within a narrow pre-equatorial tract, spanning the linear extent of 20 to 30 RPE cells (~200 µm) the retina changed from a structure dominated by a completely degenerated photoreceptor layer with massive gliosis (characteristic of the equatorial region), to a retinal architecture retaining a substantial number of photoreceptor inner segments, many short outer segments, and some long (~20 µm) outer segments. The latter,
well-organized architecture was characteristic of the majority of the peripheral retina. Photoreceptors possessing well-organized outer segments were retained in most of the peripheral retina, with the exception of the inferior temporal quadrant, in which outer segment disorganization was observed up to the ora ser-
Fig. 15. Epifluorescence micrograph of the parafoveal region of the RP donor (A) and the 12-year-old normal eye (B). Lipofuscin granules are observed brightly fluorescing, within the RPE (A, B) and within pigmented cells found in the outer retina of the RP donor (A). There was no observable quantitative difference in the amount of lipofuscin within the RPE between the two eyes in this, or any other region (×680).

178,329, whereas in our previous study we counted 168,923 in a 35-year-old normal retina and 138,000 in a 67-year-old. A larger decrease due to aging is observed in the cone population. We counted 117,551 cones in the fovea of the 26-year-old control retina, whereas Osterberg estimated the number of cones/mm² in the fovea of a 16-year-old retina to be 147,300. In the estimate of Marshall and Lattes, by 71 years, it had been reduced to 40,000. Assessment of the retinal degeneration by tabulation indicates that the loss of receptor density is more widespread in the peripheral region of the inferotemporal quadrant, and more confined to the equatorial retina in the inferonasal quadrant. The morphology, however, suggests that the most severe degeneration was in the equatorial region of the inferonasal quadrant. Taken together, the changes in the inferior nasal quadrant probably reflect longer-standing disease, and an ante-
ior progression of degeneration in the inferior temporal quadrant more recently. The quantitative and morphologic data together suggest more recent and less severe disease in the superior hemisphere.

Overall, our morphologic observations correlate well with the generalized clinical characteristics of RP, and the clinical information available for this patient. We found changes implying longer-standing disease, as shown by bone spicule pigmentation, in the inferior nasal fundus compared with the superior temporal fundus; this pattern is a common feature of RP.24 Well-preserved rod photoreceptors were observed from the pre-equatorial out to the far-peripheral region of the superior temporal quadrant of the RP donor. This progression of degeneration corresponds with the clinical observation of conservation of the extreme peripheral temporal visual field, another common characteristic of RP. Our morphological finding of many well-organized photoreceptors in the periphery of the superior temporal retina of the RP donor does not, however, necessarily suggest that there was good visual function in the peripheral field of the donor. The location of these well-organized receptors corresponds to a region of the inferior nasal visual field, which is normally visually insensitive, as it subserves the extreme periphery (beyond 50° of eccentricity).22

Ophthalmic examination of the donor at 15 years (2 years prior to death) described the visual acuity as normal, despite our identification of loss and disorganization of foveal receptors. These findings are compatible since it has also been shown that good visual acuity can be retained in the presence of marked changes in photoreceptor cell morphology.8 Normal dark adaptation was also found clinically, however, losses of visual sensitivity of ½ log unit may not be detectable with conventional clinical testing. A ½ log unit loss of visual sensitivity corresponds to a loss of two-thirds of sensitivity, which is quite compatible with some loss of photoreceptor cell function at the time of testing, 2 years prior to death. In summary, the ophthalmoscopic and histopathological abnormalities imply long-standing retinal degeneration, with apparently good preservation of some visual functions. Although dominantly inherited retinitis pigmentosa is usually considered mild, early onset of symptoms is well-recorded.23

The nature, intensity, and distribution of morphological changes in the eye that we have studied are remarkably similar to those described in the autosomal dominant RP eye examined by Kolb et al.3 However, our findings differ from the other published descriptions of RP in some respects. Very few of the ultrastructural abnormalities of the inner segment, such as autophagic vacuoles and filamentous bundles described by Szamier and Berson,4,24 were observed in the retina of this donor. This difference may be due to several factors. These cases may represent a different disease, a different stage of the same disease, or different phenotypic expression of the same genetic abnormality. Szamier and Berson suggested that ultrastructural changes in the inner segment may be due to an “imbalance in the synthetic and degradative mechanisms” of the photoreceptors,4 and that these inner segment abnormalities may reflect a specific, genetically determined metabolic abnormality. However it was not clear to the authors whether these changes were due to the primary disorder or to the chemotherapeutic agents that the patients had received. Thiopeta, Adriamycin, and 5-fluourouracil had been given for prolonged periods prior to death for metastatic disease. Subsequent investigations of the effects of these chemotherapeutic agents have demonstrated severe histologic changes in the inner and outer segments of the photoreceptors. In the light of these studies and our findings it appears that some of the ultrastructural changes observed by other authors are not directly related to retinitis pigmentosa.

Atrophy and hypoplasia of the RPE is common in advanced cases of retinitis pigmentosa, and individual RPE cells often become hypopigmented or hyperpigmented.24 Overall, it is interesting that despite the broad range of degeneration observed in the photoreceptors of this affected retina, encompassing near-normal regions, transition zones, (in which the number of viable photoreceptors dramatically changed), and areas devoid of photoreceptors, there was comparatively little morphologic change in the RPE. We did not observe any regions in which there were expansive aggregations of RPE cells, such as the continuous band of RPE extending from Bruch’s membrane to the internal limiting membrane described by Rodrigues et al29 in a case of autosomal dominant RP in a 66-year-old. Abnormal RPE morphology in our donor was observed only within a few isolated regions of the equatorial retina. The origin of these RPE cell aggregations is unclear, and may be the result of either migration or proliferation of RPE. RPE proliferation may be a nonspecific response to disease since similar morphologic appearance to that seen in this donor has been documented in response to various stimuli, such as several forms of electromagnetic radiation,30-32 tumors,33 and retinal detachment.34,35 The paucity of change in our case probably reflects the youth of the patient and, therefore, a shorter course of disease. The loss of the normal daily complement of shed photoreceptor outer segment disc membrane might be expected to result in a reduced amount of lipofuscin within the RPE. Conversely, abnormal accumulations of phagosomal material might be observed within the RPE cytoplasm if lysosomal activity was abnormal.36 We observed lipo-
fuscin which was quantitatively equivalent in this RP donor to that present in a 12-year-old normal donor eye. Additionally, the subretinal space contained very little debris in any region. These findings suggest that the RPE retained the capability to phagocytize and degrade ingested fragments. This observation is interesting when contrasted with the RCS rat animal model in which the retinal degeneration is a direct result of failure in the phagocytosis of photoreceptor outer segments.

We were unable to discriminate between choroidal capillaries bordering severely degenerate retina and near-normal regions in terms of the quantity or proportions of fenestrations in this young donor retina. This is in contrast to several reports, the most recent of which is that of Rayborn et al, who found normal capillary fenestrae to be restricted to the fovea and in the surrounding, nondegenerate areas of the eyes obtained from autosomal dominant RP donors. In their report, fenestrae were reported to be totally absent above the more peripheral and nasal regions of the retina of a 79-year-old female and her 51-year-old son. That the donors described by Rayborn et al had a different form of RP from this donor may not account for the disparate results, since it is most likely that the changes in the choriocapillaris represent a secondary response to retinal atrophy, rather than being a result of a primary disorder. The differences in findings could be explained by the longer-standing disease in their donors. It is also the case that the primary subject of their study, a 51-year-old male, had severe hypertension from renal failure which could have affected the choroidal vessels. A greater overall change in RPE structure would also be anticipated if cell death in the photoreceptor layer were a direct consequence of primary disease of the choroidal circulation, as has been suggested by other authors in studies on other variants of human RP. In this case, the presence of a relatively normal choroid most probably reflects the youth of the donor and therefore the relative brevity of the progression of the disease. The choroidal alterations observed by other authors occurred in the context of prolonged disease. In some cases these choroidal modifications may have been secondary to retinal changes, possibly long-term responses to the reduction in metabolic load resulting from the loss of the photoreceptor layer. We did not find significant abnormalities in the inner nuclear and ganglion cell layers or in the optic nerve of the RP donor retina. The attenuation of these layers observed in other reports of dominant RP may reflect long-standing disease in these patients, rather than indicating a fundamentally different pathogenic mechanism.

Retinitis pigmentosa is a term used to denote a collection of different disorders which presumably stem from different causes. The fact that some cases differ from others in their histopathology is to be expected. Our observation of normal inner retina, RPE and choroid suggest that the primary defect in this specific case of autosomal dominant retinitis pigmentosa lies within the photoreceptor layer. Our results cannot exclude the possibility that the RPE is the site of the primary defect and this causes photoreceptor cell death by a secondary effect. We can conclude, however, that the metabolic defect, whatever the source, causes cell death at the level of the photoreceptor cells initially.

The nature of the morphological changes observed do not allow us to draw conclusions as to the fundamental metabolic abnormality occurring here. Analysis of the zones of transition from well-organized to severely degenerate photoreceptors will, it is hoped, reveal a metabolic vulnerability that is unique to the photoreceptors of the affected site. The changes in the retinal layers of older donor eyes described by other investigators are not observed here because they are most probably secondary effects of the degeneration, possibly compounded by age-related changes. There appears to be a final, common pathway of the disease which causes the different disorders ultimately to appear similar, but the fact that there are differences, at least in the early stages, is encouraging for the investigator. We hope that biochemical and immunocytochemical analysis of the tissues from this donor will provide more information as to the precise metabolic vulnerability responsible for this localized degeneration.

Key words: retinitis pigmentosa, photoreceptors, pigment epithelium, inherited retinal degeneration, choriocapillaris

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