Electron microscopic observations of human retinitis pigmentosa, dominantly inherited

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Formalin-fixed eyes of a patient with autosomal dominant retinitis pigmentosa were studied by light and electron microscopy. The only photoreceptors present were foveal cones. The outer segments of these cones were shorter and wider than normal and their discs were disoriented even at their base. Foveal pigment epithelium cells contained excessive amounts of lipofuscin in large spherical clusters, reduced amounts of melanin, and were in different stages of migration away from Bruch's membrane. The existence of such quantities of lipofuscin in foveal pigment epithelium implies that these cells are capable of phagocytizing outer segment material in this form of retinitis pigmentosa. In nonfoveal retina patches of a different type of pigment epithelium occurred which contained no lipofuscin, but instead, large amounts of melanin. These latter cells appear to be solely responsible for the bone corpuscle pigmentation in this disease.

Key words: retinitis pigmentosa, electron microscopy, rods, cones, pigment epithelium, lipofuscin, melanin, phagosomes.

There has been only one electron microscopic study of the retina in any of the various forms of human retinitis pigmentosa. This involved the eyes of two subjects both of whom appear to have had an autosomal recessive form of the disease, although a precise genetic description of these cases was lacking.1

This report describes electron microscopic observations on a retina of a patient with retinitis pigmentosa inherited as a dominant trait and identifiable in this family over four generations. Young and old affected members of this family have been studied psychophysically and electrophysiologically providing a unique description of the course of this disease.2,3

Methods

The eyes were obtained within 10 hours after death from a 68-year-old female with retinitis pigmentosa. Her father had photophobia and nyctalopia. Her only child, a son, and three of his four children have retinitis pigmentosa. When the diagnosis was made at age 46, her visual fields were contracted concentrically to 15 degrees with a 1 mm. target. Acuity was correctable to 20/30 in each eye. There was some arterial attenuation in the peripheral retina but no abnormal pigmentation. She had posterior polar cataracts. Seven years later, her fields had constricted to almost 10 degrees and peripheral retinal pigmentation had formed. Subsequently, she also developed angle closure glaucoma for which

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Fig. 1. Foveal cones of the retinitis pigmentosa eye. The cones look normal at this magnification. The outer nuclear layer contains less nuclei than normal. Toluidine blue, epon section, ×560.

Fig. 2. Foveal slope photoreceptors of the retinitis pigmentosa eye. The cones are swollen and stunted and the outer segments absent or degenerate. There is only one layer of cone nuclei. There are no rods. Just beyond this region of foveal slope there are no more photoreceptors. Toluidine blue, epon section, ×560.

She underwent bilateral iridectomies. At age 65 she had bilateral intracapsular cataract extractions. When examined six months before death her glaucoma was well controlled. Her visual acuity was 20/30 in the right eye and 20/25 in the left eye, but her visual fields had contracted to 8 degrees and there was marked arteriolar narrowing and extensive peripheral pigmentation of the retina.

The eyes were placed in 10 percent formalin. The right eye was embedded in paraffin, sectioned, and stained with hematoxylin and eosin and examined by light microscopy. Unstained sections were mounted in a nonfluorescent medium and examined by fluorescence microscopy. Some sections were bleached with 0.25 percent KMnO₄ for 30 minutes and washed in 10 percent hydrobromic acid in order to test for melanin. Six normal formalin-fixed eyes from subjects 6-, 25-, 53-, 62-, 75-, and 76-years-old were also examined by fluorescence microscopy.

The left eye was removed from formalin, washed repeatedly, and cut into small pieces. The retina with choroid attached was dissected off the sclera and placed in 2 per cent osmium in veronal buffer for 1.5 hours. The tissue was dehydrated and block-stained in 2 per cent uranyl acetate in 70 per cent alcohol for two hours before embedding in epon. One micron sections were stained with toluidine blue and examined by light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate before examination in a JEM T-8 electron microscope.

In order to control for the effects of age, fixation, or handling procedures the eyes of two normal subjects of comparable age were also examined. One specimen was acquired after an enucleation for retinoblastoma and immediately fixed in ice-cold osmium tetroxide in veronal acetate buffer (courtesy of J. E. Dowling). The other was fixed in formalin several hours postmortem before being processed for electron microscopy.

Results

The posterior of the eye showed a pattern of advanced retinitis pigmentosa, forme tacheté. There were variegated strands of pigment lying in the neural retina in bone corpuscular-like patterns. In addition, round clumps of pigments were seen at a
Fig. 3. Foveal cones of the normal control eye to show autolytic and fixation changes occurring in autopsy specimens for electron microscopy. The inner segment contains very swollen mitochondria. The outer segment may be swollen and the discs split and pulled apart. Many vesicles are present but the intact discs are oriented normally. Fig. 7, ×16,000.

Fig. 4. Higher magnification of a portion of outer segment of a foveal cone from the eye with retinitis pigmentosa. The discs formed at the base of the outer segment are disoriented. ×16,000.

Fig. 5. Foveal cones just out of the very center of the fovea. The inner segments are normal in length but slightly wider than normal. The outer segments are disorganized. Portions of discs have assumed unusual angles to each other, a feature not seen in the normal eye so close to the base of the outer segment. ×3,200.

deep level producing the forme tacheté appearance. There were also numerous pale crater-like patches with hyperpigmented rims scattered about the more peripheral retina which could be "pavement" degeneration of aging.

Light microscopy revealed that the only photoreceptors were in the fovea contiguous to what appeared to be normal pigment epithelium. The number of cell nuclei in the fovea, especially in the outer nuclear layer, was reduced. Outside of the fovea the retina became atrophic with disorganization of the cellular layers, gliosis, and pigment-containing cells scattered about. From the equatorial zone to the periphery there were many areas in which the pigment epithelium was absent so that the neural retina was directly apposed to Bruch's membrane. The choriocapillaris appeared to be normal.

In one micron thick sections prepared for electron microscopy the outer and inner segments of the foveal photoreceptors could be more clearly seen by light microscopy (Fig. 1). The only photoreceptors were cones; no rods could be found anywhere in the retina. Near the foveola, the cones appeared normal at this magnification but on the foveal slope they became
Fig. 6. Pigment epithelium of the foveal region of the retinitis pigmentosa eye. The cells are sliding over each other to form a bilayer. The cytoplasm is almost obliterated by the dense accumulation of lipofuscin granules. Frequently, balls of lipofuscin granules are seen (arrow). Toluidine blue, epon section, ×800.

Fig. 7. Pigment epithelium of the peripheral retina. The cells form a thin ragged sheet with a thick basement membrane and are filled with granules. Toluidine blue, epon section, ×880.

markedly abnormal. Electron microscopy revealed that all of the cones, even those near the foveola, were abnormal (Figs. 4 and 5). The outer segments were all shorter than normal and had discs which were disorganized and disoriented even at the base of the outer segment. The inner segments were wider than normal being 4.5 μm in width compared to a normal of 1.5 to 2.5 μm. In contrast, the photoreceptors of the formalin-fixed control eye have discs which are disrupted and ballooned out (Fig. 3), but which do not show the unusual configurations seen in the retinitis pigmentosa eye.

Thin sections of foveal pigment epithelium revealed that some cells were sliding over one another and migrating away from this layer (Fig. 6). Each cell was filled with enormous accumulations of dense granules often clumped into large balls (Fig. 6, arrow). Electron microscopy showed that these granules were lipofuscin and not melanin (Fig. 8). Melanin granules were extremely rare in this foveal pigment epithelium. No recently phagocytized outer segments were unequivocally found within these cells.

Much of the pigment epithelium in the more peripheral retina, though superficially resembling that of the fovea under the light microscope (Fig. 7), turned out to be totally different (Fig. 9). These cells contained no lipofuscin but were filled with dense, round melanin granules distributed throughout the cytoplasm of the cell. This peripheral pigment epithelium differed from the foveal variety in other ways. The basement membrane was folded in many places and invaginated deeply into a cytoplasm filled with rough endoplasmic reticulum.

The bone corpuscle-like pigmentation within the neural retina was composed of this same type of pigment epithelium (Fig. 10). These cells were also devoid of lipofuscin but filled instead with the same dark, round melanin granules. Each cell had dense cytoplasm, rough endoplasmic reticulum, and adjacent basement membrane. Each cell made contact with its neighbor by zonulae adherens and gap junctions, both of which occur between normal retinal pigment epithelium cells.

The fluorescence of lipofuscin provided a vivid way to distinguish these two types of epithelium (Fig. 17). In the fovea (Figs. 17, A and E) and parafovea (Fig. 14, B), the pigment epithelium fluoresces an intense orange-yellow, whereas the
Fig. 8. Electron micrograph of lipofuscin granules in the foveal pigment epithelium of the retinitis pigmentosa eye. ×8,000.

Fig. 9. Pigment epithelial cells of the peripheral retina of the retinitis pigmentosa eye. The melanin granules are round to hexagonal in shape and uniformly electron-dense compared with the lipofuscin granules of Fig. 8. There are no lipofuscin granules in this peripheral pigment epithelium. Basement membrane material projects into the cytoplasm of the cells (arrow). Large vacuoles are seen and stacks of rough endoplasmic reticulum appear at the apical border (r.e.). ×7,000.

melanin containing epithelium (Figs. 17, C [arrow] and F) and the bone corpuscle-like deposits (Fig. 17, D, double arrow) remain dark black. Large areas of the retina could be easily examined by fluorescence microscopy in a way that is impossible to do electron microscopically. This revealed that the melanin-containing epithelium appeared in discreet patches all over the peripheral retina interspersed between areas of atrophic lipofuscin-containing epithelium and areas in which the pigment epithelium layer had completely disappeared. The transition between each area was ab-
Fig. 10. Electron micrograph of a portion of bone corpuscular pigmentation. The cells comprising the pigment clump appear to be of the same type as the cells of the peripheral pigment epithelium. The surface is folded due to the projecting cells, each of which has basement membrane material (b, thick arrows). Each cell contains melanin granules and rough endoplasmic reticulum (r.e.) and is joined to neighboring cells by zonulae adherens and gap junctions (thin arrows). x4,400.
rupt (Fig. 17, C, arrow). Bleaching removed all the melanin from the nonfluorescent epithelium, but had no effect on the fluorescent, lipofuscin-containing cells. The retinal pigment epithelium of the youngest control eye, age 6, fluoresced weakly; by age 25, the fluorescence had increased considerably and the pigment epithelium of all the older retinas showed intense fluorescence confirming a conclusion derived originally from histochemical studies\(^7\) that lipofuscin increases with age. The fluorescence of the foveal pigment epithelium of the retinitis pigmentosa eye was as intense as that of the oldest control eye.

It seemed important to us to know whether lipofuscin represents the degradation products of phagosome digestion as has been suggested.\(^5\)\(^-\)\(^10\) Although this hypothesis is probably correct it is not easy to prove. We have attempted to obtain support for it by searching the pigment

Fig. 11. Photoreceptor outer segment enveloped by the ciliary processes of the pigment epithelial cells. Portions of detached outer segment material (arrows) are passing up within the extension of the cytoplasm of the pigment epithelial cell toward the perinuclear area of the cell. \(\times27,500\).

Fig. 12. Phagocytosed outer segment material as a membrane-bound phagosome in the cytoplasm of the pigment epithelial cell. Notice the discs can still be recognized and the outer segment membrane is still complete within the whole membrane-bound structure of the phagosome. \(\times27,500\).

Fig. 13. The phagosome contents become vesicular although some discs are still seen. \(\times27,500\).
epithelium of the optimally fixed control eye for transition forms between phagosomes and lipofuscin granules. Fig. 11 (right) shows a rod outer segment being engulfed by the cytoplasm of a pigment cell and Figs. 12 and 13 show a progressive dissolving of the disc material forming more homogeneous particles of the same size as lipofuscin granules (Fig. 14). Figs. 15 and 16 illustrate that melanin is also incorporated into lipofuscin as others have already demonstrated. 8

Discussion
This study shows that in this relatively late form of autosomally dominant retinitis pigmentosa the only photoreceptors left are in the fovea and these are exclusively cones. This parallels clinical tests of younger affected members of this family who develop defects in rod function before any deficit in cone function. 2 Most of the cones in this retina have degenerated, however, and this goes along with the fact that adult members of this family lose all

Figs. 17, A and B. Show, respectively, photographs of fluorescence from foveal and parafoveal pigment epithelium (arrow). In the parafovea (B), fluorescent cells are seen breaking from the pigment epithelium and migrating into the neural retina. ×175.

Fig. 17, C and D. Show photographs of fluorescence from more peripheral retina. The arrow in C shows the abrupt transformation from the fluorescent type of pigment epithelium characteristic of the foveal region to the nonfluorescent melanin-containing variety characteristic of the periphery. The double arrow in D shows bone corpuscle pigmentation in the peripheral retina. ×175.

Figs. 17, E and F. Show respectively higher-power photographs of fluorescence from foveal (E) and peripheral (F) pigment epithelium to emphasize the strong fluorescence of the lipofuscin-containing cells of the fovea in comparison to the nonfluorescent melanin-containing cells of the periphery. ×1,000.
nonfoveal vision. The other electron microscopic study of a presumably autosomally recessive form of the disease found a similar tolerance of cones and this fact has often been reported in light microscopic descriptions of retinitis pigmentosa. What impressed us with these electron microscopic observations was how rapidly the pathology changed from totally degenerate cones on the foveal slope to those near the foveola whose pathology was too subtle to appreciate by light microscopy. This preservation of foveolar cones could be due to their relative isolation from degenerating rods.

It was a surprise to discover that the foveal pigment epithelium which appeared normal by conventional light microscopy was so abnormal by electron microscopy. The most striking change in these cells was the enormous quantities of lipofuscin and the almost total absence of melanin. Aging retinal pigment epithelium also accumulates large amounts of lipofuscin presumably due to the continuing phagocytosis of outer segment material during life as the intense fluorescence of this layer in old normal eyes indicates. The number of lipofuscin granules which could be counted in electron micrographs of the retinitis pigmentosa eye was greater than that found in the optimally fixed control eye of comparable age; in addition, none of the large clumps of these granules so striking in the retinitis pigmentosa epithelium were found in the control eye. Nevertheless, it may be necessary to sample many older eyes to ascertain whether this amount of lipofuscin could form normally. What seemed remarkable to us was that so much lipofuscin could accumulate in a retina in which there were no longer any rods and which had been undoubtedly devoid of rods for a good part of adult life. These observations suggest to us two alternative explanations for the pathology seen in this form of retinitis pigmentosa. Either the pigment epithelium in this retina is fully capable of phagocytizing and digesting outer segment material, which does not appear to be the case in an important animal model for retinitis pigmentosa, the Royal College of Surgeons strain of rats. Thus, sometime during the life-time of this patient there was either an overproduction of outer segment material or excessive phagocytosis by a greedy pigment epithelium, in order to have built up such a final store of lipofuscin. The alternative explanation could be that the excessive accumulation of lipofuscin in this case of retinitis pigmentosa is an expression of a pathology in the pigment epithelial cells which leads to the eventual death of the photoreceptors as has been suggested by some authors to occur in Refsum's disease.

The last observation of interest is the extraordinary transformation that has taken place in much of the peripheral retinal pigment epithelium. These cells have synthesized enormous quantities of melanin and have either lost or never formed lipofuscin. The cells resemble uveal melanocytes and it is possible that such melanocytes moved in to fill the gap after the lipofuscin-containing cells migrated into the neural retina. A similar sort of process may be occurring at a much smaller scale near the ora serrata of normal retina. It is also curious that the lipofuscin-containing cells seen to be entering the neural retina by fluorescence microscopy appear to make no contribution to the formation of bone corpuscle-like deposits which are only formed by melanin-containing cells.

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