Pigment epithelial windows and drusen: an animal model

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Aging rhesus monkeys, both controls and those undergoing long-term administration of investigational oral contraceptive steroids, developed widespread hyperfluorescent dots at the posterior pole. The dots were considered to represent drusen. Histologic (including electron microscopic) study showed the "drusen" in some of the animals to be almost exclusively pigment epithelial windows produced by a lipoidal degeneration of the pigment epithelial cells. The experiment provided a fortuitous model for direct correlation of clinical and histologic observations of myriad uniform, tiny, depigmented, hyperfluorescent, nonleaking spots at the level of the retinal pigment epithelium.

Key words: pigment epithelium, drusen, windows, lipoidal degeneration, rhesus monkey

When fluorescein angiography is used to study the myriad tiny yellowish white dots often seen in the fundus of an eye, with or without disease, pinpoint areas of hyperfluorescence (without leakage) appear at the site of the dots, hyperfluorescence that slowly decreases in the late phases of the angiography. Currently to be considered in the differential diagnosis for these dots are the following: (1) drusen of Bruch's membrane, (2) "windows" of the pigment epithelium.

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and, more recently, (3) blebs of the pigment epithelium.

In human material it is extremely difficult to correlate precisely such histologic changes with the clinical features, particularly in eyes that have no other disease processes which would require enucleation of a globe.

Fortuitously, in a large experiment in which there was long-term administration of investigational contraceptive steroids to rhesus monkeys, myriad yellowish white dots of variable extent and severity were observed in the fundi of a number of the animals (Fig. 1). The dots were considered to represent drusen clinically and were hyperfluorescent on fluorescein angiography (Fig. 2), thus providing a good animal model for study.

Since the dots were very numerous in the region of the clinical macula (fovea), a natural marker was also available to us for accurate clinicohistologic correlation. Untreated animals of similar age and with clinically similar observations (Figs. 3 and 4) were also examined as controls for the treated animals.
Fig. 5. Section through fovea and foveola. The arrows indicate two vacuolated pigment epithelial cells lying at the edge of the foveola. All other retinal layers and the choroid (ch) are normal. G, Ganglion cell layer. (Toluidine blue, ×80; treated animal; AFIP neg. 78-7310-2.)

Fig. 6. Inset shows presence of vacuolated pigment epithelial cells (arrows) near foveal periphery. F, Foveola. G, Ganglion cell layer. (Toluidine blue, ×165; treated animal; AFIP neg. 78-7310-3.) The electron micrograph shows a vacuolated pigment epithelial cell resting on a normal thin basement membrane (bm) and attached to adjacent normal epithelial cells by zonulae adherentes (arrows). A few of the vacuoles contain a material of very low electron density. Ch, Choriocapillaris. (×10,000; treated animal; AFIP neg. 78-7310-3.)
Fig. 7. Inset shows foveal region from a control animal. The retinal layers (i.e., \( G \), ganglion cell layer; \( IP \), inner plexiform layer; \( IN \), inner nuclear layer; \( OP \), outer plexiform layer; \( H \), Henle fiber layer; \( ON \), outer nuclear layer; \( X \), external limiting membrane; and \( PH \), layer of rods and cones) are all normal, as is the choroid. Vacuolated pigment epithelial cells are present (arrows) in the region of the fovea (at the margin of the foveola, \( F \)). (Paraphenylenediamine, \( \times 165; AFIP \) neg. 78-7310-4.) The large figure shows a vacuolated pigment epithelial cell with normal thin basement membrane (\( bm \)) and normal apical attachment (zonula adherens, \( ZA \)) to adjacent normal cell. Choriocapillaris (\( CH \)) and Bruch's membrane are normal. Note photoreceptor outer segment undergoing digestion within the normal pigment epithelial cell (arrow). (\( \times 13,000 \); untreated animal; AFIP neg. 78-7310-4.)

Materials and methods
The experimental animals were female rhesus monkeys which were part of an FDA-sponsored experiment to study the long-term effects of oral contraceptive steroids in animals. The monkeys were sexually mature at the beginning of the experiment and had been under experimentation for approximately 8 years. They were from a group that received 2.20 mg/kg of anagostone acetate plus mestranol in a 10:1 ratio, in a cyclic manner of 21 days treatment and 7 days without treatment. The dosage represented approximately 50 times the human dose. Ophthalmologic examination was performed semiannually under full mydriasis with a binocular indirect ophthalmoscope. Four eyes from two monkeys were selected for electron microscopic evaluation. The eyes of one monkey had clinical alterations regarded as severe (grade IV; Fig. 1), whereas those of the other animal had mild changes. The eyes were enucleated immediately upon sacrifice, opened equatorially, and immersed in cold (\(-5^\circ C\)) 2% glutaraldehyde or a mixture containing 4% formalin and 1% glutaraldehyde. The foveal region and samples of parafoveal regions and parapapillary regions were removed under direct microscopic observation. They were osmicated in Dalton’s chrome-osmium fixative for \(~40\) min. Dehydration was carried out through ascending concentrations of ethyl alcohol with final embedment in Epon.
For light microscopic study, serial sectioning of Epon-embedded tissue was carried out at 1 to 2 μm; the sections were then stained with either paraphenylenediamine or toluidine blue.

Sections for electron microscopic study were stained with uranyl acetate and lead citrate.

Four eyes from two nontreated or control monkeys of similar age and with ophthalmoscopic and angiographic findings of approximately similar severity (grade 3; Fig. 3) were prepared in the same manner for comparison.

Results

The fixation was good, and all retinal and choroidal layers sampled, except for the pigment epithelium, were normal by light and electron microscopy.

In the foveolar, foveal, and parafoveal regions, vacuolated pigment epithelial cells were observed in all eyes of both treated (Figs. 5 and 6) and nontreated (Figs. 7 and 8) animals. The vacuolated cells were in normal position within the epithelial layer and were normally attached to adjacent, unaffected cells (Figs. 6 and 7). Although many of the vacuoles appeared empty, a number were completely or partially occupied by a material of poor electron density.

During the sampling procedure, a single druse was found by electron microscopy at the posterior pole in one of the eyes of a treated animal (Fig. 9). This druse consisted mostly of homogeneous basement membrane containing the characteristic irregular arrangements of disordered, banded basement membrane.7' 8 Parapapillary drusen (Figs. 1 and 10) were also examined for comparison.

Fig. 8. Inset shows region of parafovea. Arrows indicate three vacuolated pigment epithelial cells. Retinal and choroidal layers are normal. H, Henle fibers. X, External limiting membrane. (Paraphenylenediamine, x400; untreated animal; AFIP neg. 78-7310-5.) Large figure shows vacuoles in another pigment epithelial cell. Many vacuoles contain relatively electron-lucent material. Choriocapillaris (CH) and adjacent Bruch’s membrane are normal. (x17,000; untreated animal; AFIP neg. 78-7310-5.)
Fig. 9. Single druse (D) found in the tissues of the posterior pole. The druse is here composed of homogeneous, vacuolated basement membrane and contains many irregular strands of disordered, banded basement membrane. The basement membrane or druse material here is in direct contact with the cell basal plasmalemma (arrows). (×17,000; treated animal; AFIP neg. 78-7310-6.)

and found here to be composed mostly of vacuolated basement membrane.

Discussion

There are few reports on naturally occurring retinal lesions in nonhuman primates. Rubin in his atlas shows a few fundoscopic photographs of rhesus monkeys which depict spots of depigmentation in the foveal area, but additional details are not given. Stafford reported on a drusenlike alteration of the fovea in an aged female rhesus monkey. Using fluorescein angiography, he noted no leakage of dye or staining, but he did observe a transmission or show-through effect similar to that observed in our cases. The lesion was believed to be limited to the retinal pigment epithelium. Since histologic and electron microscopic studies were not carried out, it was unknown whether the lesions were drusen or whether they represented pigment epithelial windows. To our knowledge there are no reported histologic descriptions of drusen in rhesus monkeys, but drusen have been described in a young female baboon. The report was considered to be the first record of this condition in an animal. Morphologically, the lesions were discrete, eosinophilic structures lying on Bruch's membrane, beneath the pigment epithelium.

From our own observations, it is clear that the yellowish-white hyperfluorescent dots seen clinically in the monkey eyes examined are due to pigment epithelial "windows," the window effect here being produced by a vacuolization of the individual pigment epithelium-
Fig. 10. Large parapapillary drusen (see Fig. 1) are seen in inset (arrows). The horizontal separation within the pigment epithelium is an artifact. g. Large granules. (Toluidine blue, ×575; treated animal; AFIP neg. 78-7310-7.) The electron micrograph shows the large druse here to be composed almost entirely of vacuolated basement membrane. A thin homogeneous basement membrane lines the cell basilar plasmalemma (arrows). The large granules in the inset appear to represent the large dense granule (G). CH, Choriocapillaris. (×13,500; treated animal; AFIP neg. 78-7310-7.)

The pigment epithelial cell vacuoles apparently transmit the wavelengths of excited fluorescein in a manner somewhat analogous to the transmission of light from the fundus of the eye through the larger vacuoles produced by diabetes mellitus in the iris pigment epithelium of some diabetics. The vacuolated cells have very few. The vacuolated pigment epithelial cell appears to contain a lipoidal material not unlike that observed in the "balloon" cells that may be seen in malignant melanomas, a form of lipoidal degeneration. Variation in lucency of the vacuoles suggests that some extraction of content occurred during preparation of the tissue. Similar changes have been recently reported in astrocytes in angiomatosis retinae, a lipidization due to leakage of lipids from the adjacent vasculature. Since no obvious leakage of lipids is seen (or has yet been demonstrated) for the "balloon" cells of the malignant melanoma or for the vacuolated pigment epithelial cells seen here, the older term "lipoidal degeneration" appears useful in distinguishing the latter changes from those in angiomatosis retinae.

Similar lipoidal degeneration has been ob-
served in human retinal pigment epithelial cells.16

From the animal studies, it appears that the vacuolization of the pigment epithelial cell, here also referred to as lipoidal degeneration, is a normal aging process. It is, of course, possible that such an aging change can be aggravated by chronic administration of drugs or by associated disease. Any possible drug-related effect therefore must be determined by statistical methods. (For example, the observation of apparently similar changes in almost 7% of a large series of normal aging rhesus monkeys11 suggests that a much higher incidence in a series of treated monkeys may be drug-related.)

Fig. 11. Inset A shows irregular dots along papillomacular bundle. Note large, irregular patch (arrow) near center of fovea (foveola). Inset B (PD × 160) shows the large patch in inset A is drusen material (D) beneath the pigment epithelium near the foveola. Note presence of vacuolated pigment epithelial cell (small free arrow) nearby. The electron micrograph shows edge of large, flat druse lying on remainder of Bruch's membrane (BR). The druse here is composed mostly of homogeneous and disordered banded basement membranes.7 Note focus of calcification (Ca) in basement membrane. PEP, Pigment epithelial cell. (×17,000.)
In another study within a free-breeding colony, however, the incidence of drusenoid changes was much higher, 34%, concentrated mostly in or around the fovea. The incidence could be directly correlated with age, i.e., 12% in 0 to 4 years of age; 42% in 5 to 9 years of age; and 82% in 10 to 20 years of age. With these limited histologic findings clarified, a review of some of the still-living treated animals was made, and it became clear that at least three clinical pictures could be observed: (1) the myriad, tiny, uniform white dots; (2) yellowish-white, generally irregular dots with considerable variation in size (Fig. 11, inset A); and (3) a mixture of the other two.

The second group appeared more likely to be drusen when examined clinically and could easily be demonstrated as such histologically and by electron microscopy (Fig. 11).

The clinical observation of myriad dots in the fundus of the eye therefore may represent not only drusen on Bruch's membrane (actually of the pigment epithelium), but also lipoidal degeneration of pigment epithelial cells ("windows" of the retinal pigment epithelium). The two are relatively distinguishable clinically, the latter being limited in size to one or two pigment epithelial cells. If the dot in question clearly possesses a slightly hyperpigmented periphery on ophthalmoscopic examination, then the diagnosis of a druse over that of a depigmented cell may be more convincingly made. When drusenoid (i.e., basement membrane of the pigment epithelium) materials are produced in excessive quantities, they may be observed clinically as large irregular patches of yellowish material. Such patches of material may also be confused with similar large irregular areas of pigment epithelial cell loss or hypopigmentation (i.e., depigmented hyperfluorescent lacunae).

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