

# Drusen, an Age Related Change in the Retina of the Fisher Rat

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## Abstract

*The formation and progression of drusen was studied in the retinas of Fisher rats. The incidence of drusen increased with age. More numerous and advanced lesions were formed in males than in females. In general, there were more lesions in lower peripheral retina than upper peripheral retinas. Early lesions appeared as an accumulation of amorphous material between the retina pigment epithelium and Bruch's membrane, which later led to the separation and disruption of retinal pigment epithelium. Increased thickness of Bruch's membrane, areolar atrophy of retinal pigment epithelium, and choroidal neovascularization were evident in advanced lesions. Drusen have been caused by many factors. To understand the etiology of these lesions, it is important to study the effect of each factor. The changes reported in this study are specifically age-related.*

**Key words:** *Drusen; Bruch's membrane; retinal pigment epithelium cells*

The pathogenesis of age-related retinal degeneration has not been clearly understood. Clinical and experimental findings suggest the involvement of many etiological factors, acting alone or in combination, to result in similar clinical manifestations known as aging retinal or macular degeneration. The presence of drusen, localized depositions of hyaline-like material between the retinal pigmented epithelium and Bruch's membrane, has been shown to precede retinal degeneration,<sup>1,2</sup> areolar retinal pigmented epithelial atrophy,<sup>1,2,4</sup> disciform scarring of the

macula,<sup>1,5</sup> and choroidal neovascularization.<sup>6,7</sup> The morphology of drusen-like lesions has been studied extensively,<sup>8-10</sup> but the pathogenesis of the drusen is still not clear.

To understand the nature and the underlying processes involved in this complex disease, it is important to study the changes which could be caused by one specific etiological factor at a time. We report here some of the age related changes and their progression in the retina of the Fisher rat. The gradual loss of photoreceptor cells in Fisher 344 rats has been shown to be age related.<sup>11</sup> Furthermore, peripheral retinal degeneration in aged Fisher rats histologically mimics this common disorder in humans. These animals, therefore, serve as an important model for the age related changes of the retinal pigment epithelium and Bruch's membrane and the formation of drusen.

## Materials and methods

Forty albino Fisher 344 rats (Charles River Breeding Laboratory, Wilmington, MA), 5 males and 5 females in each age group of 9, 12, 18, and 24

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months, were housed in a specific pathogen-free, controlled-barrier facility and were fed ad libitum. Ambient light was provided by ceiling mounted fluorescent lamps regulated on a 12-hour on/off cycle. Reflected light intensity was checked at regular intervals with a Luna-Pro electric system exposure meter. The retinas of 3-month-old rats served as controls.

Rats were anesthetized by intraperitoneal injection of sodium pentobarbital and were perfused through the left ventricle with 2.5% glutaraldehyde in 0.1M phosphate buffer, Ph 7.4, under a constant pressure of 60mm Hg for 15 minutes. Eyeballs were removed and bisected transversely just behind the limbus. The posterior eye cups were further fixed in glutaraldehyde buffer solution for 2 hours at room temperature, and then were washed briefly in 0.13M phosphate buffer. After washing, the specimens were post-fixed in 1.0% osmium tetroxide in 0.1M phosphate buffer for additional 2 hours. The fixed specimens were embedded in Spurr's<sup>1,2</sup> medium. One-micron thick sections of the hemisphere of the eye cups, including a full length of the retina between two sides of ora-serrata and optic disc were cut. These sections were stained with Azur II-methylene blue for light microscopy. At least five sections of each eye from each animal were examined. Ultrathin sections of selected regions were prepared for electron-microscopic observations.

## Results

Thickness of retinas, especially the photoreceptor cell layer, decreased progressively with age (Figure 1). Although the changes described here were observed on entire retina, because of the higher incidence of drusen, the peripheral retina was selected for this report. Distribution and incidence of drusen are reported in Table 1. Number of drusen-like lesions observed in 9-month-old rats was 42, 23 in males and 19 in females; in 12-month-old rats was 50, 27 in males and 23 in females; in 18-month-old rats was 60, 32 in males and 28 in females; in 24-month-old rats was 82, 49 in in males and 33 in females. Overall, in each group the retinas of the males had more numerous and advanced lesions than did the female retinas. The lower peripheral retinas were more affected than were the upper peripheral retinas.

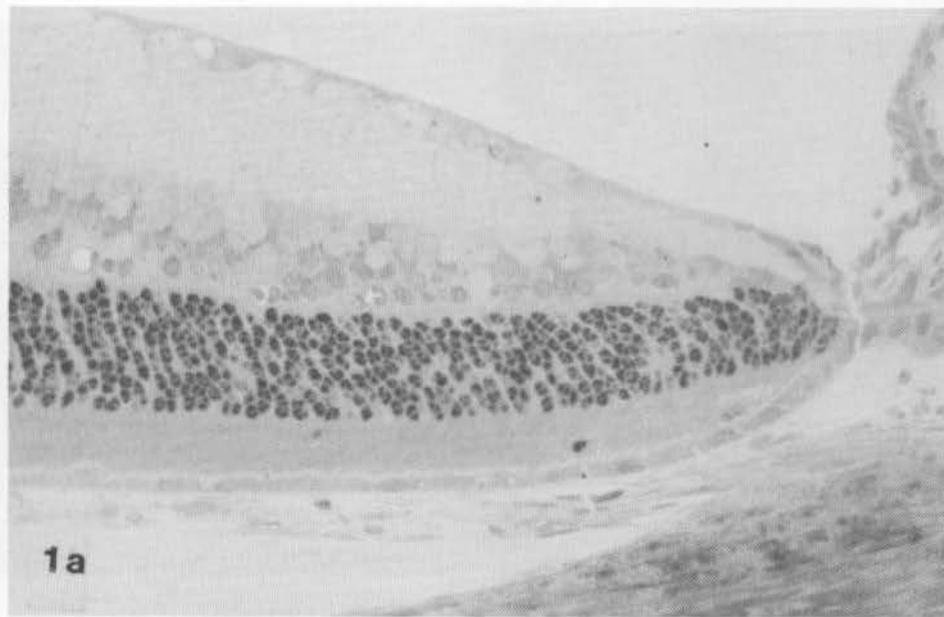
Histological, early drusen-like lesions appeared as accumulation of the amorphous material between the retinal pigment epithelium and Bruch's membrane (Figure 1). Further accumulation of this material produced a dissection type of separation of retinal pigment epithelium from the Bruch's membrane (Figure 2) which was followed by total disruption of the retinal pigment epithelium cells (Figure 3). A decrease in number of retinal pigment epithelium cells and the presence of some cellular debris in this material indicated some cell degeneration. In advanc-

ed cases areolar degeneration of retinal pigment epithelium and choroidal neovascularization were apparent (Figure 4 and 5). Finally, the retinal pigment epithelium was transformed into a layer of amorphous type material which was invaded by blood vessels (Figure 6).

Five distinct layers were observed where Bruch's membrane associated with unaffected retinal pigment epithelium: 1) basement membrane of retinal pigment epithelium, 2) inner loose collagenous zone, 3) clumps of elastin, 4) outer loose collagenous zone, and 5) the basement membrane of endothelium (Figure 7). The areas associated with lesions showed an increased amount of collagen which produced overall thickening and disruption of the Bruch's membrane layers (Figures 7 and 8). There was a loss and migration on photoreceptor cells in the subretinal space, which was reported in our earlier paper.<sup>11</sup> In the late or final stage, the choroidal capillaries were seen in all the retinal layers. In these cases, no photoreceptor cells were observed.

## Discussion

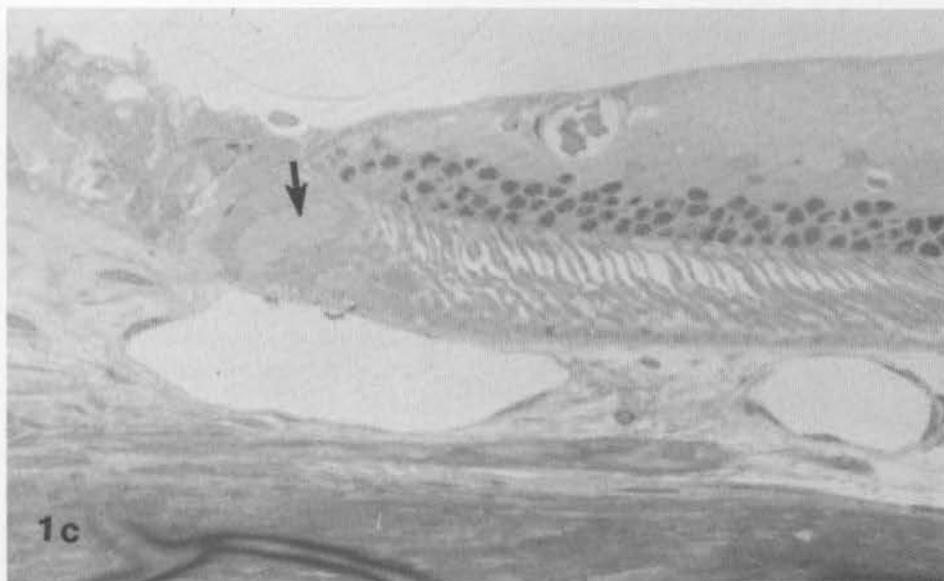
Drusen were first described by Donders,<sup>13</sup> who postulated that they were formed by the transformation of pigment epithelium. Since then, many theories have evolved describing the lesion and its origin. Recently, Hogan<sup>14</sup> emphasized that these are indeed products of retinal epithelium. Burns and Feeney-Burns<sup>9</sup> proposed some of the drusenoid material to be pieces of shed retinal epithelial cells formed by apoptosis. Fine and Yanoff,<sup>15</sup> on the other hand, postulated that drusenoid material was laid down on the scleral aspect of the choriocapillaris away from the retinal pigment epithelium; they did not believe that pigment epithelium is the primary source of drusen. Friedman et al.<sup>6</sup> and Tso<sup>16</sup> reported that drusen were deposited in an arrangement which conformed to the choriocapillaris pattern and proposed that drusen is probably formed as a pathologic manifestation of disturbance of the interaction of choriocapillaris, Bruch's membrane and pigment epithelium. Ishibashi, et al.<sup>10</sup> agreed in general with the observation by Burns and Feeney-Burns<sup>9</sup> and further hypothesized that the budding or evagination of retinal pigment epithelial cells is the initial event in drusen formation. Farkas et al.<sup>8</sup> observed progressive alteration in pigment epithelial cells as drusen developed in patients with intraocular neoplasm. The accumulation of drusenoid material elevated the degenerating pigment epithelium, and degenerating pigment epithelial cells eventually were incorporated into the developing drusen. Kenyon et al.<sup>17</sup> postulated that a diffuse abnormality of the retinal pigment epithelium resulted in an elaboration of membranous reticulum and basement membrane material. These greatly thickened the inner aspect of the Bruch's membrane and weakened the attachment



1a



1b



1c

Figure 1. Histological sections of peripheral retina of Fisher rats; a) section from 3-month-old rat retina showing normal morphology, b) retina of 9-month-old rat with drusen (arrow); thickness of all cellular layers is considerably decreased, vacuolation in retinal pigment epithelium and marked dilation of blood vessels is apparent, c) retina of 12-month-old rat with drusen (arrow); thickness of all cellular layers is further decreased with displaced photoreceptor cells; and the architectural organization of the retina is disrupted. (X450).

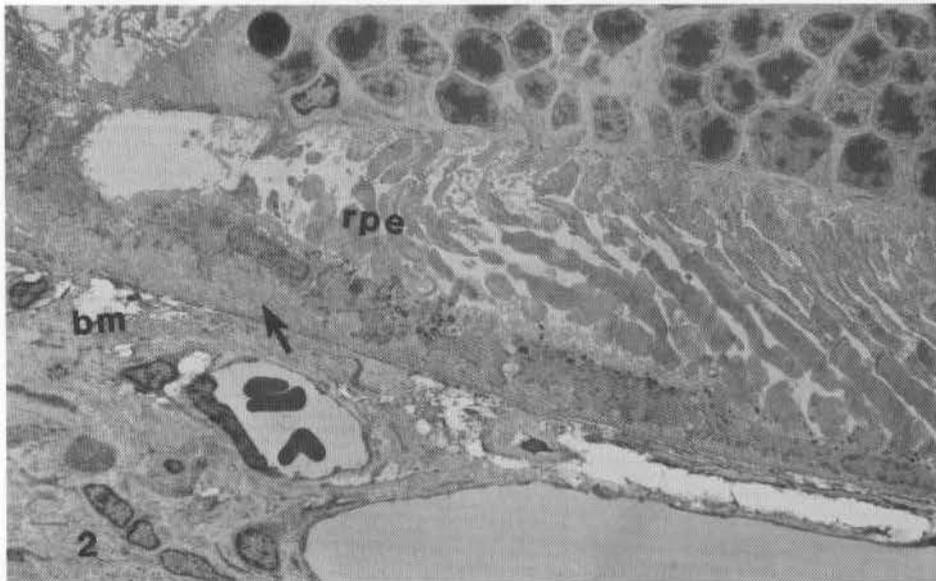
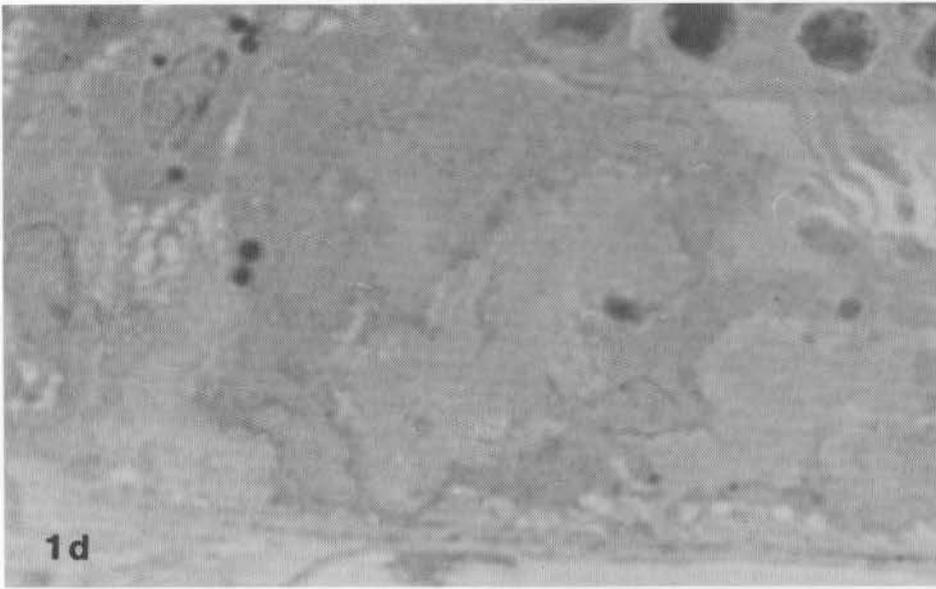


Figure 2. Electron micrograph of peripheral retina with drusen (arrow). The accumulation of drusenoid material has lifted the retina pigment epithelium (rpe) from the Bruch's membrane (bm). (X3000).

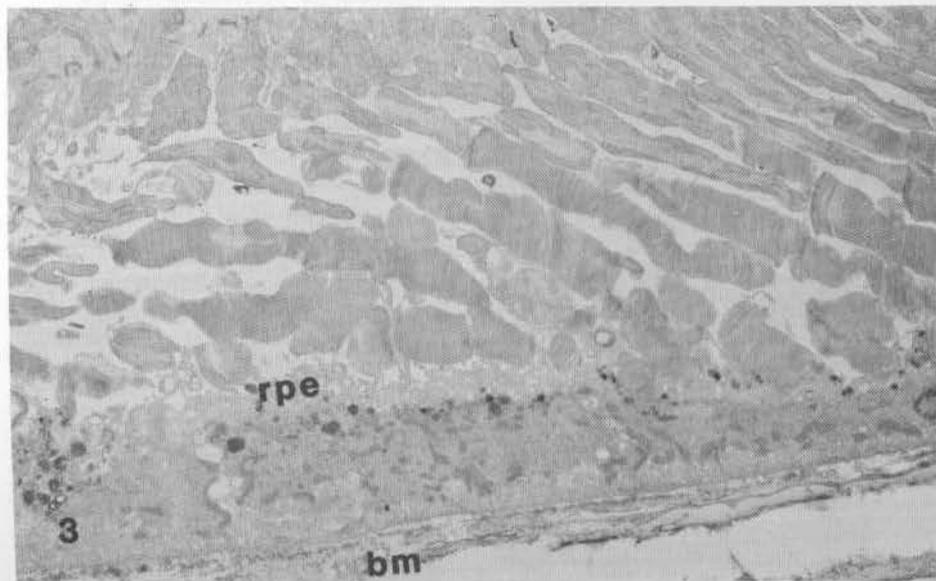


Figure 3. Electron micrograph of retina showing separation of the retina pigment epithelium (rpe) from the Bruch's membrane (bm). The morphology of retinal pigment epithelial cells is totally disrupted. (X6000).

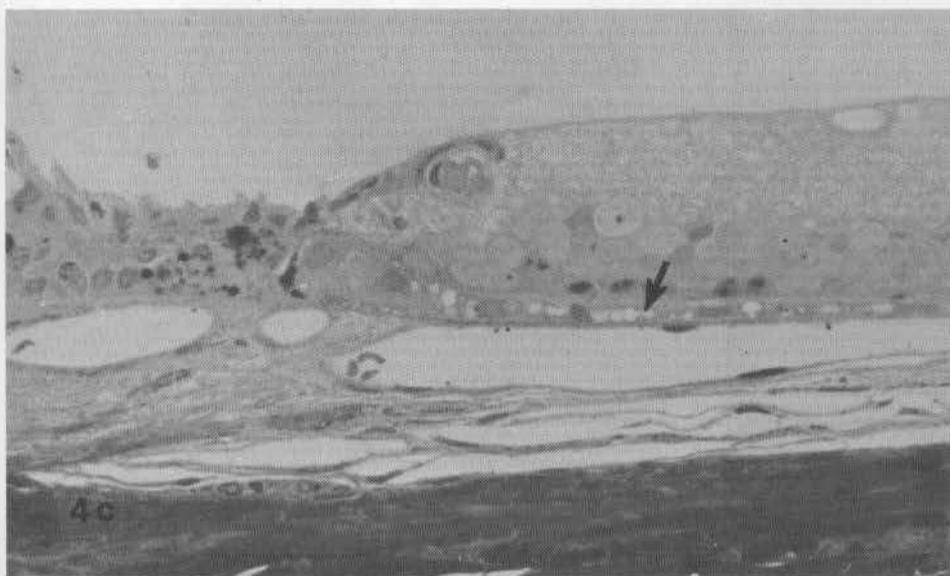
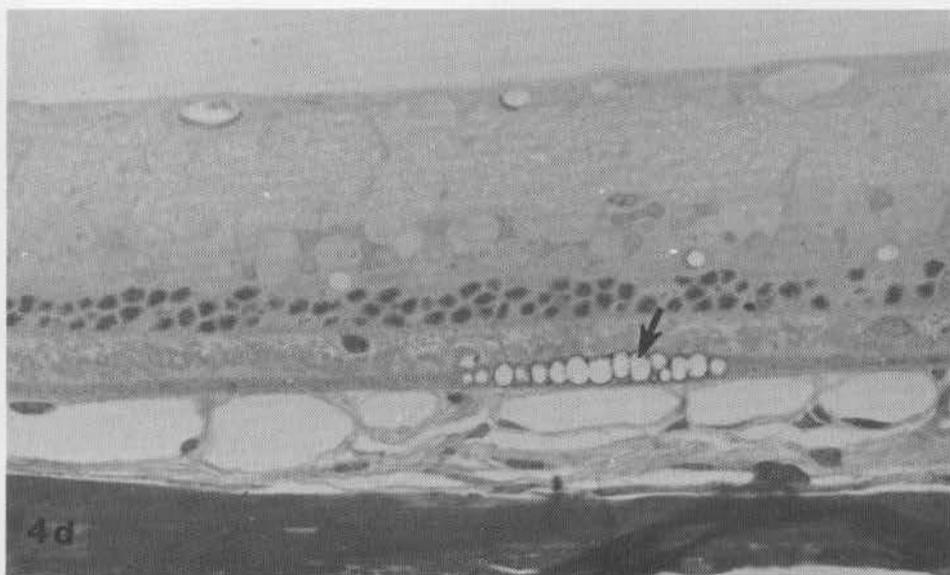
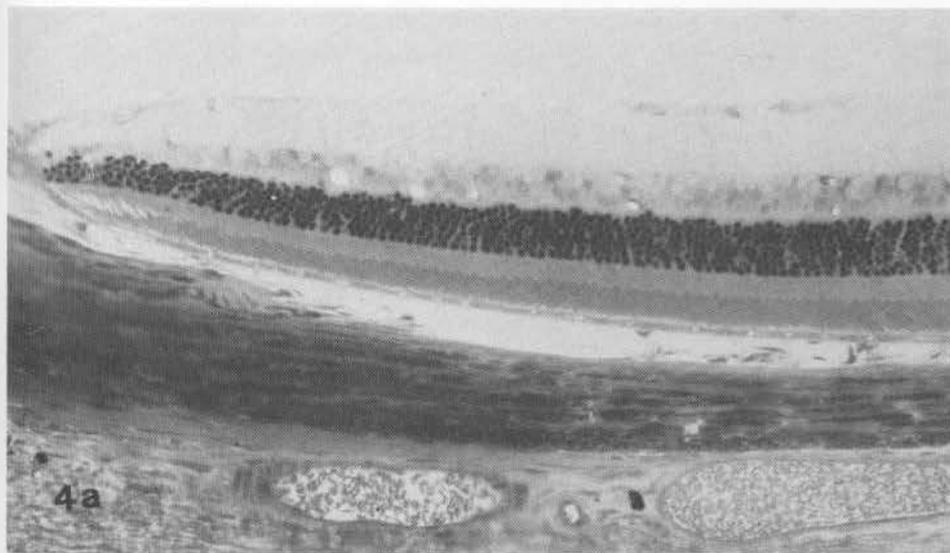


Figure 4. Histological section of retina; a) retina of 3-month-old rat showing normal morphology, b) retina of 9-month-old rat with drusen (arrow); some dilation of blood vessels is apparent; c) retina of 12-month-old rat with drusen and vacuolation (arrow) in retina pigment epithelium, d) retina from 18-month-old rat showing areolar degeneration (arrow) of retinal pigment epithelium and marked choroidal neovascularization. (X400).

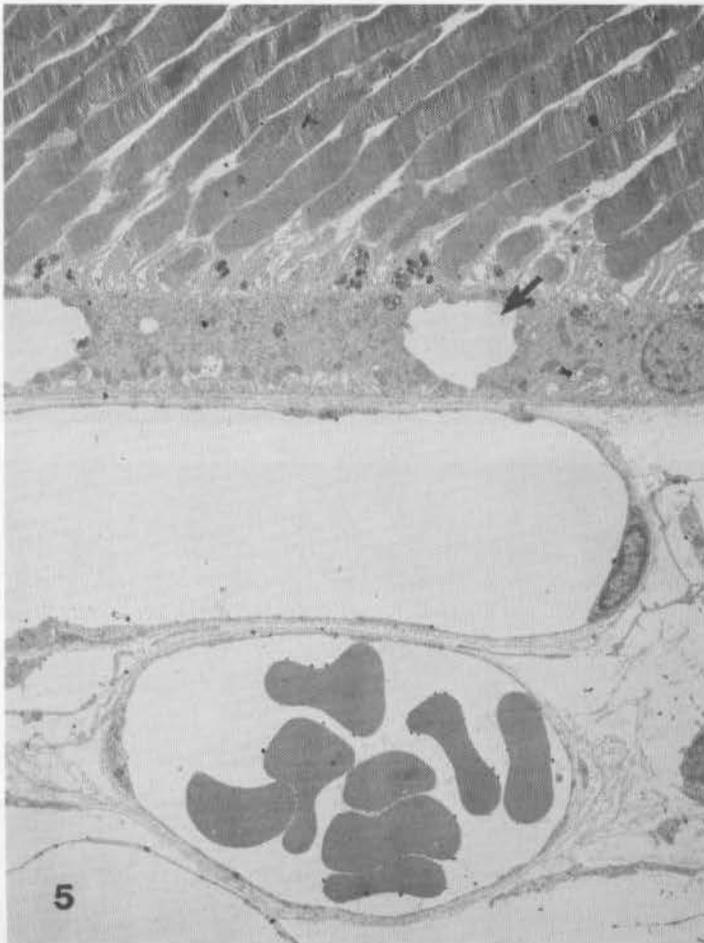
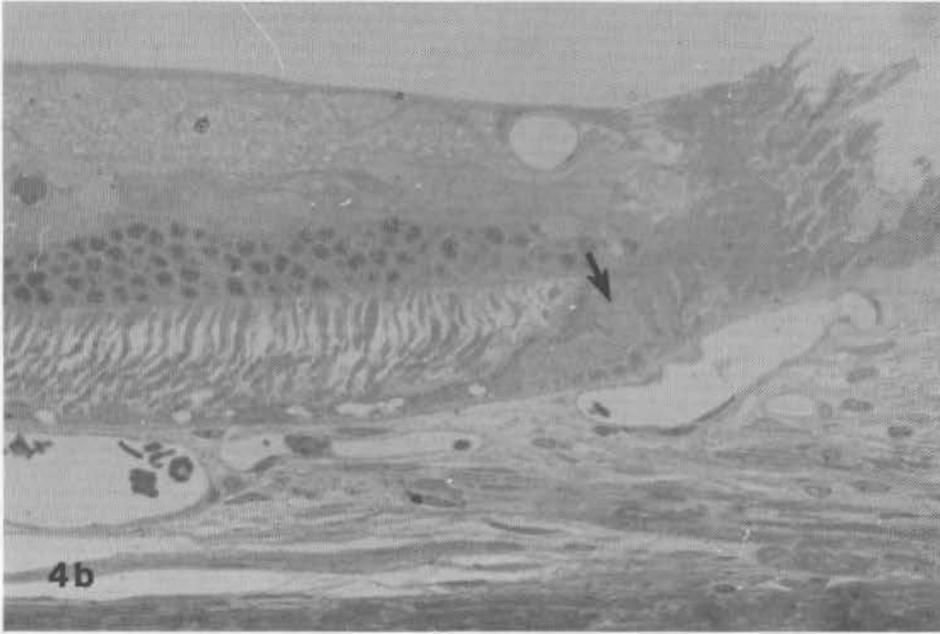


Figure 5. Electron micrograph showing vacuolation in retinal pigment epithelium (arrow) and choroidal neovascularization. Capillaries (cap) are markedly dilated. (X6000).

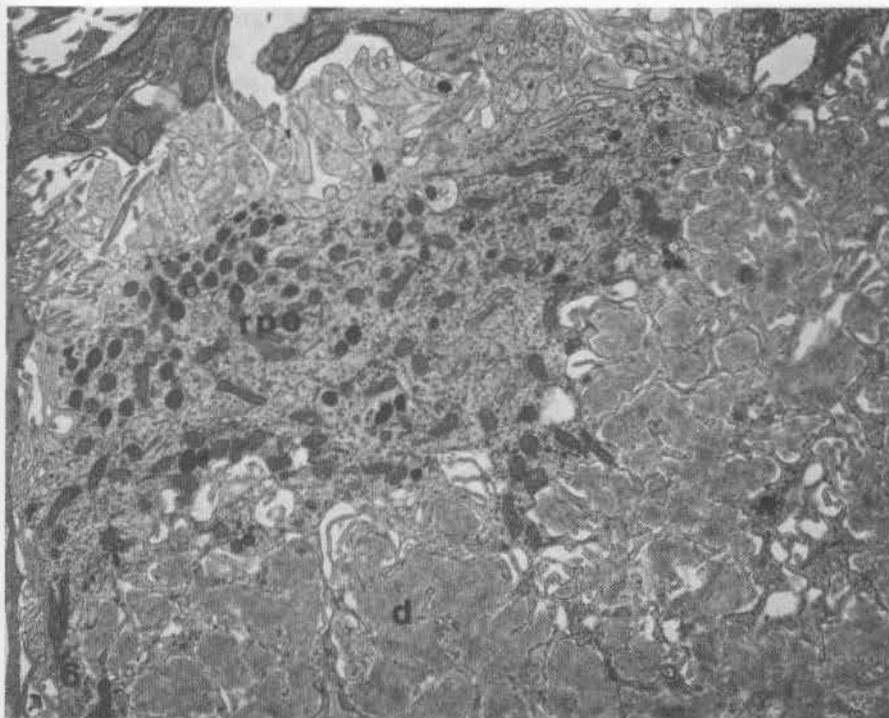


Figure 6. Electron micrograph of retinal pigment epithelium (rpe). The cells are transformed into drusenoid material. (X15000).

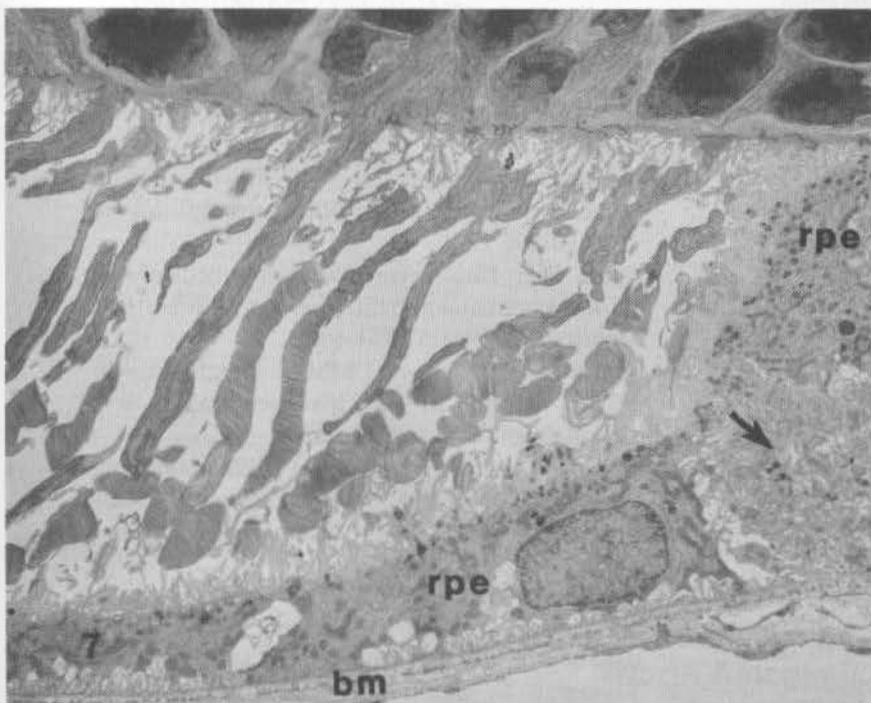


Figure 7. Electron micrograph of retina showing unaltered Bruch's membrane (bm) and the portion of Bruch's membrane associated with drusen (arrow). Increase in thickness of this portion of Bruch's membrane is apparent. (X6000).

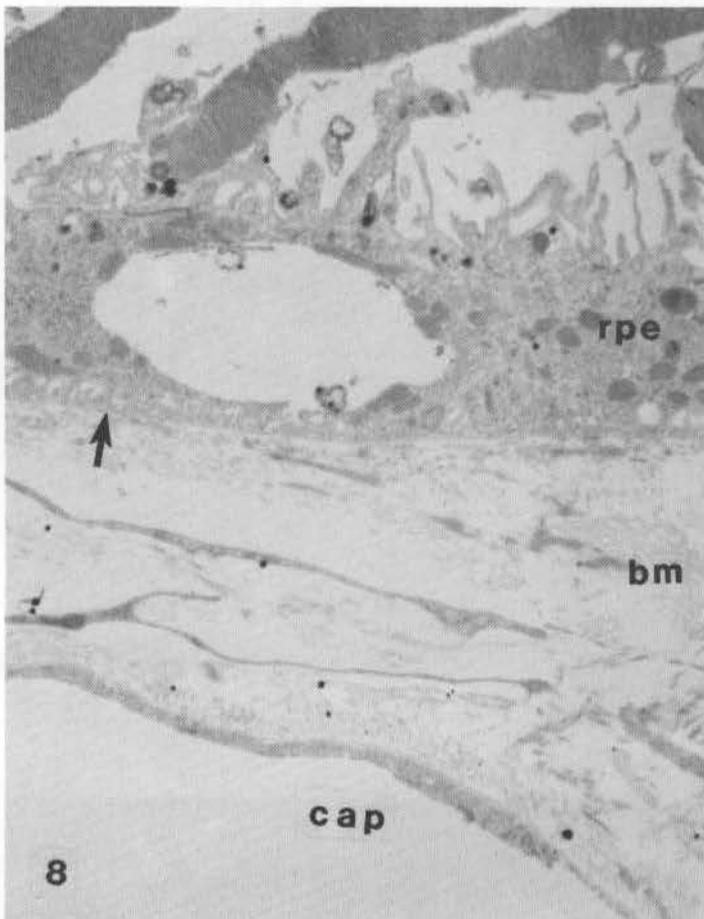


Figure 8. Electron micrograph showing vacuolation in retinal pigment epithelium (rpe) and thickening of Bruch's membrane (bm). The distance between the capillary (cap) and the basement membrane of retinal epithelium (arrow) is greatly increased. (X15000).

of the membrane at this point. In this study, we observed the drusenoid material being deposited between the pigment epithelium and the Bruch's membrane; and the pigment epithelium was displaced, almost intact, toward the subretinal space (Figure 1), especially in early stages. We did not observe any budding or evagination of any retinal pigment epithelium cells towards Bruch's membrane. We believe that drusenoid material is initially deposited by the affected retinal pigment epithelium cells. As more and more drusenoid material accumulated, the retinal pigment epithelium cell prayer was separated from its basement membrane. Later on, most likely because of this separation, the degeneration of retinal pigment epithelium cells contributed to this material. Although clinicopathological studies have convincingly shown the changes mentioned in other reports, it is difficult to rule out the effect of system diseases, i.e. diabetes, hypertension, and systemic infections in human patients. The changes observed in Fisher 344 rats used in this study are age-related. These rats were raised in a very controlled environ-

ment and were not subject to any treatment, therefore serving as a good model to study the age-related retinal degenerative processes. The most noticeable process associated with drusen was the loss of retinal pigment epithelium cells, leading to vacuolation and eventually to areolar atrophy. After the loss of pigment epithelial cells, the drusen disappeared, as reported by Green et al.<sup>18</sup> The increase in thickness of Bruch's membrane and/or the areolar atrophy of pigment epithelium is followed by choroidal neovascularization. Green and Key<sup>19</sup> reported 34.8% cases with areolar atrophy also had choroidal neovascularization. Although the exact cause of choroidal capillary ingrowth is not fully documented, Smiddy and Fine<sup>20</sup> suggested that the thickening of pigment epithelial basement membrane may stimulate choroidal vascular ingrowth. This may well be in response to failure of the elimination of metabolites and incomplete digestion of degenerating cell material. All of these factors may be directly or indirectly involved. In late or final stages, capillary ingrowth invades all of the retinal layers, which is followed by total degeneration of the retina. We hope that this report demonstrates some of the changes in the retina which are solely age-related. We have developed a method to extract the materials from sub-retinal and sub-pigment epithelial spaces. The chemical nature of these will soon be reported.

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**Table 1**  
Distribution of Drusen in Fisher 344 Rat Retina

Age in Months*	Male					Female						
	No.	Upper half Periph/Ctrl.	Lower half Periph/Ctrl.	Mean Occur.		No.	Upper half Periph/Ctrl.	Lower half Periph/Ctrl.	Mean Occur.			
9	28	8	3	13	4	5.6 ± 1.02	25	10	1	12	2	5.0 ± 1.025
12	31	9	5	14	3	6.2 ± 0.4	24	8	2	10	4	4.8 ± 1.16
18	34	10	5	14	5	6.8 ± 0.9	28	7	5	11	5	5.6 ± 1.35
24	38	12	6	17	3	7.6 ± 0.49	33	12	5	10	6	6.6 ± 1.43

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