Basal Linear Deposit and Large Drusen Are Specific for Early Age-Related Maculopathy

Christine A. Curcio, PhD; C. Leigh Millican

Objective: To determine the distributions of basal laminar and basal linear deposits in Bruch membrane (BM) with respect to age and early age-related maculopathy (ARM).

Methods: The foveas of 41 human eyes (<60 years [n = 9]; ≥60 years [n = 32]), preserved no later than 3.5 hours post mortem, were examined using light and electron microscopy. Ten eyes met histopathologic criteria of the Alabama Age-related Macular Degeneration Grading System for early ARM. We calculated the specificity, sensitivity, and odds ratios for the association of basal laminar and basal linear deposits with early ARM.

Results: Both deposits occurred only in eyes older than 60 years. The highest specificities and sensitivities for early ARM were attained for eyes that had basal linear deposits or large (>125 µm) drusen, followed by eyes with any quantity of basal laminar deposits that also contained membranous debris. Eyes with ARM were 24 times more likely than age-matched control eyes to have basal linear deposits or large drusen ($P = .002$).

Conclusions: Basal linear deposits and large drusen with membranous contents constitute different morphologic forms of the same ARM-associated lesion and may be significant for progression to late ARM.

Arch Ophthalmol. 1999;117:329-339

From the Departments of Ophthalmology (Dr Curcio and Ms Millican) and Physiological Optics (Dr Curcio), University of Alabama at Birmingham.
The authors have no proprietary interests in the products mentioned in this article.

AGE-RELATED maculopathy (ARM)[1] is a major cause of vision loss in the elderly.[2] Although its causes are poorly understood, it is agreed that the most prominent clinical and histopathologic features of ARM are lesions that involve retinal pigment epithelium (RPE) and Bruch membrane (BM). Bruch membrane is a 5-layer connective tissue sandwich interposed between the basal surface of the RPE and the choriocapillaris, the blood supply to the photoreceptors (**Figure 1**). Moving outward from the RPE, these layers are the RPE basal lamina (RPE-BL), the inner collagenous layer, the elastic layer, the outer collagenous layer, and the choriocapillaris BL. Debris in inner BM, variably called basal linear (BlinD)[3-5] or basal laminar deposits (BlamD),[6-10] have figured prominently in hypotheses of ARM pathogenesis for more than 2 decades.[3] Green and Enger[9] proposed that the terms BlamD and BlinD refer to 2 lesions with distinctive morphologic characteristics and positions relative to the RPE-BL (Figure 1). Basal laminar deposit consists primarily of fibrous long-spacing collagen (FLSC) and an amorphous material similar in electron density and texture to BL[3,4,7] located between the RPE and the RPE-BL (ie, internal to the RPE-BL). In contrast, BlinD consists primarily of membranous material located between the RPE-BL and the inner collagenous layer (ie, external to the RPE-BL). Similar membranous debris is also found in soft drusen, ie, large focal deposits with sloping sides that are also external to the RPE-BL.[6] Therefore, BlinD is sometimes referred to as diffuse drusen.[9] We herein use the generic term "basal deposits" to denote BlamD or BlinD.

The role of basal deposits in the development of late ARM, characterized by choroidal neovascularization (CNV) and geographic atrophy of the RPE, remains controversial. That eyes with large, soft, or confluent drusen are at risk for CNV has been established by clinical studies using fundus photographs.[11-13] In contrast, basal deposits are not visible in the fundus.[14] As inferred through postmortem histopathologic examination, the presence of BlinD is thought to place an eye at risk for late ARM.[14,15] Clinicopathological correlations have led to the following 3 related hypotheses about basal deposits: (1) Membranous debris in BM is associated with ARM[15]; (2) BlinD and soft drusen are diffuse and focal deposits, respectively, of the same membranous material[16]; and (3) BlamD is not specific for ARM, because its most prominent component, FLSC, is also found elsewhere.[3,7] Determining the relative risk for BlamD and BlinD is important for

SUBJECTS AND METHODS

Our results are based on the analysis of 41 eyes from 18 women and 24 men aged 17 to 92 years. Eyes were obtained from 34 donors and 7 patients requiring orbital exenteration for the removal of craniofacial tumors. No donors or patients were diabetic. Clinical records were obtained through contact with donor families and follow-up with ophthalmologists and optometrists. Use of human tissues and clinical records was approved by the institutional review board at the University of Alabama at Birmingham.

After removal of the cornea and lens, globes were fixed by immersion for a minimum of 24 hours in 0.1 mol/L phosphate-buffered 1% paraformaldehyde and 2.5% glutaraldehyde (n = 35), 4% paraformaldehyde and 0.5% glutaraldehyde (n = 5), or 4% paraformaldehyde (n = 2). Different fixatives gave similar results. Median time to preservation was 2 hours 25 minutes (range, 1 hour 5 minutes to 4 hours 2 minutes) for 34 donor eyes and 23 minutes (range, 6 to 54 minutes) for the 7 surgical specimens. Eyes were inspected grossly and photographed as previously described.[20] The macular retina, RPE, choroid, and sclera were divided horizontally with a razor just superior to the foveal depression. An approximately 2-mm-wide block containing the fovea was osmicated, dehydrated using ethanol and propylene oxide, and embedded in epoxy resin (Epon-Araldite; Electron Microscopy Sciences, Fort Washington, Pa) using standard procedures. Blocks were sectioned serially at 1 µm and stained with 2% toluidine blue in 2% sodium borate. Sectioning was terminated at the foveal center, recognized by the absence of ganglion cells and the presence of laterally traveling processes in the Henle fiber layer. Ultrathin (gold) sections were cut, stained with uranyl acetate and lead citrate, and examined using an electron microscope (JEOL1200 EXII; JEOL USA, Peabody, Mass). We scanned a single section (median length, 1.5 mm) through the foveal center of each eye along the entire RPE-BL at 5000× magnification with 10× binoculars. Representative photographs were taken at 5000× and printed at 15 000× magnification. Basal laminar deposit was considered present in an eye if FLSC or amorphous material was found internal to the RPE-BL. Basal linear deposit was considered present if irregular membranous debris was found just external to the RPE-BL.

Two to three 1-µm sections at least 60 µm apart through the foveal rod-free zone were evaluated for histopathologic features. We assigned semiquantitative grades to drusen size,[11,21] RPE changes,[11,13] and total basal deposits.[3,4,8,9] Focal deposits that raised the RPE to half of its typical height were considered drusen. Cross-sectional diameter was measured across the druse base, and individual foci were measured where drusen were confluent. Sections of 1 µm through eyes with ARM had numerous drusen (median, 8; range, 1-10). Not all drusen could be examined using electron microscopy, however, because drusen contents were sometimes extracted by processing.

Our overall strategy was to determine the number of eyes with early ARM or with no ARM, with and without ultrastructurally identified BlamD and BlinD, then subject these raw data to 2 different analyses. Both analyses are critically dependent on our case definition of early ARM. Maculopathy status was determined for all eyes using the histopathologic criteria of the Alabama Age-related Macular Degeneration Grading System.[20] To make our histopathologic case definition logically resemble those used by epidemiological studies for fundus appearance,[22-24] we use as primary criteria only features that are typically visible in the fundus (ie, drusen and RPE change) and not those that are visible only microscopically (ie, photoreceptor degeneration[9] and basal deposits[25]). The presence of basal deposits was used as a secondary criterion to distinguish ARM from other conditions with RPE change. Unlike epidemiological studies, we used drusen size rather than type (eg, hard or soft) as a primary criterion, because size is an objective measure, and most soft drusen are at least 63 µm.[14] Early ARM[20] was defined as the absence of late ARM (ie, CNV, geographic atrophy, disciform degeneration) and the presence of 1 druse greater than125 µm or severe RPE change (ie, heaping, migration, or atrophy) in any graded section. Eyes with RPE change also had to have 1 or more drusen of any size or a continuous layer of total basal deposits (ie, BlamD and BlinD combined). Ten eyes from donors or patients aged 60 to 90 years (mean age, 73.7 years) (**Table 1**) met these criteria.

Our first analysis was to test our 3 related hypotheses by calculating the specificity and sensitivity of BlamD and BlinD for early ARM.[26] These measures allow us to determine if either basal deposit is a good marker for true ARM. Such a marker would be a lesion or combination of lesions that yielded high specificity, ie, absence from eyes without ARM, and high sensitivity, ie, presence in many eyes with ARM. We computed these values using the following formulas:

$$\text{Specificity} = d/(b + d)$$
$$\text{Sensitivity} = a/(a + c)$$

where *a* indicates the number of eyes with ARM and with deposits present; *b*, without ARM and with deposits present; *c*, with ARM and with deposits absent; and *d*, without ARM and with deposits absent. We did not compute these values for eyes with only large drusen, because these lesions were part of the case definition, and therefore specificity and sensitivity are expected to be high. The first hypothesis (that membranous debris is associated with ARM) predicts that the highest specificities will belong to lesions containing membranous debris. The second hypothesis (that BlinD and large drusen are 2 forms of the same lesion) predicts that the sensitivities calculated using the total number of eyes containing BlinD and large drusen should be greater than the values calculated using the number of eyes with either lesion alone, provided that large drusen contain membranous debris. The third hypothesis (that BlamD is not associated with ARM) predicts that the specificity for any quantity of BlamD will be low.

Our second analysis was to estimate the associations between histopathologically defined ARM and basal deposits and drusen, using odds ratios (OR) and 95% confidence intervals (CI).[27-29] Odds ratios were calculated using the following equation:

$$(a \times d)/(b \times c)$$

The OR is a measure of comparative risk that compares the odds that ARM occurs among persons with a particular characteristic with the odds that ARM occurs among those lacking the characteristic. This analysis enabled us to calculate the strength and statistical significance of associations and compare them for individual or combined lesions. Because of the small sample size, exact methods were used to calculate CIs and *P* values. All statistical tests were conducted at the .05 level (2-sided).

identifying the fundus markers of the highest-risk lesions and for guiding development of animal models of ARM.

Testing hypotheses about the specificity of different basal deposits requires demonstrating their relative absence from eyes without ARM and their presence in eyes with ARM. Electron microscopy is required to distinguish BlamD from BlinD definitively,[3,4,7-9,14,17] and unfortunately, studies that examined eyes without ARM using electron microscopy had few total specimens or few eyes with ARM or did not look for both basal deposits.[3,4,18,19] In our study, we identified basal deposits using electron microscopy in eyes with ARM, age-matched eyes without ARM, and young donor eyes. All eyes were quickly preserved and specifically prepared for electron microscopy to reduce fixation and processing artifacts. Diagnosis of ARM was ascertained using criteria for fundus appearance and histopathologic characteristics. We confirmed the hypothesis that BlinD and soft drusen are 2 forms of the same ARM-associated lesion.

RESULTS

An electron micrograph from an eye lacking basal deposits (**Figure 2**, A) shows the basal surface of the RPE and the 3 inner layers of BM. The ultrastructure of BlamD is illustrated in Figure 2, parts B to F, and its component frequencies in the 41 eyes are summarized in the following tabulation:

Component	No. of Eyes
FLSC	25
Amorphous light material	25
Amorphous dark material	25
Membranous debris	14
Pigmented debris	3
Space	13
Total with BlamD	26

The most frequently seen components of BlamD were FLSC (Figure 2, B) and an amorphous basement membranelike material that had 2 distinct electron densities. The less dense material (Figure 2, C) was identical to normal BL in electron density and texture, and it typically enveloped the denser material (Figure 2, C). Membranous debris identical to material accumulating external to the RPE-BL was also present within BlamD (Figure 2, D and F). Atypical BlamD at the sloping margin of 1 large druse[16] contained mostly amorphous material and electron-lucent circular profiles (Figure 2, E). A narrow rim of electron-lucent space occasionally surrounded other BlamD components but never occupied more than a small fraction of the area between the RPE and the RPE-BL (Figure 2, B).

Of ultrastructurally detectable, diffuse abnormalities external to the RPE-BL, the 2 most common features were membranous debris and non–membrane-bounded electron-lucent droplets. These findings are illustrated in **Figure 3** and **Figure 4** and summarized for the 38 gradable eyes in the following tabulation:

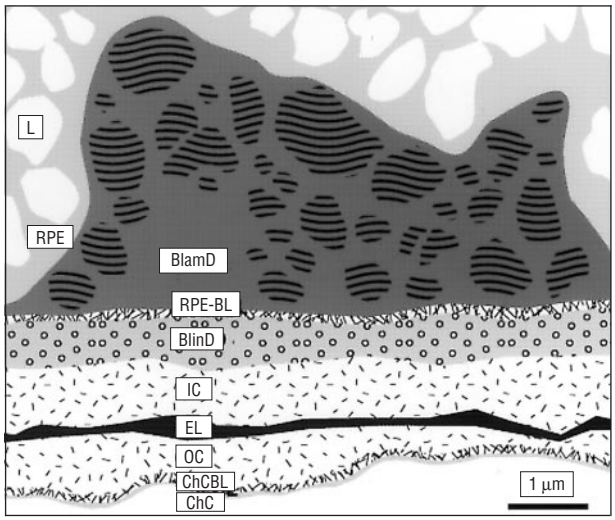

Figure 1. *Schematic of retinal pigment epithelium (RPE), Bruch membrane (BM), and basal deposits. L indicates lipofuscin granules. For layers of BM, RPE-BL indicates RPE basal lamina; IC, inner collagenous layer; EL, elastic layer; OC, outer collagenous layer; and ChCBL, choriocapillaris basal lamina. For deposits, BlamD indicates basal laminar deposit, internal to (above) RPE-BL; BlinD, basal linear deposit, external to (below) RPE-BL.*

Table 1. Eyes With Early ARM*

Patient No./ Sex/Age	Eye	OE	LED, mo	VAcc	Lens†	Phot/ FA‡	Significant Macular History§	Status of Fellow Eye	
								Chart	ALARMDGS
1/M/60	R	Yes	0.0	20/30	2	x/–	Drusen, mild ARM	Yes	NA
2/F/63	L	No	9.7	20/25	2	x/–	Dry ARM	Yes	Yes
3/M/64	L	No	NA	NA	NA	NA	No visual complaint as per family	NA	Yes
4/M/74	R	No	23.1	20/40	3	x/x	Drusen and/or pigment changes	Yes	Yes
5/M/74	L	No	18.7	20/20	2	x/–	Dry ARM, drusen, pigment clumping	Yes	No
6/M/74	R	Yes	0.0	20/50	3	x/–	Probable ARM, pigment clump, no drusen	Yes	NA
7/M/75	L	Yes	0.6	20/60	2	NA	Drusen	Yes	NA
8/M/80	L	Yes	0.3	20/40	0	x/–	Few drusen, areas RPE atrophy	Yes	NA
9/F/83	R	No	7.0	20/60	0	NA	Giant drusen, ARM	Yes	Yes
10/F/90	R	No	NA	NA	NA	NA	NA	NA	Yes

**ARM indicates age-related maculopathy; OE, orbital exenteration; LED, last examination before death or surgery; VAcc, visual acuity, corrected; Phot/FA, color fundus photographs or fluorescein angiogram; ALARMDGS, postmortem fundus findings using Alabama Age-related Macular Degeneration Grading System[20]; R, right; L, left; NA, not available; and RPE, retinal pigment epithelium.*

†0 indicates clear natural lens or posterior chamber intraocular lens; 2, moderate cataract; and 3, significant cataract.

‡x indicates yes; minus, no.

§Indicates quotes from chart.

Abnormalities	No. of Eyes
Membranous debris	9
Electron-lucent droplets	13
Heterogeneous debris	3
Pigmented debris	1
Cells	2
Other	6
Total eyes with changes	20

Heterogeneous debris resembles contents of hard drusen[6,30]; "other", includes fluid (n = 3), FLSC, reduplicated BL, and amorphous granular material.

Closed membranous profiles with empty interiors (Figure 3, A and B), typical of BlinD,[9,15] were large, irregular, and variable in size (mean diameter, 135 nm; range, 33-267 nm). They resembled the debris in some large drusen (Figure 3, B), but formed a thin sublamina external to the RPE-BL (Figure 3, A, and Figure 4, A and C) rather than a mound. Eyes with BlinD also had membranous debris within BlamD internal to the RPE-BL

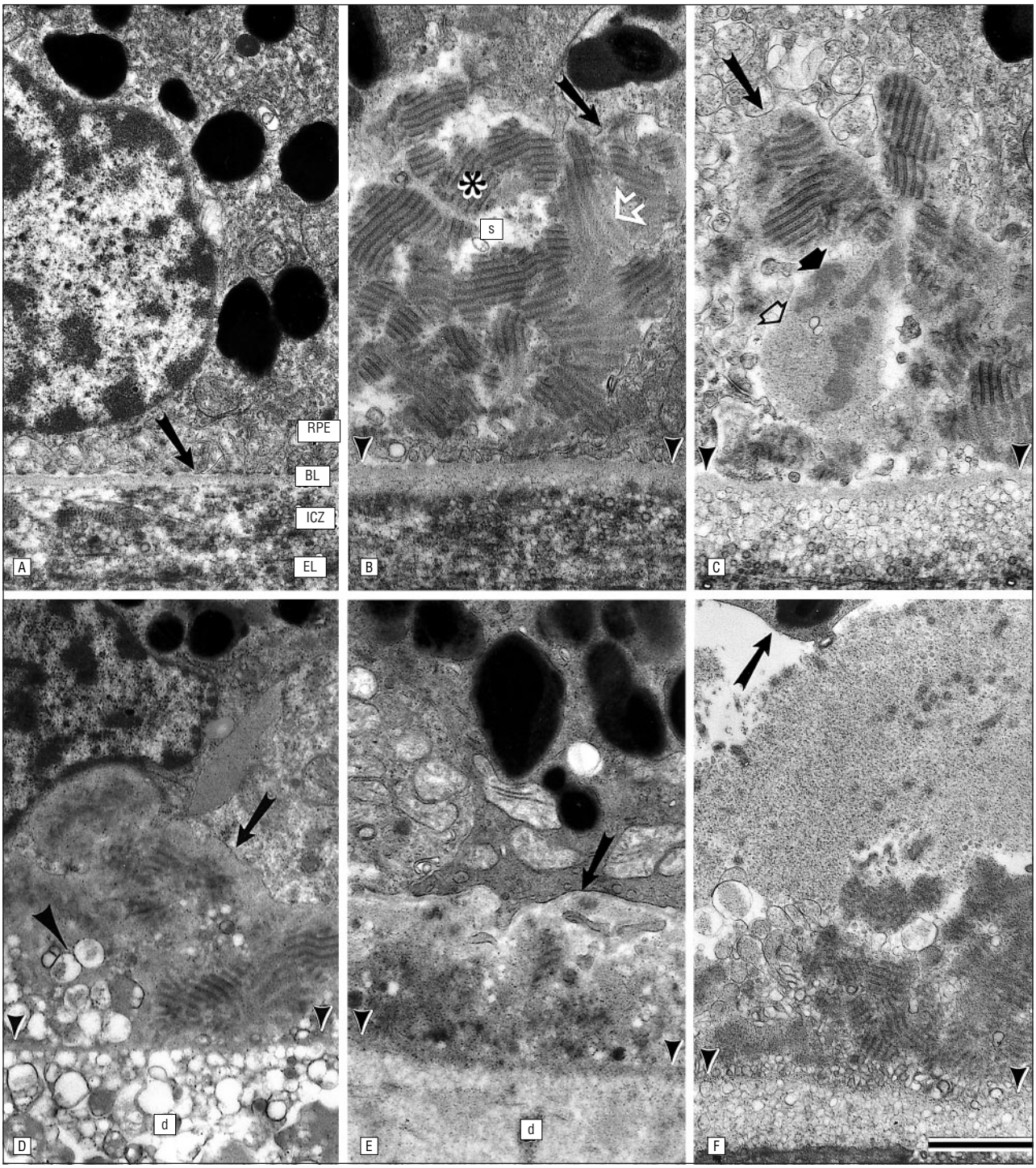

Figure 2. *Ultrastructure of basal laminar deposit (BlamD). Retinal pigment epithelium (RPE) is at the top of all panels. Black arrows indicate RPE plasma membrane; black arrowheads in parts B to F, RPE basal lamina (RPE-BL); d, druse (superficial surface only); s, electron-lucent space; and bar, 1 µm. A, Normal Bruch membrane (See Figure 1 for explanation of abbreviations). B, Asterisk indicates fibrous long-spacing collagen (FLSC); white arrow, obliquely sectioned FLSC. C, Black and open wide arrows indicate amorphous material of 2 discrete electron densities. D, Long arrowhead indicates membranous debris. E, Sloping margin of a large druse contains flocculent material, presumably fluid.[16] F, BlamD contains FLSC and membranous debris near the RPE-BL and amorphous material and coated vesicles near the RPE.*

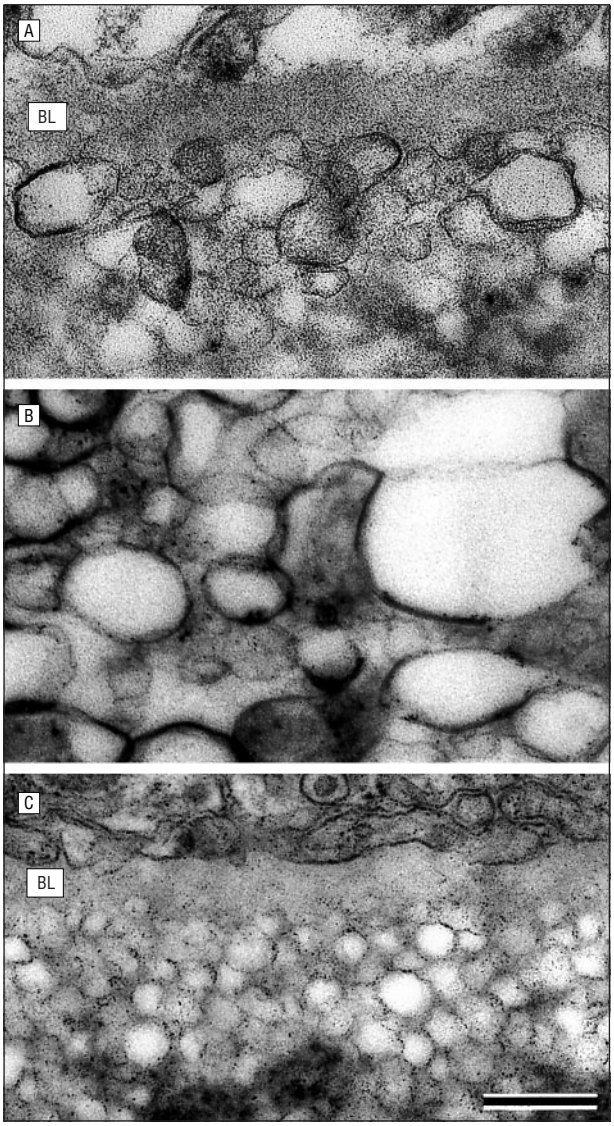

Figure 3. *Ultrastructure of membranous debris and electron-lucent droplets. Retinal pigment epithelium (RPE) is at the top of panels A and C. BL indicates RPE basal lamina; bar, 0.25 µm. A, Diffusely distributed membranous debris. B, Membranous debris in a small druselike mound. C, Non–membrane-bounded droplets.*

(n = 7) (Figure 4, C and E), sometimes forming distinctive linear tracks (Figure 4, E). Eyes without a separate layer of BlinD had isolated membranous profiles only within BlamD (n = 4). Retinal pigment epithelial somata also contained debris as individual profiles (Figure 4, C) or within large vacuoles (**Figure 5**) (n = 3).

In contrast to membranous debris, electron-lucent droplets lacked a distinct membrane, but were occasionally surrounded by a single thin electron-dense line (Figure 3, C). They were also smaller, rounder, and more uniform in size (mean diameter, 75 nm; range, 33-117 nm) than debris profiles. Droplets were rarely seen on the internal side of the RPE-BL (Figure 4, D), but they were scattered throughout both collagenous layers of most eyes older than 40 years. Droplets formed a distinct sublayer just external to the RPE-BL in 13 eyes (Figure 3, C, and Figure 4, D). Some eyes (n = 5) had 2 discrete sublayers of membranous debris and droplets, with the debris closer to the RPE-BL (Figure 3, A, and Figure 4, C). In 2 eyes, droplets were embedded in moderately electron-dense homogenous substance, forming a diffuse (Figure 4, B) or small focal deposit.

By comparing the appearance of basal deposits in ultrathin and adjacent 1-µm sections, we determined that BlamD can be detected reliably using light microscopy, but BlinD cannot (**Figure 6** and **Figure 7**). In 1-µm sections (Figure 6, A), BlamD is a bushy light blue material between the base of the lavender RPE somata and the pale edge of RPE-BL,[4] forming isolated patches or a continuous layer (Figure 6, B-D). In contrast, BlinD forms a burgundy or desaturated grayish pink layer external to the RPE-BL, tinctorially distinct from RPE cytoplasm, BlamD, and the inner collagenous layer (Figure 7). Basal linear deposit was most easily detectable when it was continuous with drusen (Figure 7, A and B) or obviously irregular (Figure 7, C and D). Basal linear deposit was difficult to detect in 1-µm sections when it formed a thin smooth layer that blended in with the RPE-BL (Figure 7, E and F). In no case, however, was an abnormality in the inner collagenous layer graded as BlamD or vice versa by light microscopy.

To justify combining eyes with BlinD and eyes with large drusen (>125 µm) in the calculation of lesion specificity and sensitivity for ARM, we first demonstrated that drusen in these eyes contain membranous debris (**Figure 8**). Large drusen in 6 eyes resembled descriptions of soft drusen.[16] All drusen examined in these eyes contained heterogeneous vesicular profiles similar to those in BlinD but larger (Figure 8, A), as well as varying proportions of other components. These components included lakes of a homogeneous, moderately electron-dense material (Figure 8, B and C) and intermixed electron-dense granules and circular electron-lucent spaces[31] (Figure 8, D). Large drusen in a seventh eye had progressed to almost complete calcification[15] (not shown[20]) and did not contain membranous debris.

Both basal deposits increased with age (**Table 2**), as they were ultrastructurally detectable only in eyes 60 years and older. Within the older group, however, their distributions were markedly different. Basal laminar deposit was present in 26 (81%) of eyes, and BlinD in only 9 (28%). There also was evidence of an age-related increase within the older eyes. Basal laminar deposit was present in 8 (73%) of seventh-decade eyes and 7 (88%) of ninth-decade eyes. Percentages of eyes with BlinD are less reliable due to smaller numbers, but 4 (50%) of ninth-decade eyes contained BlinD.

Specificities, sensitivities, and ORs for basal deposits in eyes older than 60 years are shown in **Table 3**. As stated earlier, our goal was to find a lesion or combination of lesions with high specificity and sensitivity for ARM. In 3 eyes with ARM, the inner collagenous zone split, and its contents washed out during processing. Although we could infer the presence of BlinD from indentations along the RPE-BL, we did not include these eyes in our calculations to ensure that hypothesis testing was conservative. Three of the 4 most specific lesions (0.68-0.73) contained membranous debris (BlinD, BlinD or large drusen, and BlamD with membranous debris). The fourth specific lesion (0.83) was a continu-

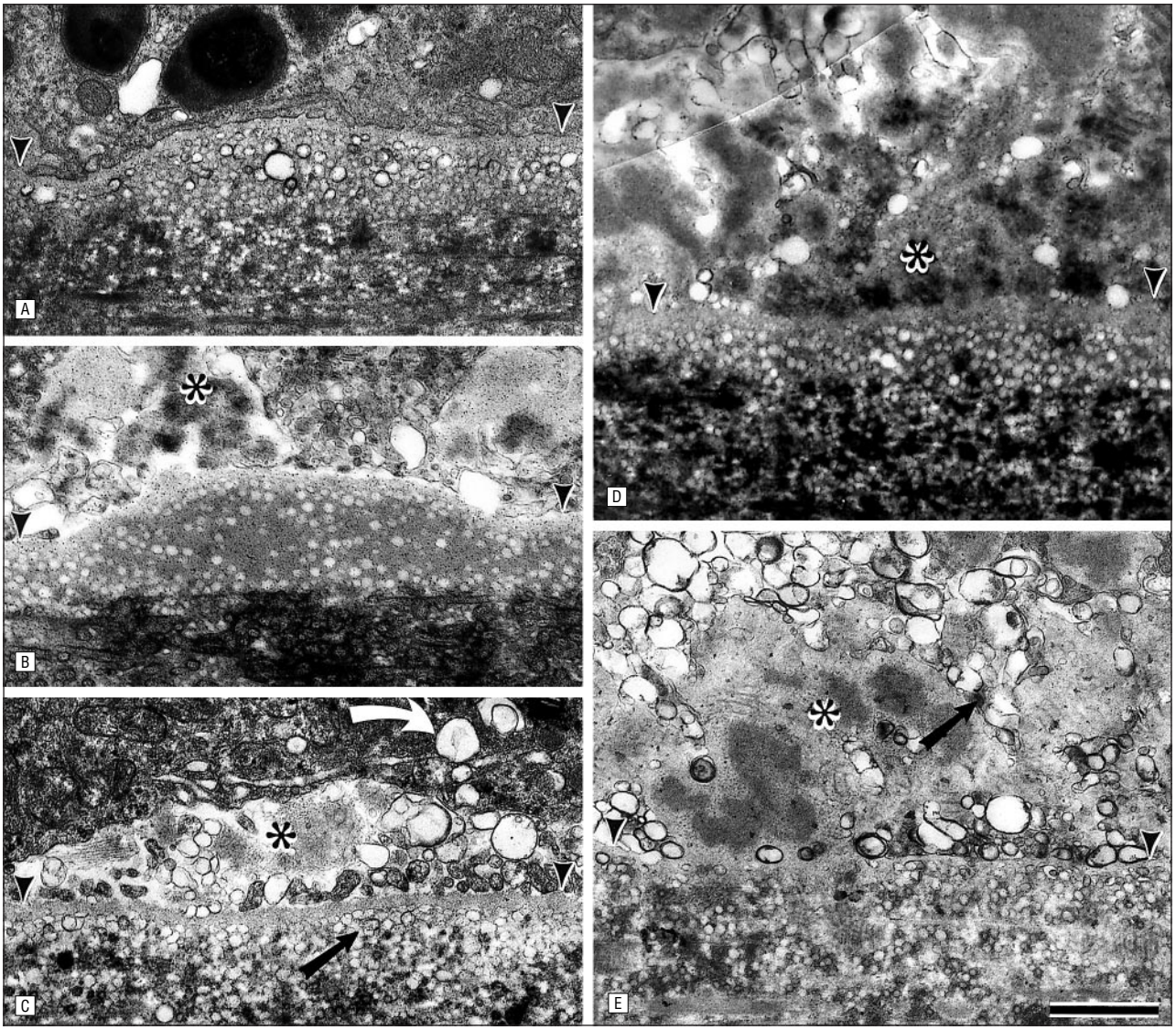

Figure 4. *Membranous debris, electron-lucent droplets, and basal laminar deposits (BlamD). Black arrowheads indicate retinal pigment epithelium basal lamina (RPE-BL); asterisks, BlamD; and bar, 1 µm. A, Basal linear deposits. B, Droplets within a moderately electron-dense substance. C, Membrane-bounded profiles within RPE (white arrow) and external to the RPE-BL (black arrow). D, Rows of electron-lucent droplets external to the RPE-BL. E, Tracks of membranous profiles (arrow) are present within BlamD.*

ous layer of BlamD. Of these 4, the highest sensitivity was found for eyes that had BlinD or large drusen (0.90). The second highest sensitivity was found for eyes with any quantity of BlamD that also contained membranous debris (0.70). Basal linear deposit alone or a continuous layer of BlamD, both of which were quite specific for early ARM, were found in only some eyes with ARM, and therefore had only moderate sensitivity (0.43 and 0.30, respectively). Finally, the specificity of 0.18 for any quantity of BlamD was the lowest observed, despite the fact the sensitivity of this lesion was high (0.80) due to its presence in many eyes with ARM.

The calculation of ORs (Table 3) emphasized the strong association between early ARM and BlinD or large drusen. Eyes with ARM were 24 times more likely than age-matched control eyes to have these lesions ($P = .002$). Although the 95% CI is wide due to the small number of eyes, its lower bound (3.52) is much larger than unity, indicating a reliable effect. Also consistent with the specificity calculations, eyes with ARM were 5 times more likely than controls to have BlamD that contained membranous debris, but this effect did not reach significance ($P = .06$). Odds ratios for other lesions did not achieve significance.

COMMENT

Our results support the hypothesis that BlinD and large drusen with membranous contents constitute different morphologic forms of the same ARM-associated lesion.[9,14,15] Electron microscopy enabled us to detect small basal deposits, and until a marker molecule is found for BlinD, this specific lesion is best identified by this method. Because our case definition for early ARM used only features visible in the fundus as primary criteria, we conclude that large drusen and severe RPE changes are fundus markers for membranous debris. By establishing membranous debris as a salient feature of ARM, our results also have implications for assessing the validity of animal models, which to date have

exhibited BM abnormalities resembling BlamD but not BlinD.[32-34] To our knowledge, specificity and sensitivity have not been calculated in previous histopathologic studies of ARM, and therefore it is important to consider assumptions and limitations of our analysis. First, determining that membranous debris is strongly associated with early ARM does not prove causality. In fact, it is more likely that membranous debris is a specific manifestation signifying that the RPE has been sufficiently damaged[4] by other processes to produce funduscopically visible lesions. Second, our conclusions regarding specificity are based on the relative, not complete, absence of BlinD from eyes without ARM. It is possible that the few eyes without ARM and with membranous debris actually had incipient ARM that did not meet the criteria of large drusen or severe RPE change. Third, ARM may be a group of genetically heterogeneous diseases.[35,36] That 1 lesion is highly specific (absent from eyes without ARM) and highly sensitive (present in many eyes with ARM) can indicate that 1 ARM genotype dominated the sample or that basal deposits constitute a final common phenotype for multiple ARM genotypes. Our analysis cannot distinguish between these possibilities.

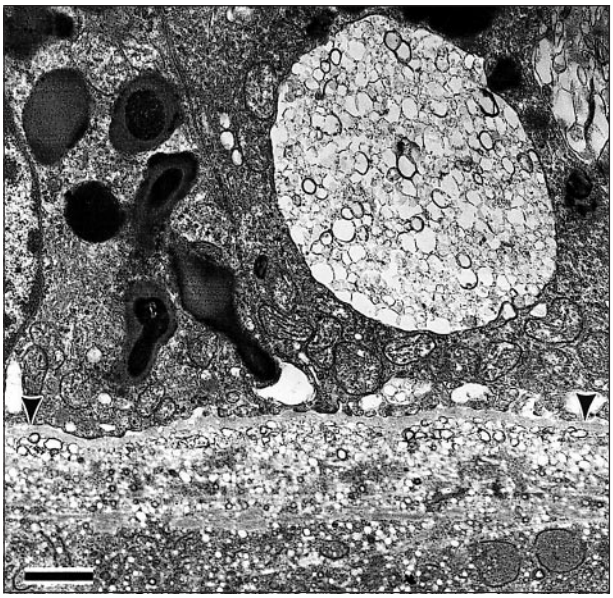

Figure 5. *Vacuole of membranous debris in retinal pigment epithelium (RPE) cytoplasm. Arrowheads indicate RPE basal lamina; bar, 2 µm.*

Our data are consistent with the idea that membranous debris in diffuse or focal forms places an eye at risk for visual loss due to late ARM.[14,15,32] After early uncertainty about the plane invaded by choroidal blood vessels in ARM,[3,17,37,38] it is now clear that they ramify in the plane of BlinD and drusen (ie, external to the RPE-BL) and not in the plane of BlamD.[16,25,39-42] Previous histopathologic studies did not determine the relative risk associated with BlinD and BlamD, as these disagree on what proportion of eyes with ARM and CNV also have membranous debris.[9,39] In 2 large series of surgically excised neovascular membranes,[43,44] BlamD was present in virtually all ARM specimens, and BlinD was present in very few ARM specimens. However, the group without ARM was younger than patients with ARM, and therefore specificity could not be established for basal deposits in the absence of age matching. Furthermore, in intact eyes and surgically excised membranes, BlinD may be underestimated due to its low detectability using routine histological methods.[9,16,45] Fi-

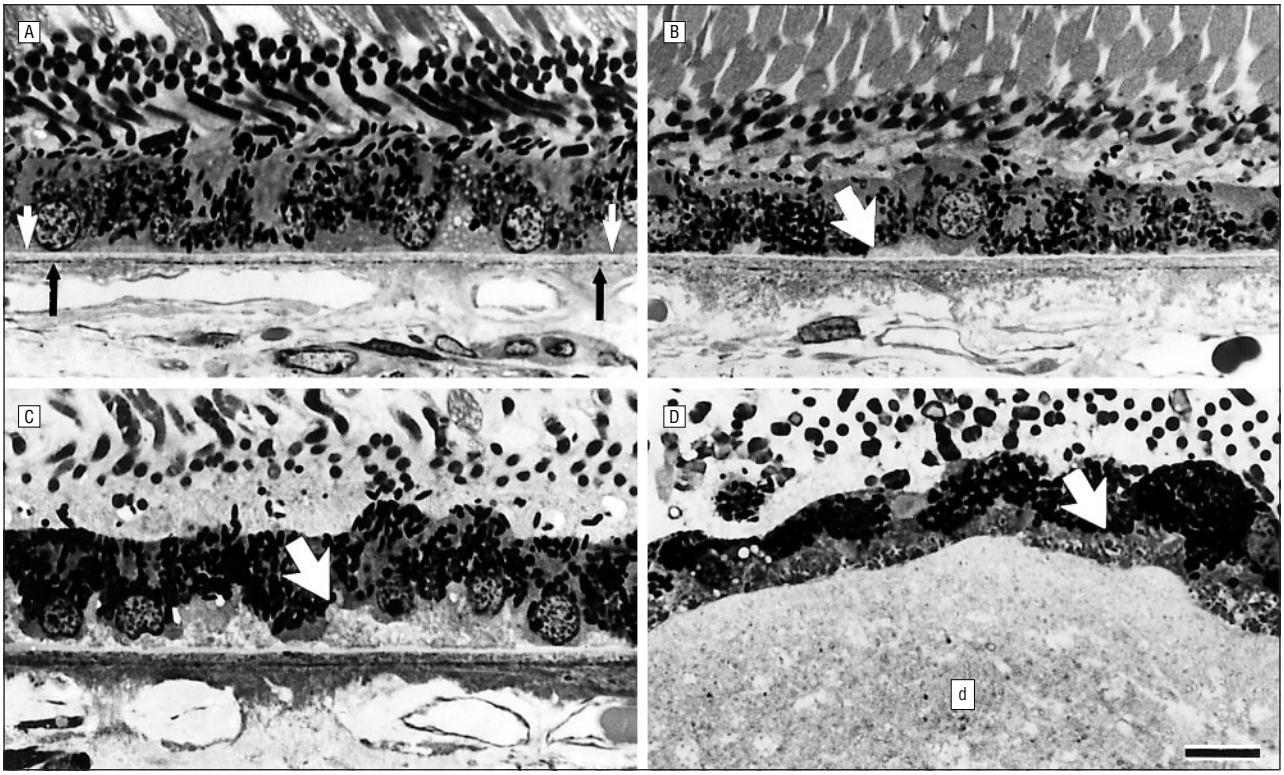

Figure 6. *Bruch membrane (BM) and basal laminar deposit (BlamD) in toluidine-blue–stained 1-µm sections. Bar indicates 10 µm. A, Normal retinal pigment epithelium (RPE) and BM. White arrows indicate the RPE basal lamina; black arrows, the elastic lamina. B, Patch of BlamD (arrow). C, Thin continuous layer of BlamD (arrow). D, BlamD (arrow) overlying a druse (d) 150 µm in diameter.*

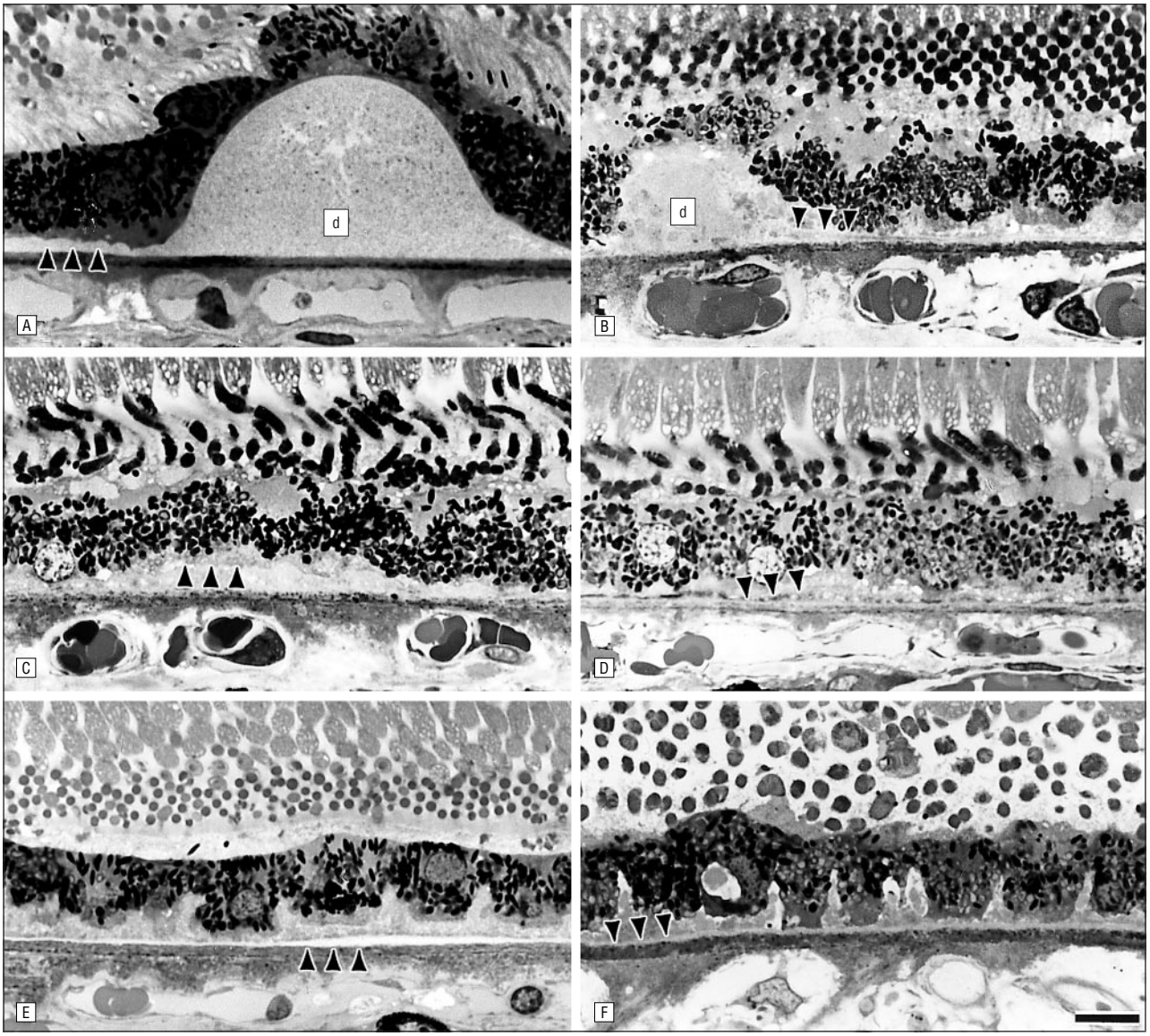

Figure 7. *Basal linear deposit (BlinD; triple arrowheads) in toluidine-blue–stained 1-µm sections. Bar indicates 10 µm. A and B, Continuous with small drusen (d). C and D, Irregularly thickened layer. E and F, Blended with retinal pigment epithelium basal lamina.*

nally, BlinD may be degraded by vessels growing in the plane external to the RPE-BL, lowering its detectability even further. The mechanisms by which membranous debris facilitates CNV are unknown, but may include providing a cleavage plane for opportunistic vascular buds[16] or providing factors that stimulate and/or attract cells.[46,47]

Our morphologic observations of BlinD are consistent with previous reports of a layer of membrane-bounded, round-to-oval, electron-lucent profiles external to RPE-BL in normal eyes and those with ARM.[16,18] Membranous debris is reportedly confined to central macula, and its occasional presence in the subretinal space suggests that it is ultimately derived from photoreceptor outer segments.[6,15,25,46] On the basis of our results, we suggest that the RPE produces membranous debris and deposits it in BM in the following 2 ways: a steady accumulation of individual vesicles[15,18] resulting in diffuse deposits (BlinD) and an episodic delivery of large vacuoles[15,31,48] resulting in focal deposits (soft drusen). The balance between both mechanisms presumably determines whether an eye has soft drusen, BlinD, or both.[6,9] Sub-RPE material other than membranous debris may exist in focal and diffuse form, including an electron-dense, finely granular substance with numerous droplets (Figure 3, C[4,14,40]) and debris seen in type II mesangiocapillary glomerulonephritis.[49] The amorphous material accompanying membranous debris in large drusen is suggestive of yet another process involved in drusen formation. Irregular lakes of a moderately electron-dense material, previously interpreted as disintegrating hard drusen,[25] appear to grow in situ by aggregation of debris or other components.

Our morphologic observations in well-preserved eyes confirmed that FLSC and amorphous basal lamina–like material in 2 electron densities are major components of BlamD.[3,4,7,18,48,50,51] Electron-lucent space, a previously described component,[4,48,52] was minimal, perhaps because our specimens were opened quickly after death and pre-

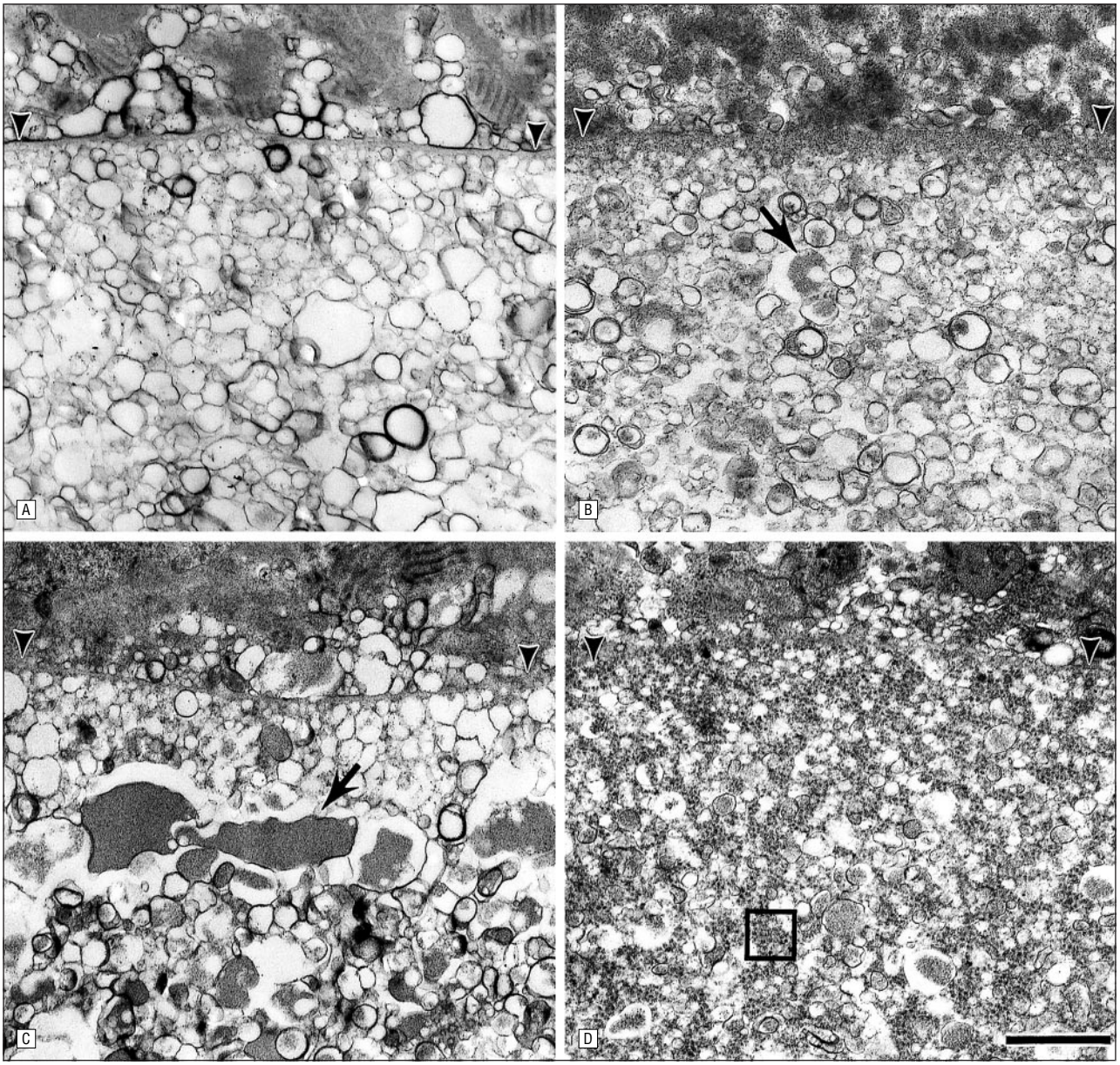

Figure 8. *Contents of large drusen. Retinal pigment epithelium (RPE) is at top of all panels. Arrowheads indicate RPE basal lamina; bar, 1 µm. A, Membranous debris. B and C, Homogeneous material (arrow) between membranous debris. D, Membranous debris, homogeneous material, small dense granules (within box), and electron-lucent circular spaces.*

Table 2. Distribution of Basal Deposits With Age*

		No. (%)	
Age, y	**Total**	**BlamD**	**BlinD**
<60	9	0 (0)	0 (0)
60-70	11	8 (73)	3 (27)
71-80	13	11 (85)	2 (20)†
81-92	8	7 (88)	4 (50)
Total ≥60	**32**	26 (81)	9 (28)

**BlamD indicates basal laminar deposit; BlinD, basal linear deposit.*
†Three eyes were not gradable for BlinD.

served. Of the various forms of BlamD examined, only BlamD containing membranous debris had high sensitivity and specificity for early ARM. A continuous layer of BlamD, irrespective of debris content, was relatively specific[45] but was poorly sensitive (ie, present in a few eyes with early ARM). Although having any amount of BlamD was nonspecific, it is important to note that 8 of 9 eyes with ARM and BlinD or large drusen also had at least some BlamD. The ninth eye, with calcified drusen, was at a more advanced disease stage, when BlamD disappears.[3]

Our data are consistent with a 3-phase model of basal deposit processing that reflects the following escalating levels of RPE damage[3,4,19,48]: secretion of the less dense BL-like material by the RPE; polymerization or condensation of smaller molecules to produce FLSC and the denser amorphous material; and finally, release of membranous debris, at which point lesions are visible in the fundus. The stimulus that initiates BlamD secretion is unknown, but may be related to changes in filtration caused by the age-related ac-

Table 3. Sensitivity and Specificity of Basal Deposits and Drusen for ARM*

	BlinD† or Large Drusen‡		BlamD With Membranous Debris		Continuous BlamD§		BlinD†		Any BlamD	
	ARM	Non-ARM	ARM	Non-ARM	ARM	Non-ARM	ARM	Non-ARM	ARM	Non-ARM
Present, No.	9	6	7	7	3	4	3	6	8	18
Not present, No.	1	16	3	15	7	19	4	16	2	4
Specificity	0.73		0.68		0.83		0.73		0.18	
Sensitivity	0.90		0.70		0.30		0.43		0.80	
Odds ratio	24.00		5.00		2.00		2.00		0.90	
95% Confidence interval	3.52-163.75		1.02-24.47		0.36-11.57		0.33-11.87		0.03-6.07	
P	.002		.06		.65		.64		.99	

*ARM indicates eyes with age-related maculopathy; BlinD, basal linear deposit; and BlamD, basal laminar deposit.
†Only 7 of 10 eyes with ARM gradable for BlinD.
‡*n* = 7, including 1 with calcified drusen.
§Using light microscopy.

cumulation of debris in BM.[15,19] Our data also support the idea that the significance of BlamD in ARM pathogenesis lies in predisposing inner BM to further abnormalities.[52] We suggest that BlamD facilitates the passage of membranous debris into the inner collagenous zone by separating the RPE from its BL, which is normally closely apposed.

On the basis of our results, we make 3 suggestions regarding the nomenclature of basal deposits. First, we caution against using the term "BlinD" to denote a lesion in a specific location (ie, external to the RPE-BL) and its principal component, membranous debris. This component can be located elsewhere (ie, internal to the RPE-BL), and other material can occur in diffuse deposits external to the RPE-BL.[3,6,17,25] It has been similarly argued that position takes precedence over components in naming BlamD and its most prominent component, FLSC.[53] Second, we encourage the use of the term "diffuse drusen" for BlinD. This term, the meaning of which has evolved[32,38,52] to its present synonymity with BlinD,[9] emphasizes the similarities between BlinD and soft drusen in origin, fine structure, location, association with RPE changes, and risk for advanced ARM.[6,14] Diffuse drusen is also less likely to be confused with BlamD than BlinD. Although BlinD and BlamD are probably both products of the RPE, they differ in other important respects, a fact belied by the similarity of their present names. Finally, a recent proposal[54] to rename BlamD basement membrane deposit to emphasize its resemblance to basement membranes merits serious consideration.

A new observation is the association of the earliest stages of BlinD formation with non–membrane-bounded, electron-lucent droplets distinct in size and morphologic characteristics from membranous debris. Found only in eyes older than 40 years, droplets were common in both collagenous layers,[19,55,56] forming rows external to the RPE-BL.[55] We suggest the following 3 possible sources for droplets: membrane-bounded packets of RPE cytoplasm,[19,30,31,55] disintegration of membranous debris, or aggregation of blood-borne substances in association with BM extracellular matrix components.[57] Although further study is required to determine the source of droplets, our results point with renewed emphasis to the hypothesized role of lipids in ARM pathogenesis. Neutral fats and phospholipids appear in BM in midlife and are present in some drusen and basal deposits.[56,58-60] It has been proposed that lipid accumulation renders BM hydrophobic, impeding the outward flow of ions and fluid from the retina[56,61] and ultimately leading to RPE and photoreceptor dysfunction.[62,63] The ultrastructural correlates of neutral fats and phospholipid-rich deposits in BM are not known, because lipids are not well-preserved in routine tissue processing.[64] Even in our conventionally processed material, however, membranous debris and droplets resemble the extracellular lipid-rich material in developing atherosclerotic plaques.[65-67] A high proportion of lipids in membranous debris and droplets would explain the presence of empty space in BlamD and the inner collagenous layer, the fragility of soft drusen, and the poor correlation between ultrastructure and histochemically determined BM lipid content.[4,16,17,56] A proper evaluation of the lipid hypothesis requires the improved morphologic examination that non–lipid-extracting ultrastructural methods could provide.[64]

Accepted for publication November 9, 1998.

Supported by grant EY06109, National Institutes of Health, Bethesda, Md, and Research to Prevent Blindness Inc, New York, NY.

We thank the Alabama Eye Bank, Birmingham (A. Patrick, Technical Services Director), for timely retrieval of donor eyes. One donation was obtained from the Montana Eye Bank, Missoula. Surgical specimens were obtained through the Eye Foundation Hospital and the Tissue Procurement Program of the University of Alabama at Birmingham Comprehensive Cancer Center (NIH grant CA13148). G. McGwin, Jr, consulted on data analysis (NIH grant AG04212). Ramon F. Dacheux, PhD, provided guidance on the use of the Department of Ophthalmology Electron Microscopy Facility.

Reprints: Christine A. Curcio, PhD, Department of Ophthalmology, University of Alabama at Birmingham, 700 S 18th St, Birmingham AL 35294-0009 (e-mail: curcio@vision.vsrc.uab.edu).

REFERENCES

1. Bird AC, Bressler NM, Chisholm IH, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. *Surv Ophthalmol.* 1995;39:367-374.
2. Leibowitz HM, Maunder LR, Milton RC, et al. The Framingham Eye Study Monograph. *Surv Ophthalmol.* 1980;24(suppl):335-610.
3. Sarks SH. Ageing and degeneration in the macular region: a clinico-pathological study. *Br J Ophthalmol.* 1976;60:324-341.
4. Loeffler KU, Lee WR. Basal linear deposit in the human macula. *Graefes Arch Clin Exp Ophthalmol.* 1986;224:493-501.
5. Feeney-Burns L, Burns RP, Gao C-L. Age-related macular changes in humans over 90 years old. *Am J Ophthalmol.* 1990;109:265-278.
6. Sarks SH. Drusen and their relationship to senile macular degeneration. *Aust J Ophthalmol.* 1980;8:117-130.
7. van der Schaft TL, de Bruijn WC, Mooy CM, Ketelaars DA, de Jong PT. Is basal laminar deposit unique for age-related macular degeneration? *Arch Ophthalmol.* 1991;109:420-425.
8. van der Schaft TL, Mooy CM, de Bruijn WC, et al. Histologic features of the early stages of age-related macular degeneration. *Ophthalmology.* 1992;99:278-286.
9. Green WR, Enger C. Age-related macular degeneration histopathologic studies: the 1992 Lorenz E. Zimmerman Lecture. *Ophthalmology.* 1993;100:1519-1535.
10. Rosa RH Jr, Thomas MA, Green WR. Clinicopathologic correlation of submacular membranectomy with retention of good vision in a patient with age-related macular degeneration. *Arch Ophthalmol.* 1996;114:480-487.
11. Bressler SB, Maguire MG, Bressler NM, Fine SL. Relationship of drusen and abnormalities of the retinal pigment epithelium to the prognosis of neovascular macular degeneration. *Arch Ophthalmol.* 1990;108:1442-1447.
12. Bressler NM, Munoz B, Maguire MG, et al. Five-year incidence and disappearance of drusen and retinal pigment epithelial abnormalities. *Arch Ophthalmol.* 1995;113:301-308.
13. Klein R, Klein BEK, Jensen SC, Meuer SM. The five-year incidence and progression of age-related maculopathy. *Ophthalmology.* 1997;104:7-21.
14. Bressler NM, Silva JC, Bressler SB, Fine SL, Green WR. Clinicopathological correlation of drusen and retinal pigment epithelial abnormalities in age-related macular degeneration. *Retina.* 1994;14:130-142.
15. Sarks JP, Sarks SH, Killingsworth MC. Evolution of geographic atrophy of the retinal pigment epithelium. *Eye.* 1988;2:552-577.
16. Sarks SH, van Driel D, Maxwell L, Killingsworth M. Softening of drusen and subretinal neovascularization. *Trans Ophthalmol Soc U K.* 1980;100:414-422.
17. Green WR, Key SN. Senile macular degeneration: a histopathological study. *Trans Am Ophthalmol Soc.* 1977;75:180-254.
18. Hogan MJ, Alvarado JA, Weddell JE. *Histology of the Human Eye.* Philadelphia, Pa: WB Saunders Co; 1971:328-363.
19. Feeney-Burns L, Ellersieck MR. Age-related changes in the ultrastructure of Bruch's membrane. *Am J Ophthalmol.* 1985;100:686-697.
20. Curcio CA, Medeiros NE, Millican CL. The Alabama Age-related Macular Degeneration Grading System for donor eyes. *Invest Ophthalmol Vis Sci.* 1998;39:1085-1096.
21. Pauleikhoff D, Barondes MJ, Minassian D, Chisholm IH, Bird AC. Drusen as risk factors in age-related macular disease. *Am J Ophthalmol.* 1990;109:38-43.
22. Klein R, Klein BEK, Linton KLP. Prevalence of age-related maculopathy. *Ophthalmology.* 1992;99:933-943.
23. Vingerling JR, Dielemans I, Hofman A, et al. The prevalence of age-related maculopathy in the Rotterdam study. *Ophthalmology.* 1995;102:205-210.
24. Mitchell P, Smith W, Attebo K, Wang JJ. Prevalence of age-related maculopathy in Australia. *Ophthalmology.* 1995;102:1450-1460.
25. Sarks JP, Sarks SH, Killingsworth MC. Evolution of soft drusen in age-related macular degeneration. *Eye.* 1994;8:269-283.
26. Hennekens CH, Buring JE. *Epidemiology in Medicine.* Boston, Mass: Little Brown & Co Inc; 1987:327-347.
27. Fisher RA. The logic of inductive reference. *J R Stat Soc.* 1935;98:39-54.
28. Breslow NE, Day NE. *Statistical Methods in Cancer Research.* Lyons, France: International Agency for Research on Cancer; 1980.
29. Sahai H, Kurshid A. *Statistics in Epidemiology: Methods, Techniques, and Applications.* Boca Raton, Fla: CRC Press Inc; 1996.
30. Ishibashi T, Patterson R, Ohnishi Y, et al. Formation of drusen in the human eye. *Am J Ophthalmol.* 1986;101:342-353.
31. Green WR, Schwartz DM. Aspects histopathologiques. In: Coscas G, ed. *Dégénérescences Maculaires Acquises Liées à l'Age et Neovaisseaux Sous-Rétiniens.* New York, NY: Masson Publishing; 1991;5:90-119.
32. Gottsch JD, Bynoe JD, Harlan JB, Rencs EV, Green WR. Light-induced deposits in Bruch's membrane of protoporphyric mice. *Arch Ophthalmol.* 1993;111:126-129.
33. Katz ML, Robison WG Jr. Age-related changes in the retinal pigment epithelium of pigmented rats. *Exp Eye Res.* 1984;38:137-151.
34. Kliffen M, Lutgens E, Mooy CM, et al. Apolipoprotein-E3 transgenic mice as an animal model for age-related maculopathy [abstract]. *Invest Ophthalmol Vis Sci.* 1998;39(suppl):S882.
35. Allikmets R, Shroyer NF, Singh N, et al. Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. *Science.* 1997;277:1805-1807.
36. Gorin MB, Jackson KE, Ferrell RE, et al. A peripherin/retinal degeneration slow mutation (Pro-210-Arg) associated with macular and peripheral retinal degeneration. *Ophthalmology.* 1995;102:246-255.
37. Sarks SH. New vessel formation beneath the retinal pigment epithelium in senile eyes. *Br J Ophthalmol.* 1973;57:951-965.
38. Kenyon KR, Maumenee AE, Ryan SJ, Whitmore PV, Green WR. Diffuse drusen and associated complications. *Am J Ophthalmol.* 1985;100:119-123.
39. Sarks SH. Drusen patterns predisposing to geographic atrophy of the retinal pigment epithelium. *Aust J Ophthalmol.* 1982;99:445-459.
40. Chang TS, Freund KB, de la Cruz Z, Yannuzzi LA, Green WR. Clinicopathologic correlation of choroidal neovascularization demonstrated by indocyanine green angiography in a patient with retention of good vision for almost four years. *Retina.* 1994; 14:114-124.
41. Gass JDM. Biomicroscopic and histopathologic considerations regarding the feasibility of surgical excision of subfoveal neovascular membranes. *Am J Ophthalmol.* 1994;118:285-298.
42. Sarks JP, Sarks SH, Killingsworth MC. Morphology of early choroidal neovascularization in age-related macular degeneration: correlation with activity. *Eye.* 1997;11:515-522.
43. Grossniklaus H, Hutchinson A, Capone A Jr, Woolfson J, Lambert HM. Clinicopathologic features of surgically excised choroidal neovascular membranes. *Ophthalmology.* 1994;94:1099-1111.
44. Grossniklaus HE, Green WR. Histopathologic and ultrastructural findings of surgically excised choroidal neovascularization. *Arch Ophthalmol.* 1998;116:745-749.
45. Spraul CW, Grossniklaus HE. Characteristics of drusen and Bruch's membrane in postmortem eyes with age-related macular degeneration. *Arch Ophthalmol.* 1997;115:267-273.
46. Killingsworth MC, Sarks JP, Sarks SH. Macrophages related to Bruch's membrane in age-related macular degeneration. *Eye.* 1990;4:613-621.
47. Dastgheib K, Green WR. Granulomatous reaction to Bruch's membrane in age-related macular degeneration. *Arch Ophthalmol.* 1994;112:813-818.
48. van der Schaft TL, de Bruijn WC, Mooy CM, de Jong PTVM. Basal laminar deposit in the aging peripheral human retina. *Graefes Arch Clin Exp Ophthalmol.* 1993;231:470-475.
49. Duvall-Young J, MacDonald MK, McKechnie NM. Fundus changes in (type II) mesangiocapillary glomerulonephritis simulating drusen: a histopathological report. *Br J Ophthalmol.* 1989;73:297-302.
50. Marshall GE, Konstas AGP, Reid GG, Edwards FT, Lee WR. Type IV collagen and laminin in Bruch's membrane and basal linear deposit in the human macula. *Br J Ophthalmol.* 1992;76:607-614.
51. van der Schaft TL, Mooy CM, de Bruijn WC, Bosman FT, de Jong PTVM. Immunohistochemical light and electron microscopy of basal laminar deposit. *Graefes Arch Clin Exp Ophthalmol.* 1994;232:40-46.
52. Green WR, McDonnell PJ, Yeo JH. Pathologic features of senile macular degeneration. *Ophthalmology.* 1985;92:615-627.
53. Loeffler KU, Lee WR. Is basal laminar deposit unique for age-related macular degeneration [letter]? *Arch Ophthalmol.* 1992;110:15-16.
54. Loeffler KU, Lee WR. Terminology of sub-RPE deposits: do we all speak the same language? *Br J Ophthalmol.* 1998;82:1104-1105.
55. Killingsworth MC. Age-related components of Bruch's membrane. *Graefes Arch Clin Exp Ophthalmol.* 1987;225:406-412.
56. Pauleikhoff D, Harper CA, Marshall J, Bird AC. Aging changes in Bruch's membrane: a histochemical and morphological study. *Ophthalmology.* 1990;97:171-178.
57. Guyton JR. The role of lipoproteins in atherogenesis. In: Longenecker JB, ed. *Nutrition and Biotechnology in Heart Disease and Cancer.* New York, NY: Plenum Publishing Corp; 1995:29-38.
58. Holz FG, Sheraidah G, Pauleikhoff D, Bird AC. Analysis of lipid deposits extracted from human macular and peripheral Bruch's membrane. *Arch Ophthalmol.* 1994;112:402-406.
59. Pauleikhoff D, Zuels S, Sheraidah GS, et al. Correlation between biochemical composition and fluorescein binding of deposits in Bruch's membrane. *Ophthalmology.* 1992;99:1548-1553.
60. Haimovici R, Rumelt S, Small DM, Gantz DL, Freddo TF. In situ lipid analysis of drusen and age-related changes in Bruch's membrane, using hot stage polarizing microscopy [abstract]. *Invest Ophthalmol Vis Sci.* 1997;38(suppl):S93.
61. Bird A. Age-related macular disease. *Br J Ophthalmol.* 1996;80:2-3.
62. Chen JC, Fitzke FW, Pauleikhoff D, et al. Functional loss in age-related Bruch's membrane change with choroidal perfusion defect. *Invest Ophthalmol Vis Sci.* 1992;33:334-340.
63. Remulla JFC, Gaudio AR, Miller S, Sandberg MA. Foveal electroretinograms and choroidal perfusion characteristics in fellow eyes of patients with unilateral neovascular age-related macular degeneration. *Br J Ophthalmol.* 1995;79:558-561.
64. Guyton JR, Klemp KF. Ultrastructural discrimination of lipid droplets and vesicles in atherosclerosis: value of osmium-thiocarbohydrazide-osmium and tannic acid-paraphenylenediamine techniques. *J Histochem Cytochem.* 1988;36:1319-1328.
65. Guyton JR, Bocan TMA, Schifani TA. Quantitative ultrastructural analysis of perifibrous lipid and its association with elastin in nonatherosclerotic human aorta. *Arteriosclerosis.* 1985;5:644-652.
66. Bocan TM, Schifani TA, Guyton JR. Ultrastructure of the human aortic fibrolipid lesion: formation of the atherosclerotic lipid-rich core. *Am J Pathol.* 1986;123: 413-424.
67. Guyton JR, Klemp KF. The lipid-rich core region of human atherosclerotic fibrous plaques: prevalence of small lipid droplets and vesicles by electron microscopy. *Am J Pathol.* 1989;134:705-717.