ARTICLE

Photoreceptor topography in ageing and age-related maculopathy

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Abstract

The relative rate of rod and cone degeneration is a fundamental characteristic of any disorder affecting photoreceptors, including ageing and age-related maculopathy (ARM). The macula consists of a small cone-dominated fovea surrounded by a rod-dominated parafovea. In donor eyes with grossly normal maculas, the number of foveal cones is stable and the number of parafoveal rods decreases by 30% over adulthood. These trends continue in early ARM. In exudative ARM, the photoreceptors that survive over disciform scars are largely cones, and rods decline precipitously in relation to thick subretinal pigment epithelium deposits. The preferential vulnerability of rods over cones has been confirmed by recent functional studies showing that the loss of scotopic sensitivity is greater than the loss of photopic sensitivity throughout adulthood and in patients with early ARM. A hypothesis that these effecfs are due to to retinoid deficiency at the level of the photoreceptors is proposed. The topography of rod loss in ageing and ARM is consistent with the location of early ARM lesions described in population-based studies and is not consistent with the location of fundus autofluorescence due to lipofuscin.

Age-related maculopathy (ARM)[1] is a major cause of new vision loss among the elderly of the industrialised world.[2–4] Early ARM is characterised by drusen and changes in retinal pigment epithelium (RPE) pigmentation, associated with minimal or mild vision loss. Late ARM is characterised by geographic atrophy of the RPE with or without choroidal neovascularisation, associated with severe vision loss. Although the most prominent clinical and histopathological lesions of ARM involve the RPE and Bruch's membrane, it is the degeneration, dysfunction and death of photoreceptors, through an atrophic process or a neovascular event and its consequences, that account for the vision loss associated with ARM. The functional status of photoreceptors is the most direct bioassay of the significance of changes in the RPE/Bruch's membrane complex.[5] Further, understanding photoreceptor function and survival in age-related disease begins with a firm understanding of how photoreceptors function and survive in normal aged eyes.

It is important to determine which photoreceptors are most affected by ageing and ARM, not only to target potential interventions to the most affected cells, but also to target mechanistic studies towards investigating the earliest disease-related changes. As is well known from the study of inherited retinal degenerations,[6,7] the rate of rod and cone degeneration is a fundamental characteristic of any disorder affecting photoreceptors. Because rods and cones have distinctly different biology, the rates at which they die provide important clues to the events that initiate their demise. In order to determine the relative rate of degeneration, however, one must obtain comparable information for both rods and cones at matched locations in the same well-characterised study eyes. Few studies of ageing and ARM have met these criteria. To date, only studies of photoreceptor number in donor eyes and visual function in living subjects have directly addressed differences between rod and cone biology in the same aged or ARM eyes.

The anatomical macula is a 6 mm diameter eye centred on the fovea.[8] The macula contains two subregions with distinctly different photoreceptor content: a small cone-dominated fovea, only 0.8 mm (2.75°) in diameter, and a surrounding rod-dominated parafovea. In young adults, rods outnumber cones in the macula by 9:1, so the macula is cone-enriched compared with the eye as a whole (20:1), but not cone-dominated.[9,10] Fig. 1 shows the inner segments of cone photoreceptors in the fovea and cones and rods in the parafovea of human retina. As revealed in unstained flat-mounts viewed with Nomarski differential interference contrast microscopy and video, photoreceptor inner segments are tightly packed in a mosiac that efficiently covers the retinal surface. The fovea contains small cone inner segments, and the parafovea contains large cones surrounded by numerous small rods. In histological studies

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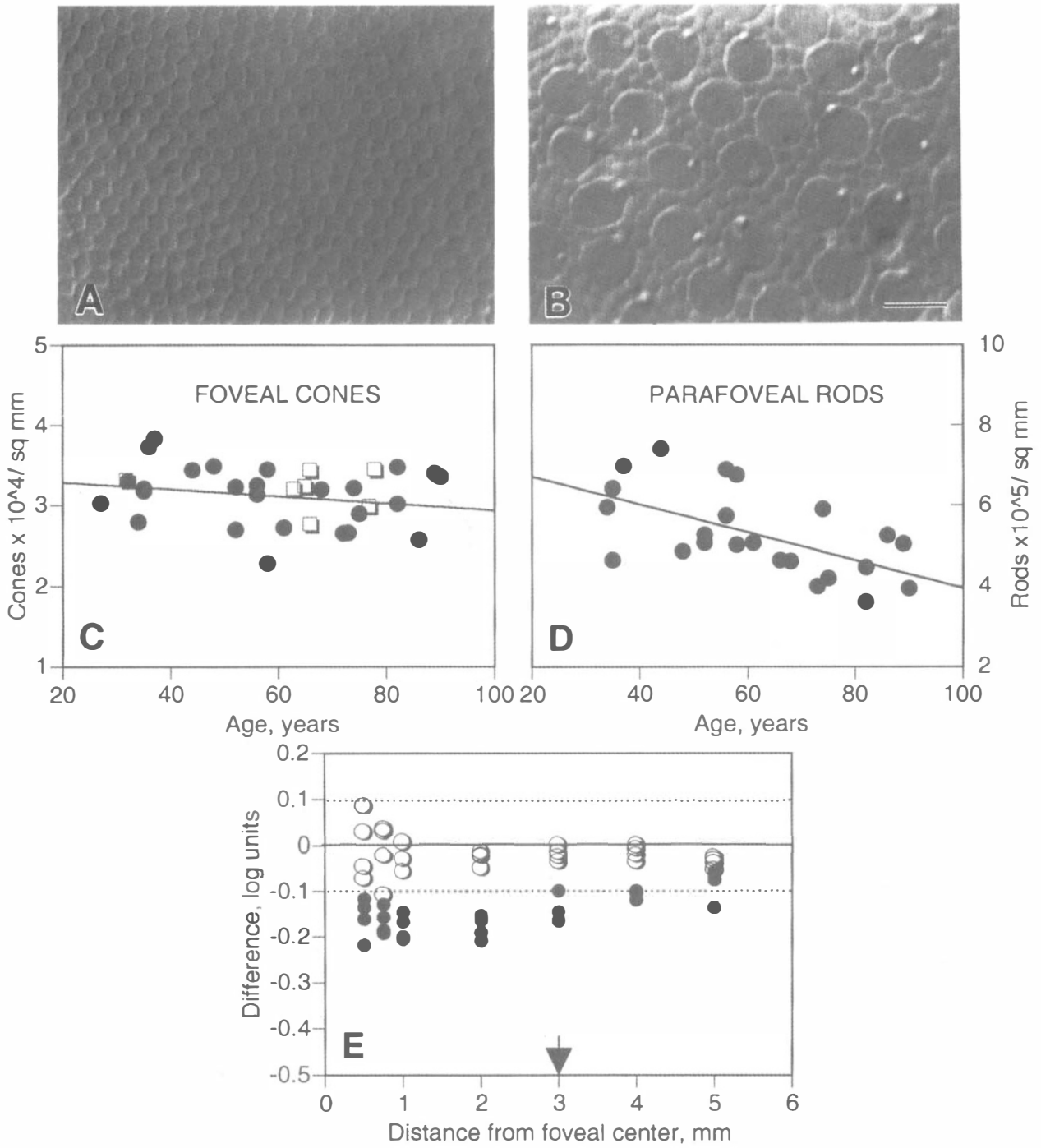

Fig. 1. *Age-related changes in photoreceptor density of grossly normal human macula. (A), (B) Mosaic formed by photoreceptor inner segments in normal retina (73-year-old). Nomarski differential interference contrast and video imaging of an unstained whole mount. Scale bar in (B) represents 10 μm. (A) Cone inner segments in the foveal centre. (B) Cone and rod inner segments (large and small profiles, respectively) in the parafovea. Refractile bumps on cones represent individual lipofuscin granules. (C), (D) Total number of macular photoreceptors. Photoreceptors were counted in systematically sampled whole mounts, and the total number was determined by numerical integration. (C) Total number of cones in a 0.8 mm (2.75°) diameter area centred on the fovea. Filled circles, eyes obtained from donors within 4 h of death;[11] open squares, eyes obtained within 45 min of surgical enucleation from patients with craniofacial tumours not involving the eye.[50] Regression versus age was not significant (r = –0.227). (D) Total number of rods within a 4 mm diameter area centred on the fovea in donor eyes. Regression versus age was significant (p < 0.05, r = –0.587). (E) Loss of cones (open circles) and rods (filled circles) in ageing, as a function of distance from the foveal centre. Five eyes from donors 82–90 years old were compared with 7 eyes from donors 27–37 years old.[11] Loss is expressed as the mean pair-wise difference (in log units) between the younger eyes and the older eyes at matched retinal locations.[11,21] The dotted lines represents the limits of normal variability determined by comparing eyes within each eye group with each other in the same way. Outer limit of the macula is 3 mm radius (arrow).*

of photoreceptor loss, it is important to localise counting samples accurately with respect to the foveal centre, where there are steep gradients in photoreceptor density.[9,10] For this reason, studies using retinal flat-mounts,[11,12] horizontally oriented histological sections[13] or vertically oriented sections through the foveal centre[14] provide more readily interpretable estimates of rod and cone loss in ageing and disease than studies using vertical sections at unspecified macular locations (e.g.[15,16]).

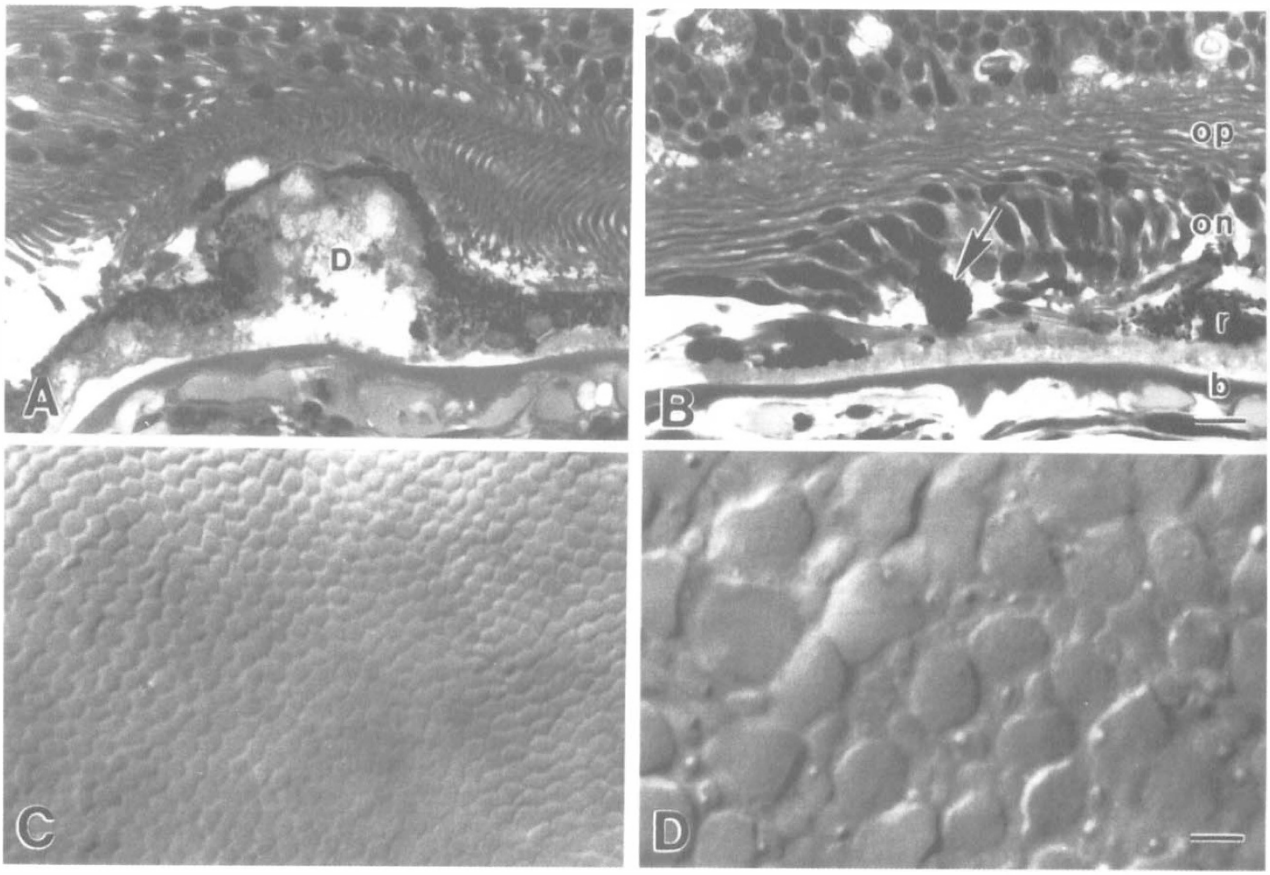

Fig. 2. *Photoreceptor morphology and topography in the eyes of a 79-year-old woman with non-exudative age-related maculopathy (drusen and thick sub-RPE deposits, RPE clumping and small circles of geographic RPE atrophy). (A), (B) Histopathology, 3 μm JB-4 sections, Richardson's stain, left eye. (A) Medium druse (D) with partially extracted contents underlies foveal cones. (B) Photoreceptor atrophy, sloughed RPE cell (arrow) and thick basal deposits in the parafovea. op, outer plexiform layer; on, outer nuclear layer, r, retinal pigment epithelium (RPE); b, Bruch's membrane. (C), (D) Mosaic formed by inner segments, whole mounted retina of right eye. (C) Foveal cone inner segment appears normal. (D) In the parafovea, cones are large and deformed, and few rods remain.*

We counted rods and cones in whole-mounted retinas with grossly normal maculas obtained from 27 donors within 4 h of death.[11] Although the peak density of cones can vary considerably between individuals, the total number within the 0.8 mm diameter cone-dominated fovea is remarkably stable throughout adulthood at a mean of 32 000 (Fig. 1C). The 4 h post-mortem interval to fixation was short enough to permit accurate cell counts in the delicate fovea, because the number of foveal cones obtained in donor eyes (Fig. 1C, filled circles) was very similar to the number obtained in more rapidly preserved eyes that had been surgically removed from patients with craniofacial tumours (Fig. 1C, open squares). These results were supported by another study that did not detect an age-related change in peak foveal cone density.[13] On the other hand, the foveal centre of eyes from donors older than 90 years have fewer cone nuclei than mid-life donors, suggesting that cone loss may occur at very advanced ages.[14] Foveal cone spacing is an important determinant of visual resolving power.[17] Sampling theory indicates that a 75% decrement in cone number would be required to account for a decline in resolution from 6/6 to 6/12. Clearly, a loss of this magnitude does not occur in normal ageing.

In contrast the number of rods in the parafovea of the same eyes was decreased by 30% (Fig. 1D).[11] Another study detected significant loss of rods but not cones,[12] and we demonstrated rod loss in another series of eyes (unpublished data from eyes in[18]). Notably, the loss of rods throughout the lifespan is very slow, only 2 rods/mm^2 of retina per year. A small number of apoptotic photoreceptor nuclei appear throughout adulthood in macaque monkey retina.[19] Within the human macula, age-related rod loss was not spatially uniform. It was most prominent in an annulus at 0.5–2 mm from the fovea and declined to undetectable levels by 8 mm from the fovea (Fig. 1E). The relative rate of rod and cone loss outside the macula remains to be determined, because the three studies that examined comparable areas of temporal retina disagree.[11–13] Interestingly, there were no gaps in the parafoveal photoreceptor mosaic or intrusions of other cells into the mosaic in the older eyes. Rather, the surviving rod inner segments expanded to fill the space vacated by the dying rods, and cones were unaffected, so that the photoreceptor mosaics in young and elderly eyes were qualitatively indistinguishable. Thus, the rods and cones actively regulate their space allocation in the mosaic of inner segments throughout adulthood. These results suggest that if other factors are equalised, the same

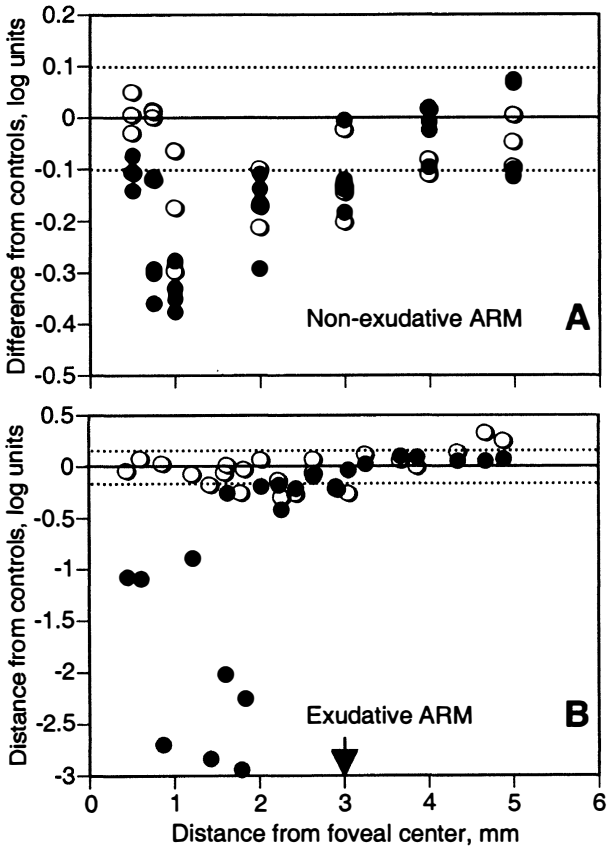

Fig. 3. *Loss of cones (open circles) and rods (filled circles) in non-exudative ARM (A) and exudative ARM (B), as a function of distance from the foveal centre. Outer limit of the macula is 3 mm radius (arrow in B). Loss is expressed as the log of the mean pair-wise differences between an ARM eye and a control group at matched retinal locations.[1,21] The dotted lines represent the limits of normal variability determined by comparing eyes in the control groups with each other in the same way. (A) An eye of an 81-year-old man with thick sub-RPE deposits and RPE clumping (see fig. 1C, D of Curcio* et al.[21]*) was compared with 4 age-matched controls with grossly normal maculas. (B) An eye of a 76-year-old man with disciform degeneration and geographic atrophy of the RPE was compared with age-matched controls with grossly normal maculas.[22] Photoreceptors overlying the disciform scar in this eye did not form a countable mosaic.*

number of photons will be captured by wave-guiding inner segments for rod-mediated phototransduction and, therefore, any age-related declines in scotopic sensitivity will not be related to the location of rod loss. This prediction was borne out by a study of scotopic sensitivity in young and elderly adults with healthy maculas.[20]

If age-related parafoveal rod loss were actually due to RPE/Bruch's membrane pathology that was not visible in the fundus, it follows that rods are preferentially affected in ARM as well as normal ageing. To address this question, we analysed the topography of rods and cones in 12 eyes with ARM.[21,22] Six eyes had non-exudative ARM (drusen, pigment change and geographic atrophy) and 6 had exudative ARM (geographic atrophy and disciform degeneration subsequent to choroidal neovascularisation). In each eye, we systematically sampled photoreceptors in flat-mounted retinas and determined the loss relative to age-matched controls. The fellow eye was prepared for histopathological evaluation and, in 6 cases, carbonic anhydrase histochemistry[23] was used to identify the surviving photoreceptors. Clinical records were available for most eyes and indicated better vision in the non-exudative ARM group than the exudative ARM group.

Fig. 2 shows RPE/Bruch's membrane pathology and the photoreceptor mosaic for a pair of eyes with non-exudative ARM. Despite the presence of drusen and thick deposits (Fig. 2A,B), the foveal cone mosaic of the ARM eye appeared remarkably normal (Fig. 2C; compare with Fig. 1A). The total number of foveal cones in 5 non-exudative ARM eyes (1 eye had mechanical damage to the fovea) fell within the normal range (Fig. 1C). In contrast, the parafovea was distinctly abnormal, with few rods, broadened cone inner segments and wide spaces among cells (Fig. 2D; compare with Fig. 1B). At systematically sampled locations throughout the parafovea, loss of rods could be detected, particularly at 0.5–1 mm from the foveal centre (Fig. 3A). These changes did not result in a significant reduction in the total number of macular rods relative to age-matched controls, however, because of the localised nature of the loss.[22]

In exudative ARM eyes (disciform degeneration and geographic RPE atrophy), we observed two distinct patterns of photoreceptor loss (Fig. 4). First, despite severe disease many photoreceptors survived, typically in pockets of subretinal space enclosed externally by a leaflet of fibrovascular scar (Fig. 4A,B). These surviving cells did not form a countable mosaic, but using carbonic anhydrase histochemistry we determined that they were virtually all cones (Fig. 4B), a reversal of the normal rod:cone ratio in the macula. Second, just peripheral to the geographic RPE atrophy associated with the disciform scar was a transitional zone of thick deposits and RPE degeneration (Fig. 4C). In this area, photoreceptors formed a mosaic (Fig. 4D–F) and could be counted. Across the transition, in conjunction with thick deposits and RPE degeneration, both of which were more severe near the scar, the number of rods dropped dramatically (Fig. 3B). In contrast the number of cones changed very little (Fig. 3B). As in ageing, photoreceptor loss in exudative ARM occurs by apoptosis.[24]

At each location where counts were made in ARM eyes, we calculated rod loss and cone loss relative to controls (Fig. 4A, B). From the number of sites with loss, we determined the number of sites where rod or cone loss was greater. In 4 of 6 non-exudative ARM eyes and 5 of 6 exudative ARM eyes, there were more sites where rod loss exceeded cone loss. In other words, rod loss predominated in three-quarters of our sample of ARM eyes. It remains to be determined whether the 3 of 12 eyes where cone loss predominated constitute a distinct subtype of ARM. In summary, although the macula is a cone-enriched retinal region, it is the rods which show the earliest signs of degeneration in most eyes, and the last photoreceptor in an exudative ARM macula is a cone.

Recent functional studies by Jackson, Owley and colleagues have supported the histological evidence for preferential vulnerability of rods in ageing and ARM.[25,26]

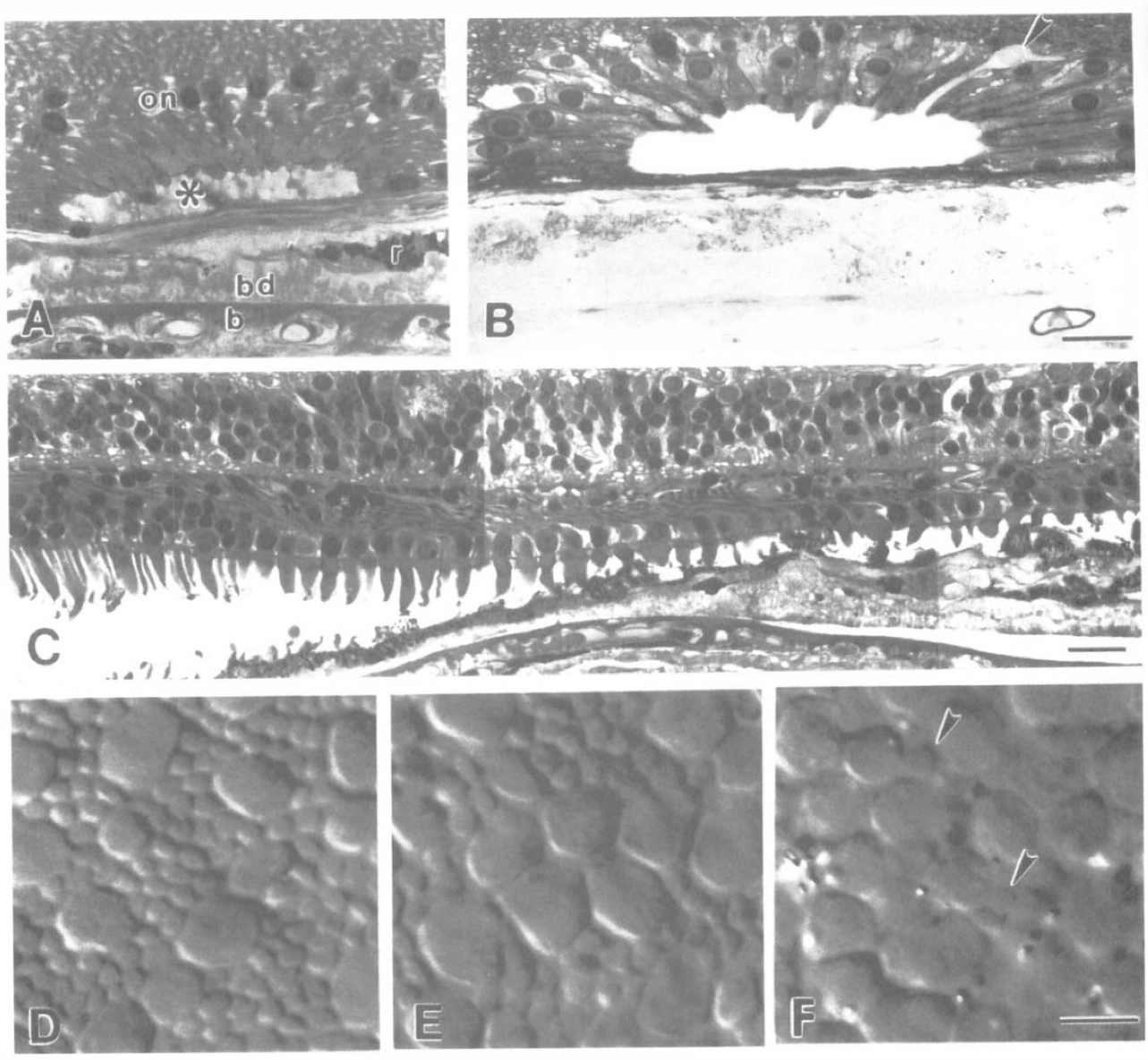

Fig. 4. *Photoreceptor morphology and topography in eyes with exudative age-related maculopathy. (A)–(C). 3 μm JB-4 sections. (A), (B) Left eye of an 81-year-old man. Scale bar in (B) represents 10 μm. (A) A pocket of subretinal space (asterisk) with surviving photoreceptors over a disciform scar and RPE atrophy. Periodic acid–Schiff stain. on, outer nuclear layer; r, retinal pigment epithelium; bd, basal deposits; b, Bruch's membrane. (B) Almost all surviving photoreceptors are positive for carbonic anhydrase (red/green cones).*[23] *One carbonic-anhydrase-negative blue cone is indicated (arrowhead). The maculas of control eyes have one to three rows of carbonic-anhydrase-negative rod nuclei, which are absent from the exudative AMD eye. (C), (D) Right and left eyes, respectively, of a 90-year-old woman. (C) Montage showing transition from intact photoreceptor layer (at left of panel) to degenerate photoreceptors associated with RPE atrophy and disciform degeneration (at right of panel) Scale bar represents 10 μm. (D)–(F) Transition from normal photoreceptor mosaic to cone-dominated mosaic near the margin of a disciform scar with geographic RPE atrophy. Nomarski differential interference contrast-video images of unstained retinal whole mount, at 392 (D), 280 (E) and 112 (F) μm from the limits of the intact photoreceptor layer. Few rods are present in (F) (arrowheads). Scale bar in (F) represents 10 μm.*

These functional studies met the criteria listed at the beginning of this review, in that they determined photopic and scotopic sensitivity at matched retinal locations in the same well-characterised eyes. The studies were large, involving 106 normal subjects from seven decades of adulthood and 80 early ARM patients. Significantly, macular health was ascertained objectively in all subjects by grading fundus photographs, and the effect of lens density, which reduces retinal illuminance in older persons, was accounted for on an individual basis. These studies showed that scotopic and photopic sensitivity both decline throughout adulthood, but in 80% of adults the rod dysfunction was greater. Further, scotopic and photopic sensitivity loss occurred in early ARM patients, but in 87% of patients the rod dysfunction was greater. Although ARM is a complex multifactorial disorder, these results suggest a final common pathway at the level of photoreceptor function.

The most direct evidence linking sub-RPE deposits to photoreceptor loss is the transitional zone around geographic RPE atrophy, as shown in Fig. 4. A similar relationship also occurs around the optic nerve head of normal eyes, where there is clumping of RPE cells and atrophy that spreads slowly with age, Bruch's membrane thickening, focal or diffuse sub-RPE deposits, and preferential loss of rods over cones (peripapillary chorioretinal atropy).[27] Prominent rod dysfunction is the primary clinical manifestation of several inherited

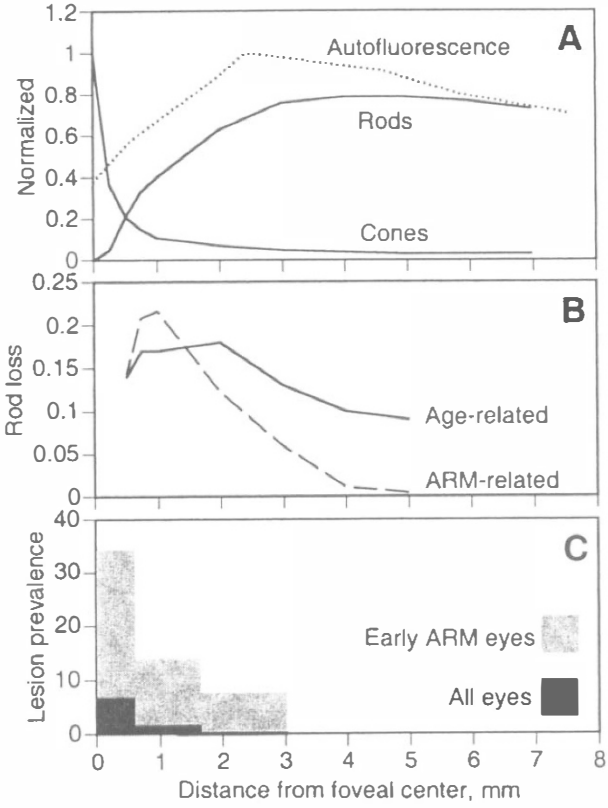

Fig. 5. *Photoreceptor density and funduscopically visible autofluorescence (A), rod loss (B) and early ARM lesions (C), as a function of distance from the foveal centre. (A) Mean spatial density of cones and rods (310^5/mm^2) in 8 young adults[10] is normalised to mean peak cone density. Mean autofluorescence due to lipofuscin along the horizontal temporal meridian is normalised to the maximum of 7–15° (2–4 mm) in three observers.[44,51] (B) Loss of rods in log units is calculated as described in Fig. 4 and averaged across four meridians. Age-related loss wsa determined by comparing eyes from 82–90 year old donors with eyes from 27–37 year old donors (see Fig. 4A). ARM-related loss represents the mean loss in 4 eyes with early ARM.[22] (C) The distribution of early ARM lesions (soft indistinct drusen and/or RPE hypo- and hyperpigmentation) was determined for participants in the Beaver Dam Study.[46] The bars indicate the area-weighted prevalence for lesions in retinal regions 0–0.5 mm, 0.5–1.5 mm and 1.5–3.0 mm, respectively, from the foveal centre. These regions correspond to the central subfield, the ring of four inner subfields and the ring of four outer subfields, respectively, of the Wisconsin Age-related Maculopathy Grading System grading grid.[52] 'All eyes' indicates prevalence in the right eye of 4350 participants in a population-based sample; 'early ARM eyes' indicates prevalence in 247 participants with early ARM lesions in the right eye.*

diseases featuring extensive sub-RPE deposits, including Sorsby's fundus dystrophy,[28] membranoproliferative glomerulonephritis type II[29,30] and dominant late-onset retinal degeneration.[31,32] These findings indicate that preferential rod loss in relation to thick deposits is not unique to the macular deposits of ARM. Further, these findings are consistent with the idea that diffuse deposits, which differ ultrastructurally and probably biochemically among these disorders, may serve as non-specific barriers to the resupply of molecules essential preferentially to rods. Patterns of photoreceptor loss over focal deposits (drusen), however, remain to be directly demonstrated.

Rods and cones are electrically coupled and share the same light exposure, humoral environment and support system. However, anatomical and functional studies in ageing and ARM eyes now agree that rods are affected earlier and more severely than cones and that the effects of ageing and ARM are qualitatively similar.[11,21,25,26] Moreover, recent studies have shown that the rod-mediated component of dark adaptation slows in both ageing and ARM.[33,34] This deficit in rod kinetics is even more striking than the deficit in steady-state scotopic sensitivity.[35] We proposed a new hypothesis to account for these phenomena.[5] As briefly summarised below, we hypothesised that age- and disease-related changes in Bruch's membrane lead to reduced retinoid transfer from the blood and localised scarcity of 11-*cis*-retinal at the photoreceptors.

The rod-mediated portion of dark adaptation is thought to represent the regeneration of rhodopsin and other aspects of recovery during the visual cycle. The visual cycle comprises biochemical reactions in the RPE and photoreceptors that produce the vitamin A derivative 11-*cis*-retinal from all-*trans* precursors delivered across Bruch's membrane by plasma proteins. Retinoids are also required for photoreceptor survival, as vitamin A deprivation leads to outer segment degeneration and photoreceptor death,[36] affecting rods first, then cones.[37] Cones have a different retinoid delivery pathway that may involve neurosensory retina.[38] According to current models,[39] slow dark adaptation indicates poor regeneration of rhodopsin. Slow dark adaptation occurs in systemic vitamin A deficiency[40] and genetic disorders affecting visual cycle components or the retinoid transport system. When insufficient vitamin A is available to regenerate rhodopsin, active intermediates desensitise the retina and reduce sensitivity.[39] Characteristic debris accumulates within Bruch's membrane from early adulthood through senescence,[41] and additional material accumulates between the RPE and Bruch's membrane in older adults and in ARM patients.[42] Together, these processes could impair the translocation of retinoids across Bruch's membrane. Rod dysfunction and degeneration occur in other late-onset conditions with sub-RPE deposits (see above), and dark adaptation improves in patients with Sorsby's fundus dystrophy given vitamin A supplements,[43] presumably overcoming the translocation deficit via mass action. Thus, this model potentially explains the slowing of rod-mediated dark adaptation and the earlier involvement of rods relative to cones in ageing and ARM, and the similar effects of ageing and ARM on photoreceptors. It also links photoreptor degeneration with age changes in Bruch's membrane and the characteristic lesions of ARM.

In addition to leading to new mechanistic hypotheses, improved measures of macular rod and cone topography in ageing and ARM also underscore the spatially heterogeneous effect of these processes. It is informative to compare the topography of rod loss with the normal distribution of photoreceptors and with other features visible in the fundus of living patients, with the

underlying assumption that causally related events should exhibit a similar topography. In the absence of multi-parametric data from many individual eyes, this question was addressed by comparing topographic data available from different studies. Fig. 5A shows the density of cones and rods in young adults[10] and fundoscopically visible autofluorescence due to lipofuscin,[44] the RPE age-pigment thought to participate in ARM pathogenesis.[45] Autofluorescence follows closely the normal distribution of rods. Fig. 5B shows the difference in rod numbers between young and elderly donors (from Fig. 1E) and the difference between non-exudative ARM and control eyes (as per Fig. 3A). In ageing and ARM, the greatest rod loss occurs 1–2 mm (3.5–7°) from the foveal centre, with the disease-related loss falling off more sharply than the age-related loss. Notably, the scotopic sensitivity loss that occurs in early ARM patients[26] also declines markedly across this same distance (not shown). Finally, Fig. 5C plots the prevalence of drusen and RPE changes determined for all participants in the Beaver Dam Eye Study and for 274 participants with early ARM.[46] The prevalence data probably underestimate the extent of Bruch's membrane pathology, because diffuse deposits are invisible in the fundus.[42,47] Nevertheless, Fig. 5C demonstrates that the soft drusen and RPE changes that typify early ARM cluster within the central 1 mm of the macula. Taken together, the graphs in Fig. 5 show that age- and disease-related rod loss occurs in a very specific part of the macula. This loss is not located at the site of highest rod density, and it occurs where Bruch's membrane pathology is present but less severe than it is at the cone-dominated foveal centre. Further, rod loss is not related to lipofuscin accumulation, which is maximal at more eccentric locations.

In summary, anatomical studies and recent functional studies have converged to demonstrate that photoreceptor degeneration and loss occur before disease in the RPE/Bruch's membrane complex progresses to late ARM. Furthermore, macular rods are affected earlier and more severely than cones in ageing and ARM. These findings are significant for both clinical and basic research. In many patients tests of rod function may permit detection of ARM at earlier stages than do standard tests of cone function such as visual acuity. The preferential vulnerability of rods in ageing and ARM is a phenomenon which should be accounted for by mechanistic theories such as the retinoid deficiency hypothesis[5] or others. These findings provide a standard against which the relevance of emerging model systems (e.g. mice bearing the gene defects causing early-onset macular disorders[48]) and other potentially pathogenic phenomena in the macula should be assessed. Finally, as rods secrete factors that enhance cone survival,[49] early inventions that target rod photoreceptors may have an indirect salutary effect on cones as well.

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