Reports

Density of Human Cone Photopigments as a Function of Age

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The density difference between fully bleached and fully dark-adapted retinas was assessed for 77 eyes (47 subjects) in the age group 13–50 yr. No significant change in density was found as a function of age. The time constant of pigment regeneration was also found to show no age effects up to the age of 50 yr. These findings are at odds with another study on the density of foveal cones. Invest Ophthalmol Vis Sci 26:1014-1016, 1985

Kilbride et al 1 announced in their ARVO abstract that they found, for subjects between 22 and 50 yr, a decrease with age in the cone pigment density in the fovea. At the conference this was substantiated as an appreciable difference in pigment density in patients less than vs those greater than age 40 (including limited data for subjects older than 50 yr). Their data were best fit by a least squares to a line of slope 0.05 per decade.

In the 5 yr that our densitometer is in operation, we had gathered a substantial amount of data on two-way densities in normal subjects with foveal fixation. Since we never had the impression that we saw much difference with age, at least up to the age of 50 yr, Kilbride’s report prompted us to plot all available data of that age group as a function of age.

Materials and Methods. The Utrecht densitometer was extensively described by Van Norren and van der Kraats. A photomultiplier attached to a modified Zeiss funduscamera (Zeiss; Oberkochen, West Germany) analyzes light reflected from the fundus. The measuring wavelength was 554 nm, 600 Td. The measuring field was 2.5 deg. Subjects fixated cross hairs centered with the stimulus field. The retina was first bleached with yellow light (λ > 530 nm) of 10^6 Td during 2 min. Thereafter, regeneration of cone pigments was followed in the dark for at least 6 min, or longer if the trace had not yet reached a stable level, and finally the bleaching light was switched on again for 2 min. The maximum two-way density change was defined by the difference between the average level of the density trace during the last minute of dark adaptation and the average of two 1-min traces obtained during bleaching. If the two traces in the bleached condition did not reproduce within 0.05 density units, the data were discarded. Such shifts are generally caused by unstable fixation. Subjects were classified as “normal” when they had no known history of visual disorders, showed no signs of retinal pathology, had clear optical media, and a corrected visual acuity of 1.0 or more. For a study emphasizing the age group 13–50 yr, the latter criterion does not introduce much of a bias since senile changes in visual acuity are known to occur only after the age of 45 yr.4 No subjects with optical disorders of more than 6 diopters were used, for their difficulty to properly fixate the cross hairs without wearing their correction. Informed consent was obtained from all subjects after the nature of the procedure had been explained fully. Color vision was not always tested. Except for a few cases all data were obtained in the first and only measurement.

Results. In Figure 1a the two-way density is plotted as a function of age for 77 eyes (47 subjects). The mean density is 0.31 ± 0.032. This standard deviation compares well with the standard deviation of 0.021 which was earlier given for 20 repeated measurements on a single subject.

There are no significant differences between eyes less than vs those equal to or greater than age 40 (Student’s t-test, P < 0.10). The data are best fit by a least square to a line of slope 0.0038 per decade. The correlation coefficient r = −0.110, which means that the decrease with age is not significant (P > 0.10).

Only when results for eyes between 20 and 30 yr are compared with those past 40 yr is a significant difference revealed (P < 0.05).

In the bottom half of Figure 1, the time constant of regeneration is given. It was obtained by taking data points from the regeneration curve at 0.5-min intervals for the first 3.5 min. The logarithm of the difference in final density and that at time t was then plotted; a best fitting straight line drawn through these points defined the time constant. Although regeneration systematically deviates from this straight line, the obtained time constant gives a reasonable first order approximation of the speed of pigment regeneration. Again, no indication is found for a
Fig. 1. Two-way density of foveal cones as a function of age for 77 healthy eyes (top). Time constant of regeneration as a function age for the same material (bottom). For a few measurements, indicated with closed circles, the time constant was not available.

change in the speed of regeneration between 13 and 50 yr of age ($r = 0.216; P > 0.05; \text{slope} 2.7 \text{ sec per decade}$).

**Discussion.** We do not understand why Kilbride et al1 find a substantial decrease of cone photopigment density with age, and we find, at most, a very slow trend. In both studies,2 deep red wavelengths were used to correct the baseline to zero optical density, and the maximum density was assessed with wavelengths (\(\lambda\) about 550 nm) which are not very sensitive for the well known senile changes in the optical media. Kilbride et al used a shorter bleach (30 sec vs our 120 sec), but with a $10^6$ Td bleaching light it would require a very substantial decrease in the rate of photolysis with age to yield significantly less bleached pigment at the end of a 30-sec period. We know of only one other study concerning densitometry of cone pigments in which age effects are discussed. Baker and Kuyk6 found in one of the author’s eyes a substantial increase in the time constant of regeneration (from 120 to 195 sec) over a period of 16 yr. This finding can be reconciled with our findings if the age of the subject at the time of the most recent measurement was well beyond 50 yr. For two subjects in our files between 60 and 65 yr (out of a total of 4 with age beyond 50 yr), we found time constants in the range 150–200 sec. Since the two-way densities of these subjects were between 0.21 and 0.24, there might indeed occur a loss of density with age, but the onset of this loss is probably well beyond 50 yr.

Anatomical studies on the loss of photoreceptors with age are scarce, and we do not know of any quantitative reports concerning the foveal cones. Gartner and Henkind7 recently noted displacement of receptor cell nuclei from the outer nuclear layer (ONL) both into the outer plexiform layer (increasing
after age 30 and most pronounced after age 50), and into the layer of rods and cones (only after age 40, most common after age 50). Accompanying the loss of nuclei in the ONL, they noted shrunken and deformed rods and cones, and a diminution in the numbers of photoreceptors. They also stated, however, that nuclear displacement was rare in the center of the fovea. Thus, although receptor cells are increasingly lost with age, no conclusive evidence is available whether or not foveal cones are significantly affected before the age of 50. The disks in the outer segments of the photoreceptors which contain the photopigment are known to be subject to a continuous process of renewal. The efficacy of this mechanism might be the reason why we find that the cones remain in fine shape up to the age of about 50 yr.

Key words: cones, pigment density, age effects, pigment regeneration


References

Retinal S-Antigen Epitopes in Vertebrate and Invertebrate Photoreceptors

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Monoclonal antibodies specific for the retinal S-antigen were obtained by hybridization of spleen cells from a BALB/c mouse immunized with bovine S-antigen and NS-1 myeloma cells. Five selected antibodies specifically labeled the photoreceptor cells of the retina by immunofluorescence. Whereas antibody S9E2 only reacted with bovine S-antigen, the other antibodies showed interspecies cross-reactivity. They were used for the characterization of specific epitopes of S-antigen in photoreceptors from a wide range of species representative of various classes of vertebrates and invertebrates. The presence of S-antigen in distant species (vertebrates, Amphioxus, nemerteans, annelids, molluscs) indicates a high phylogenetic stability and suggests an important role for this protein in photoreceptor function. Invest Ophthalmol Vis Sci 26:1016–1021, 1985

The retinal “S-antigen” is a specific component of photoreceptor cells that has been isolated and purified from the retina of several mammals. The immunologic properties of this protein include organ specificity, interspecies cross-reactivity and immunopathogenicity. Most work with S-antigen has dealt with its ability to induce experimental autoimmune uveoretinitis (EAU) in laboratory animals. This antigen is also involved in ocular autoimmune disease in man. EAU and circulating antibodies are produced after immunization with xenogenic or allogenic S-antigen and even with autologous retina. These antibodies have been used to localize S-antigen in the retinal photoreceptors of guinea pigs by immunofluorescence. Cross reactivity between S-antigen from various mammals has been demonstrated by immuno-diffusion, enzyme immunoassay (ELISA), immunofluorescence and pathogenic activity. The use of monoclonal antibodies to bovine S-antigen in ELISA studies showed the presence of two types of epitopes in the protein. Some epitopes were specific to bovine S-antigen, while others were common to S-antigen from various mammals. These nonspecies-specific epitopes were recognized in the retinas of other classes of vertebrates by immunofluorescence. In this article, we present an immunofluorescent analysis of the distribution of S-antigen epitopes in the photoreceptors of selected species representative of various classes of vertebrates and invertebrates.

Materials and Methods. Details of hybridization and of the specificities of the antibodies analyzed by...