Density of Foveal Cone Pigments at Older Age

Johan E. E. Keunen,* Dirk van Norren,† and Gerard J. van Meel*

We investigated foveal cone photopigment kinetics by retinal densitometry in 34 eyes of 29 healthy subjects with clear optical media and good visual acuity, ranging in age from 39 to 79 years. Our aim was to assess possible senile disturbances of foveal cones. To assess the effects of ocular straylight, we measured not only in subjects with a clear crystalline lens, but also in pseudophakia and aphakia. In a limited number of subjects color vision was assessed with a Nagel anomaloscope; no systematic changes with age were found. A significant decrease in two-way density and in time constant of regeneration was found to occur only after age 60, with large individual variations. There was no indication that results for subjects with their natural crystalline lens, in aphakia, or in pseudophakia were different. We argue that a reduction in the number of cones with age, rather than an increase in ocular stray light is the most likely explanation of our findings. Invest Ophthalmol Vis Sci 28:985-991, 1987

Snellen1 was probably the first in modern science to report on senile visual dysfunction. He provided a quantitative description of the decline in visual acuity with increasing age. Nowadays, many alterations during the aging process are known, relating to all levels of the human visual system.2 In the present study it was our aim to investigate, with the technique of retinal densitometry, the effects of age on the foveal photoreceptors.

Few data are available about densitometry and age. Quite recently Weale3 stated that "it is unlikely that the method of fundus reflectometry is sensitive enough to reveal age changes." He probably based this opinion on his earlier work4,5 and that of Rushton.6 In the last few years, however, densitometers have improved considerably (van Norren and van der Kraats,7 Kilbride et al,8 Faulkner and Kemp9) and new data on age effects were published. Baker and Kuyk10 found in one of the authors' eyes a substantial increase in time constant of visual pigment regeneration over a period of 16 years. For subjects between 22 and 50 years Kilbride et al11 announced a steady decrease with age in cone pigment density of the fovea. In reaction, van Norren and van Meel12 reported that they could not find significant changes in cone pigment density and time constant in 77 eyes as a function of age in an age group of 13–50 yr. Kilbride et al13 recently published a full account of the age effects that they had found on cone pigment density in the fovea. In a group of 19 subjects (19 eyes) ranging from 22 to 70 years they found a gradual decrease in cone pigment density. Their data were best fit by a line with a slope of 0.032 per decade.

Additional information on the aging of human photoreceptors can be gained from histological studies showing possible morphological modifications. Marshall14 demonstrated that with increasing age, cone outer segment membranes tend to become disordered, especially those in the fovea.15 Weale2 described a small loss of cone outer segment membranes between birth and age 40. After age 40 a rate change occurs with much more rapid loss of cone outer segment membranes. In a histological study on the macula of 104 human eyes Gartner and Hendkind16 showed in 24 eyes obvious reductions in the number of nuclei in the outer nuclear layer; they also found considerably increased displacement of nuclei from the outer nuclear layer of cones after age 40. This may point to a dysfunction of a certain fraction of the cones. Farber et al17 found a foveolar cone density of 49,600 cones/mm² in a 71-yr-old male, which they compared with the classic Osterberg18 study that reported in the foveola of a 16-yr-old male a cone density of 147,300 cones/mm². Yuodelis and Hendrickson19 calculated a foveolar cone density of 48,804 cones/mm² in a donor eye of a 72-yr-old adult, while in a 37-yr-old adult a foveolar cone density of 76,282 cones/mm² was found. Similarly, Marshall and Laties20 observed an age-related reduction in the number of foveal cones. In six eyes obducted after age 70 Caratero et al21 found no modifications of the photoreceptor outer segments, but did find nuclear and cytoplasmatic modifications in the mor-
phology of the cone photoreceptors. In rhesus monkeys (which have a lifespan of between 20 and 25 yr) the cone density drops about 20% between the ages of 5 and 20 yr.  

In summary, histological data give an indication for senile cone photoreceptor changes which accelerate after age 40, but quantitative data are scarce. Since the previous publication from this laboratory (van Norren and van Meel) summarized available data of densitometry as a function of age up to 50 years, in the present study we concentrated on older ages. Our findings point to a fairly late onset of senile changes. A decrease of two-way density and an increase of time constant as a function of age may occur in healthy subjects after age 50, but the individual differences are large.

**Materials and Methods**

**Subjects**

We investigated 55 healthy subjects (75 eyes). All subjects had a best-corrected Snellen visual acuity of 5/5 or better, clear media, and no funduscopic abnormalities. From the original group the data of only 29 subjects (34 eyes), ranging in age from 39 to 79 yr, were sufficiently accurate to be used in this study. The criteria are given under Procedure. To assess possible adverse consequences of senile opacification and color change of the crystalline lens we included in our results five eyes in aphakia and ten eyes in pseudophakia. In the 17 subjects (19 eyes) with their own crystalline lens, slit-lamp examination revealed no abnormalities.

**Retinal Densitometer**

The Utrecht densitometer used in this study has been described by van Norren and van der Kraats. Briefly, the apparatus has an optical and an electronic part. A 450-W xenon high-pressure lamp is the light source for the bleaching, reference, and measuring beam. A yellow filter in the bleaching beam eliminates wavelengths shorter than 530 nm; the wavelength of the reference beam is 711 nm and that of the measuring beam 554 nm. The three beams are converged near a sector disc that produces a repetitive sequence of 7.5 ms pulses of darkness, reference, bleaching, and measuring light. The beams are focused on an optical fiber, which leads to a modified Zeiss fundus camera. The diameter of the field projected in the eye is 3.8°. In its center a reflected field of 2.5° diameter is measured by a photomultiplier, which is used in the photon counting mode. After correction for dark counts, reference and measuring beam counts are plotted on a penwriter. A microprocessor calculates a quantity termed “density” based on the logarithm of the ratio of the measuring counts and the reference counts. The outcome of this calculation is also continuously plotted on the penwriter. The density difference between the fully bleached and fully dark-adapted condition defines the two-way density. The second parameter of importance is the speed of recovery after bleaching. This was obtained by taking readings from the density trace at 30 sec intervals for the first 180 sec after switching off the bleaching light. The logarithm of the difference between these readings and the mean reading in the fully dark-adapted condition was then plotted against time and a straight line was fitted through the data points. Such a procedure is the optimal one when the regeneration curve follows a simple exponential function; in that case all data points lie on a straight line and the slope of the line defines the time constant \((1/e\text{-value})\) of regeneration. Despite the fact that regeneration systematically deviates from an exponential function, the deviations generally are of such magnitude that the procedure described above yields a reasonable parameter to characterize the speed of regeneration. We have labeled this parameter “time constant.”

When stray light forms a non-negligible fraction of the light collected by the photomultiplier, the relation between the density recordings and the pigment density becomes more complicated. For a detailed discussion, refer to other studies from this laboratory. For the present purposes it serves to mention that the effect of increasing stray light, while producing no changes at the pigment level, will be to lower the observed two-way density and to slightly shorten the apparent time constant. This can be derived from the relation given by van Norren and van der Kraats:

\[
\Delta_D - \Delta_B = -\log (1 - \sigma) 10^{-D(1-e^{m})} + \sigma \quad (1)
\]

where \(\Delta_D\) and \(\Delta_B\) are the penwriter positions in the fully dark-adapted and the fully bleached conditions, \(\sigma\) is the fraction of stray light in the bleached condition, \(D\) is the two-way density of the visual pigment and the \(t_0\) is the time constant of regeneration. That the apparent time constant decreases with increasing stray light is not obvious from the formula given above, but follows from numerical examples.

**Anomaloscope**

Color matching was investigated with a Nagel type I anomaloscope by a standard technique. The subject was asked to make red-green (RG) settings for each eye with a constant yellow brightness (YB) setting of 12.5. The mean value of five matches for each eye was calculated. The normal matching range in-
Table 1. Clinical and densitometric data, and mean anomaloscope settings

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OD = right eye; OS = left eye.

* Not measured.

† Data too noisy to calculate time constant.
‡ Congenital deuteranomaly.

Procedure

The nature of the experiment was carefully explained and informed consent was obtained from all subjects. We dilated pupils with tropicamide (Mydriaticum®, Roche). In all cases a pupil diameter of at least 5 mm was reached. This is sufficient to allow densitometry even under bright bleaching conditions in subjects with senile miosis. A bar made of dental impression compound, and two temple pads were used to immobilize the subject's head. The fundus camera was adjusted to obtain the highest possible reflection as measured by the photomultiplier. Subjects fixated cross-hairs centered with the stimulus field. The macula was bleached for 2 min with a 6 log trolands bleaching light. After switching of the bleaching light, we followed the regeneration of cone pigments until the density trace had reached a stable level. This generally took 6 to 10 min. Then the bleaching light was switched on again for 2 min. The data were discarded if the two traces in fully bleached condition did not reproduce within 0.05 density units; larger shifts are usually caused by unstable fixation. We noted that elderly subjects often had difficulties in sitting quietly continuously, or in fixating cross-hairs during the densitometric measurement, which takes 20–30 min. Also, subjects with even a small denture fitting problem often showed movements of their head with regard to the bite bar. Some subjects showed profuse slavering with their denture which takes 20–30 min. Also, subjects with even a small denture fitting problem often showed movements of their head with regard to the bite bar. Some subjects showed profuse slavering with their denture in the bite bar. Another reason for discarding data was undesirable reflection of the measuring beam in pseudophakia and aphakia due to an intraocular implant lens surface or a clear residual cataract membrane. In these cases the measuring trace on the penwriter was capricious and not in line with periods of bleaching and dark-adaptation. In total, 41 mea-
Fig. 1. Foveal densitometry in normal subjects of different age. The measuring sequence is as follows. After a 6 log trolands bleaching exposure of two min, a dark period starts at "OFF" during which the pigment regenerates. At "ON" the bleaching light is switched on again, resulting in pigment photolysis. The top trace shows results of a 49-yr-old subject; two-way density is 0.29 and time constant 104 s. The bottom trace represents the same sequence in a 61-yr-old subject; two-way density is 0.16 and time constant is 137 s.

Results

The clinical data and results of densitometric measurements, and the mean RG anomaloscope settings of all subjects are summarized in Table 1. The data are arranged according to age. The YB settings on the anomaloscope are not given in Table 1. All data were normal except for subject number 15 who is deuteranope with an YB setting of 14.

Figure 1 gives an example of a foveal densitometric measurement in a 49-yr-old subject (top) and a 61-yr-old subject (bottom). In both subjects the visual acuity was 5/4 and the crystalline lens was replaced by an implant lens. The older subject shows a much lower two-way density than the younger subject. Also, regeneration in the older subject is much slower. This trend is also found in the rest of the data. In Figure 2a the two-way density of all subjects is plotted as a function of age. In Figure 2b the time constant of pigment regeneration is plotted as a function of age. Different symbols in Figure 2 indicate aphakia, pseudophakia or the crystalline lens. To paint a more generalized picture, we also plotted the mean of the densitometric data per decade from this paper and from the paper of van Norren and van Meel12 in Figure 3. Only the seventh and eighth decade demonstrate statistically significant reduced two-way densities, in comparison with the second up to and including the sixth decade (student t-test, P = 0.05). The time constants for the same decades demonstrate also a significant difference when comparing the seventh and eighth decade with previous decades (student t-test, P = 0.05). Because of the fairly high correlation between the data for left and right eye (two-way density r = 0.89, time constant r = 0.65), for statistical analysis we used only the data from the right eye whenever a choice was available. As our measurements are similar to the data of Kilbride et al,13 a dashed line in Figure 3a represents their least-squares regression line of cone pigment density in the fovea with progression of age.

Discussion

It was the intent of this study to reveal possible quantitative age changes of foveal cones with densitometry. Significant changes are found, but only after age 60. The causes for the observed decrease in two-way density may be of two kinds: senile changes in macular photoreceptors, and senile increase of light scatter by the ocular media.

Histological data described in the Introduction certainly allow for the first possibility; various morphological modifications after age 40 in cone photoreceptors are described. The decrease in number of foveal cone photoreceptors with age2,17,20 and the misalignment of foveal cone outer segment membranes15 may both account for a decrease in two-way density. As far as we know, no data are available from microspectrophotometry studies in which the density of foveal cone pigments per receptor was assessed as a function of age.

Our color vision data do not show a significant correlation between Raleigh match midpoint and age (r = 0.037, P = 0.13), just as Kilbride et al13 reported recently.
Several authors demonstrated in subjects without ocular abnormalities a slight shift with age of the Raleigh match midpoint towards the green end of the scale. That we did not observe this effect may be due to the limited number of subjects in the present study. Smith et al demonstrated that pseudoproptanomaly is an independent indication for reduced absorption in foveal cone photopigments. Loss of foveal cone pigment density associated with a pseudoproptanomalous shift in the Raleigh equation in patients with retinitis pigmentosa was reported by van Meel and van Norren and by Kilbride et al. Yet, in the present data we did not find a significant \( r = 0.0386, P = 0.135 \) correlation between the Raleigh match midpoint and two-way density (data of subject 15 not included because of congenital red-green deficiency). Kilbride et al, who discussed a similar finding, suggested that the lacking correlation between the Raleigh equation and two-way density may have been due to the different anatomic changes to which the two kinds of measurements are most sensitive. Foveal two-way density reflects optical density differences off all cones. The Nagel anomaloscope would be most sensitive to cone pigment density changes occurring in individual cones, such as shortened outer segments. Thus the senile decrease in two-way density is more likely caused by reduction in the number of cones than by changes in individual cone structure.

Stray light due to ocular senescence may also reduce two-way density. In order to evaluate the stray light factor due to an aging crystalline lens, we measured also in aphakia and pseudophakia. The data per age group are too scarce to draw firm conclusions, but there is no indication (cf Fig. 2) that there are systemic differences between the three groups of subjects. Of course, not only the lens contributes to stray light: scattered light may also arise from the vitreous, the retina, the vitreoretinal interface, clear residual cataract membranes, and intraocular implant lenses. If stray light played a role in the senile decrease of two-way density, however, we would have expected to find a decrease of the time constant rather than an increase (see Methods). As demonstrated in Figure 2b, the time constant rather has a tendency to increase with age.

Densitometry does not differentiate between the factors which may explain the drop in two-way density, but in view of the foregoing we strongly favour...
Fig. 3. Mean two-way density of foveal cones per decade of age for 107 healthy eyes, ranging in age from 13 to 79 yr (top). Mean time constant of cone pigment regeneration for the same material (bottom). The error bars indicate ±1 SD. The dashed line represents the least-squares regression line of cone pigment density in the fovea with progression of age, given by Kilbride et al.13

an explanation in terms of a reduction in the number of cones.

The substantial decrease of photopigment density with age, with an early onset reported by Kilbride et al.,11,13 is not in line with our results (see Fig. 3a). There is a statistically significant difference (student t-test, \( P = 0.01 \)) between the Kilbride data and the measurements with the Utrecht densitometer (reported by van Norren and van Meel12 and in this paper; because of the high correlation between left and right eyes we only used right eyes for statistical analysis when a choice was available). A possible explanation lies in the differences in field size diameter. Kilbride et al. illuminated a field of 30° diameter, while in the Utrecht densitometer a field of only 3.8° diameter is illuminated. The large field makes Kilbride's apparatus exquisitely sensitive to stray light in the optical media and the increase thereof with age.

A serious problem in studying larger numbers of subjects is reflected in our experience that nearly half of a selected group of older subjects is not capable of sitting quietly and fixing cross hairs during at least 20 min. This is a considerable disadvantage in cases of clinical indications for densitometry to assess age-related normative data for future clinical studies on senile macular disease.

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

**Acknowledgments**

The authors are indebted to Professors J. Pokorny and V. C. Smith, Eye Research Laboratories, University of Chicago, Illinois, for helpful discussions.

**References**