A Psychophysical Technique For Measuring Cone Photopigment Bleaching

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A new, clinically applicable test has been developed to measure the bleaching of the foveal cone photopigments. This noninvasive test is called steady-state color matching. Steady-state color matching is based on the dependence of a color match on the optical density of the cone photopigments. By measuring the color match as a function of retinal illuminance, it is possible to compute the optical density of the cone photopigments at each illuminance. Thus, abnormalities of photopigment bleaching can be detected. This study demonstrates that the technique is readily performed by a clinical population. Patients with central serous retinopathy and retinitis pigmentosa are shown to have low effective optical densities. Invest Ophthalmol Vis Sci 28:711-717, 1987

The healthy human visual system can adapt over an 11 log unit range of retinal illuminance. This wide range of adaptation is controlled by both photochemical and neural processes, and to a small extent by pupilary reflexes. In many retinal diseases, adaptational processes are disturbed.[1,2] This research is concerned with abnormalities in photopigment kinetics, ie, the processes of photopigment bleaching and regeneration. These processes are a series of chemical reactions, with bleaching taking place in the photoreceptors, and regeneration requiring both the photoreceptors and the retinal pigment epithelium. At present, the only widely accepted procedure for measuring photopigment kinetics in both normals and patients is retinal densitometry.[1,3-9] For instance, in patients with pathology of the photoreceptor-to-pigment epithelium interface, retinal densitometry has been used to demonstrate abnormalities in both the photosensitivity and the regeneration of photopigments.[7,9] However, retinal densitometry is a difficult and costly procedure and requires assumptions concerning stray light that are difficult to test.[8] Thus, a convergent technique for measuring photopigment kinetics is desirable.

We have developed a candidate technique, steady-state color matching, that provides rapid, easily-performed measurements of photopigment kinetics. This article presents the method, then considers factors affecting the interpretation of the results. Patient data indicating the applicability of the technique to patients with retinal disease is also shown. In the companion article we present detailed results obtained using steady-state color matching on a population of patients with insulin dependent diabetes mellitus.

Background

Color matching is a psychophysical technique that reveals photoreceptor properties. The normal observer can match any given light (the standard) with a mixture of three or fewer suitably-chosen lights (primaries). Once such a match is established, it does not change over a wide range of illuminances and adapting conditions.[10]

The color match most frequently used in the clinic is the Rayleigh match.[10] This and other red-green color matches have the advantages of being easily instrumented, minimally affected by preretinal filtering, and minimally influenced by contributions from short wavelength sensitive cones and rods. The patient is instructed to match one side of a disk, which contains a yellow or orange standard light (589.6 nm), to the other side, which contains an adjustable mixture of a red and a green primary light. By the appropriate adjustment of both the relative amounts of the red and green primaries, and the retinal illuminance of one side of the disc, a setting can be obtained for which an equal number of quanta of light are absorbed by the long wavelength sensitive cones and the medium wavelength sensitive cones on each side of the disk. Under these conditions, both sides of the disk produce identical responses at the photoreceptor stage of the visual system.
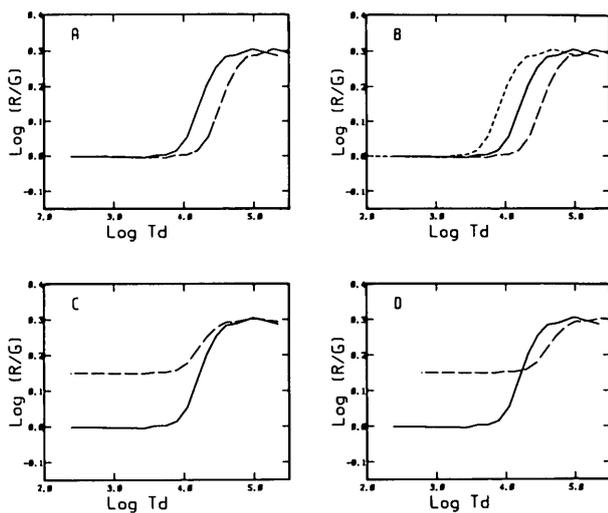
Fig. 1. The theoretical effect of various factors on the color-match-illuminance effect. (A) Neutral density filters will cause the color-match illuminance effect (solid line) to translate rightward horizontally to higher illuminances (dashed line). (B) Changes in the rate of photopigment regeneration will also cause a horizontal translation of the color-match-illuminance effect. A decrease in the regeneration rate will cause the curve to translate to the left (small dashes), while an increase in the regeneration rate will cause the function to slide to the right (long dashes). (C) A decrease in the optical density of the photopigments, with no other change, will cause the low illuminance matches to be more similar to the high illuminance color matches. Thus, the total size of the color-match-illuminance effect will decrease, but the high illuminance matches will be unchanged (dashed line). (D) The effect of misorientation of the photoreceptors (the Stiles-Crawford effect). Oblique orientation of the photoreceptors with respect to the incident light would cause a combined effect of decreasing the effective optical density of the photoreceptors, as well as of causing decreased sensitivity of the cones (dashed line).

To the remainder of the visual system, at every neural level, there is no difference between the two sides of the disk. Although later neural processing cannot make the two sides of the disc appear different, neural disease can change the appearance of the disk, or impair the ability of a patient to make an equal quantum match. The only assumption is that there are long and medium wavelength-sensitive cone photopigments; the actual numbers of cones are irrelevant as long as there are sufficient cones to make a match.

The color match is sensitive to factors that change the spectral sensitivity of the cones. The spectral sensitivity of a given cone depends upon the extinction spectrum of the photopigment, the concentration of the photopigment, and the path length of light through the cone. Previous psychophysical studies have shown that patients with certain retinal disorders make abnormal color matches at moderate retinal illuminances.[11-14] These matches are shifted so that more red primary is required for a match, a change that is consistent with a reduced optical density of the cone photopigments, due to either a decreased path length of

light through the cones, or a lower concentration of photopigment in the cones. That is, these altered color matches imply that the ability of the photoreceptors to absorb light, as indicated by the optical density, is impaired. A decreased path length through the cones may be due to receptor disorientation, or misalignment (the Stiles-Crawford effect)[15], or shortened photoreceptors. Another factor that can decrease the optical density of the photopigments is bleaching. It is well-known that at high illuminances, more red primary is required to make a match (the color-match-illuminance effect).[16-19] Theoretically, the change in the amount of the primaries is almost linearly dependent on the change in the concentration of the photopigments with bleaching.[19] For normal observers, color matches do not shift at retinal illuminances less than 8,000 td. As retinal illuminance is increased above 8,000 td, the color match requires progressively more red primary, until an asymptote is reached at about 90,000 td. This color-match-illuminance effect occurs because, as the quantal flux incident on the photoreceptors increases, there is a decrease in the steady-state concentration of the photopigments that the retina is able to maintain. Above 8,000 td, each additional increase in illuminance causes a decrease in the steady-state concentration of the photopigment. At high illuminances, the concentration of the photopigments is effectively dilute, and no further decreases in optical density are measurable. From the change in the color match at each retinal illuminance, an estimate of the optical density at that illuminance can be calculated.[19] For normal observers, the average optical density at low illuminances is about 0.3 (see below). The retinal illuminance that bleaches half of the photopigment (the half bleach illuminance or I_0) is calculated to be about 4.4 log td[18,19] and agrees with estimates made by retinal densitometry.[8,20]

Factors Expected to Affect the Color-Match-Illuminance Effect

In a clinical population, there are a number of factors that can alter the color-match-illuminance effect. We will examine the theoretical effect of four such factors: filter effects, changes in the rate of photopigment regeneration, changes in the optical density of the photopigments, and the Stiles-Crawford effect.

The first factor to be considered is the effect of pre-retinal filters. The solid line in Figure 1A shows a color-match-illuminance effect for a single observer. The dashed line shows the theoretical effect of a 0.3 log unit neutral filter on the color-match-illuminance effect. For a neutral filter, the curve will be translated to the right, since the only effect of the filter is to decrease the amount of light incident on the retina. The case for a

nonneutral filter is more complicated, since such a filter will differentially attenuate the primaries. However, since a filter cannot change the wavelength of the primaries, which are monochromatic, it cannot change the shape of the bleaching function. Thus, so long as there is no change in the mechanisms subserving the color matches (see results), any filter will simply translate the function in a combination of vertical and horizontal movements. The lights used for our match (except for the 480 nm desaturant) are all longer than 540 nm, and thus should undergo little selective filtering.

The second factor to be considered is the effect of changes in the rate of photopigment regeneration. Analogous to filter effects, changes in the rate of photopigment regeneration will also produce horizontal shifts of the color-match-illuminance effect. For instance, if a disease process slows the rate of regeneration, the whole curve should shift to the left (Figure 1B, small dashes). This can be best understood in terms of the retinal illuminance at which photopigment concentration begins to decrease. For a normal observer at this retinal illuminance, the rate of regeneration is balanced by the rate of photopigment bleaching due to light, and the resulting concentration is high. For a patient with slower regeneration, the equilibrium concentration of photopigment would be lower. Thus, the curve would be translated to lower retinal illuminances. Similarly, increases in the rate of photopigment bleaching would translate the curve to higher illuminances (to the right, Figure 1B, larger dashes). Note that these changes represent increases or decreases in the regeneration rate without a change in the nature of the regeneration process.[7] A change in the nature of the regeneration process could conceivably alter the shape of the color-match-illuminance effect.

The third factor to be considered is a change in the amount of photopigment in the cones. Such a change will also affect the color-match-illuminance effect. For instance, if the length of the photoreceptors were shortened, without any other changes, the size of the color match effect would be altered, but I_0 would not change. At low retinal illuminances more red primary than normal would be required for a match, but at high retinal illuminances the red-green ratio would be normal, since both the patients and the normals would have low optical densities at a high retinal illuminance. Thus, the size of the color-match-illuminance effect would be decreased as shown in Figure 1C. The altered low illuminance color match is the well-known pseudo-protan color match.[10,14]

The final factor to be considered is an abnormality in the orientation of the cone photoreceptors (which can produce an abnormal Stiles-Crawford effect). The Stiles-Crawford effect is the dependence of the sensitivity of the photoreceptors on the angle at which light

is incident upon them. The eye is less sensitive to light striking the photoreceptors obliquely. This is important because a wide range of retinal diseases cause abnormal Stiles-Crawford effects.[11,12,15] Patients with abnormal Stiles-Crawford effects are expected to have abnormal color-match-illuminance effects. The abnormal Stiles-Crawford effect should result in a combination of a filter effect and a change in optical density (Figure 1D). The change in optical density can occur due to the shortened path-length of light through the tilted photoreceptors. The filter effect is due to the decreased sensitivity of the cones to off-axis light (the Stiles-Crawford I effect). Thus, patients with abnormal Stiles-Crawford effects will show a combination of the above effects: decreased optical density and increased I_0.

Materials and Methods

Apparatus

The color matches were performed using the high illuminance optical apparatus described previously.[19] The apparatus has four Maxwellian view optical channels and computer-controlled light feedback circuitry for adjusting the radiance of the primary lights. By using infrared LED's, a television camera, and a video monitor, it was possible to align rapidly the patient's eye to the apparatus, as well as to monitor pupil position and size throughout the experiment. The patients' pupils, even at the highest illuminance, were larger than the 2.1 mm diameter exit pupil of the apparatus.

Stimulus

The stimulus was a 4 deg, circular field divided into two halves. On the left was the 589.6 nm standard light. On the right was a mixture of a 546 nm green primary and a 650 nm red primary. A dim 480 nm light masked the contribution of rods and short wavelength sensitive cones to the match.

The patient matched the right side to the left by the method of adjustment. By turning one knob, the patient adjusted the "color," the ratio of the red vs. green primary, without greatly changing the luminance. By turning the other knob, the observer changed the "brightness," the luminance of the red and green mixture. For patients eight illuminances were tested, from 260 to 130,000 td, with ten matches at each illuminance. Normal control observers also made an additional set of matches at 260,000 td. Each session lasted approximately 1 hr, including obtaining informed consent and making a bite bar. Following each session, the individual settings were calibrated,[19] and the log of the average ratio of red to green primaries, the (R/G) ratio, was obtained at each illuminance.
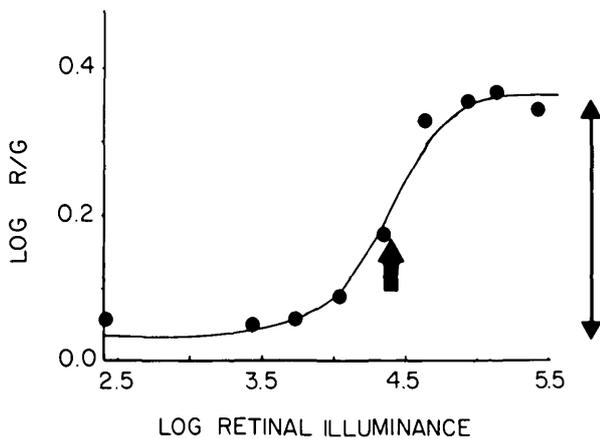
Fig. 2. An illustration of the fitting procedure used to analyze data from the color-match-illuminance effect. Filled circles are color matching data for a normal observer; solid line is a cumulative normal distribution fit to the data. Heavy black arrow is the mean of the distribution, used to indicate the retinal illuminance that bleaches half of the cone photopigments. Tall thin arrow is the difference between the low and high illuminance color matches, used to estimate the optical density of the cone photopigments.

Data Analysis

To facilitate comparisons of the data across individuals, we obtained estimates of four parameters of interest from the normal population: the baseline color-match, the optical density of the photopigments (the size of the change in the color-match), the half-bleach illuminance (the retinal illuminance which bleaches half of the photopigments), and the slope of the change (the range over which the change occurs). The baseline, or low illuminance color-match was obtained by computing a weighted average of the data at the three lowest
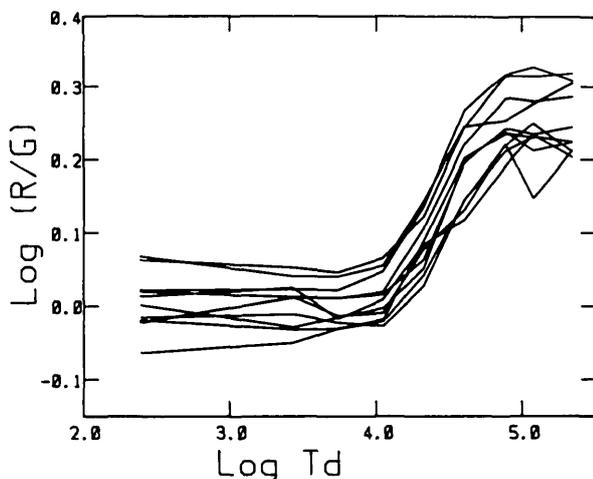

Fig. 3. Color matching data as a function of retinal illuminance for 11 normal observers and a 4 deg field size. The log (R/G) is the log of the ratio of the relative radiances of red and green primaries, as measured by a photometer with a photopic filter.

retinal illuminances. The baseline color-match is comparable to the color match that is frequently obtained in the clinic using a Nagel anomaloscope. Figure 2 shows schematically how the other three parameters were obtained. First, a cumulative normal distribution was fit by computer to the data. There are three parameters for this fit: the height, the mean, and the variance. The height provides an estimate of the optical density of the cone photopigments. Since, for a given set of photopigments, we can calculate the relation of the color-match to optical density,[19] the difference between the low-illuminance and high-illuminance color matches is predicted by the optical density. The mean of the function provides an estimate of the half-bleach illuminance, or I_0.[19] The variance controls the slope of the fit curve.

Results

Normal Data

We tested 27 normal observers on this task. Ages of the subjects ranged from 18–47 years old, with a mean age of 30. Data from 11 of the observers are shown in Figure 3. Note that despite differences in the baseline color-matches across observers, all observers show similar color-match illuminance effects. That is, the color match remains constant below 8,000 td. Above 8,000 td, however, the color-match begins to change for all observers. The mean I_0 for 27 observers was 4.40 ± 0.09 log td. The mean optical density was 0.27 ± 0.037.

Control for the effect of blur and retinal illuminance: To determine whether the color-match-illuminance effect was sensitive to factors such as optical blur and rod-intrusion we performed two control experiments. To test whether visual acuity per se would alter the results on the parameter estimates, we introduced lenses into the optical system to reduce the effective acuity of normal observers to 20/40 and 20/50. Results obtained in this experiment on two observers indicated that moderate amounts of blur did not affect the measurement of the color-match-illuminance effect. Results for the observer reduced to 20/50 acuity are shown in Figure 4. These data indicate that, for patients with 20/50 or better visual acuity, optical blur will have little effect on the data. The second control experiment was designed to detect whether, in healthy eyes, the color match was sensitive to retinal illuminance except over the range of interest. The rationale here is that if some observers show large filter effects, the retinal illuminance may be low enough to allow rod intrusion into the color-match. We measured the color-match-illuminance effect from 1–260,000 td. The color match was invariant from 1–8,000 td (a slope of only −0.004 per log unit). We also found that there was no change

in the color-match between 100,000 and 600,000 td. These control experiments confirm that under our conditions, for normal observers, color matches are invariant for low illuminances (below 8,000 td) and for high illuminances (above 90,000 td).

Patient Data

In this article, patient data is used only to illustrate the effects of retinal disease on the data, rather than to give detailed clinical descriptions of all diseases that can cause abnormalities in the color-match-illuminance effect. Such descriptions for a series of diabetic patients appear in the accompanying article.

Patient 1: Patient 1 was a 22-year-old woman diagnosed as having retinitis pigmentosa with Usher's syndrome (an accompanying hearing loss). Funduscopic examination showed bone pigment spicules, narrowing of the retinal vessels, and pallor of the optic disk. Electroretinograms were extinguished under standard testing conditions. No lens changes were noted, and the visual acuity was 20/30. Goldmann visual fields revealed only a slight constriction of the peripheral isopters. Her brother also has retinitis pigmentosa; there was no other known incidence in this family. Her data (Fig. 5) show a significant decrease in the optical density of the photopigment (a maximum optical density of 0.1), but with an I_0 of 4.38, which is indistinguishable from normal. The lower optical density is consistent with previous studies that indicate that low illuminance color matches are shifted in patients with retinitis pigmentosa.[14] There is little scatter of the individual settings, and there is no overlap of the settings made at low vs high retinal illuminances. The present results show that the low illuminance shift is due to a low optical density, and not to a shift in the entire color-match-illuminance effect.

Patients 2 and 3: Patients 2 and 3 were both women diagnosed with insulin dependent diabetes mellitus, aged 28 and 19, respectively. In this paper we present only a minimal analysis of the effects of insulin dependent diabetes mellitus; a detailed analyses is given in the following article. Patient 2 had no evidence of retinopathy on fundus examination and fluorescein angiography. The patient's visual acuity was 20/20 OU and the patient was in good health other than having diabetes. Patient 3's retinal exam on the tested eye revealed a few microaneurysms, a slight amount of macular edema, and a best corrected visual acuity of 20/25. The data (Fig. 6) show a change in I_0 without a concomitant change in the optical density of the photopigments. Estimates of I_0 were 4.72 log td for Patient 2 and 5.00 log td for Patient 3. Note that in Figure 6 these patients' data fall mainly within the 95% confidence intervals for the raw data. This is due to
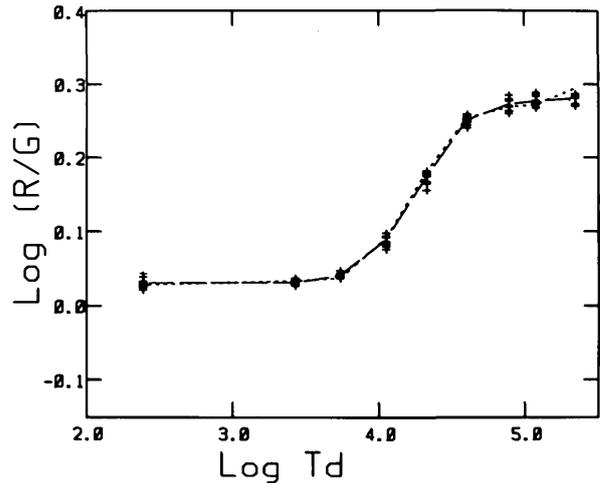

Fig. 4. The effect of blur on a normal observer. The solid line shows data collected under normal conditions. The dashed line shows data collected a week later in the presence of optical blur sufficient to reduce the observer's acuity to 20/50. The crosses are the individual data points gathered in the blurred condition.

the large variability in the baseline color-matches of the normal observers.[21] We are interested in comparing the shape of the color-match illuminance effect, not differences in individual pigments or preretinal filters. From the data in Figure 3, it is clear that, if the data of individual observers were slid vertically to produce the best fits to the mean curve, the actual shape differences between normal observers is small. The I_0 estimates for patients 2 and 3 are clearly outside of the range of the normal observers. This increase in I_0 for patients 2 and 3 could arise from a filter effect or from
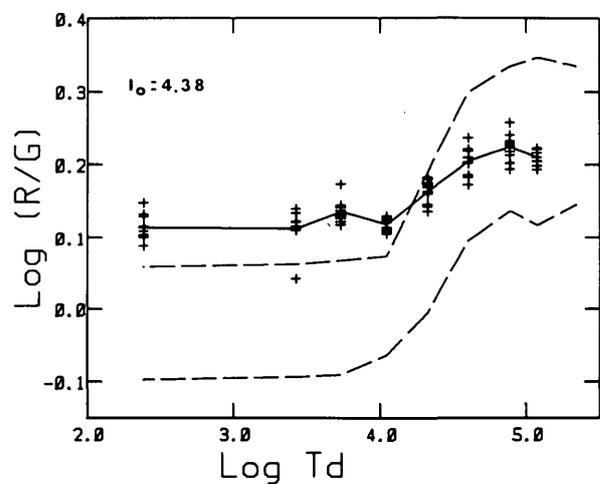

Fig. 5. Color-match-illuminance effect data for a patient with retinitis pigmentosa. The crosses are individual settings, the solid line connects the average setting for each retinal illuminance. Also shown are the 95% confidence limits for the normal population (dashed lines). The I_0 for this set of data is shown in the upper left hand corner.
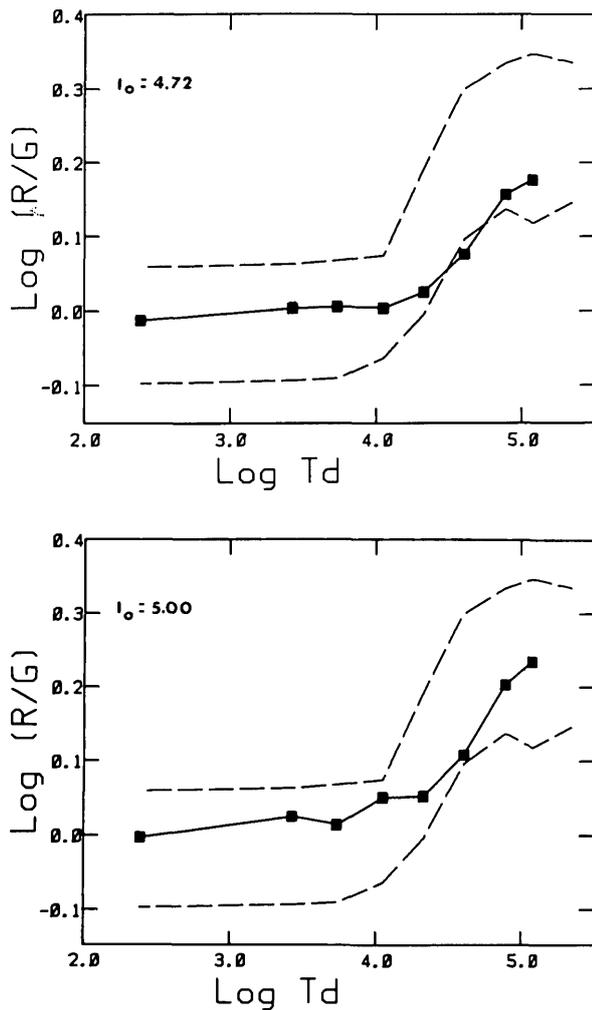
Fig. 6. Data for two patients with insulin dependent diabetes mellitus. Squares indicate average setting, dashed lines as in Figure 5. Top—Patient 2, bottom—Patient 3.

a change in photopigment kinetics (see the following article for a more detailed analysis).

Patient 4: Patient 4 is a 37-year-old male with central serous retinopathy (CSR). Data are shown in Figure 7. The area of the serous detachment was about one-half disc diameter, centered on the fovea. Data were collected both during a period of active serous detachment (VA 20/30) and 3 months later when the serous detachment was resolved (VA 20/20). During the active phase of the disease, I_0 was abnormally high (5.56 log td), while the optical density of the photopigments was low. The low initial optical density is consistent with previous findings that report a pseudo-protan effect in these patients.[11] The high I_0 could be taken to imply an increase in the rate of photopigment regeneration. However, such a conclusion is inconsistent with retinal densitometry data that show that during the active phase of CSR photopigment kinetics are slowed.[7,9] We explain this apparent discrepancy based on the ob-

served abnormalities in the Stiles-Crawford effect of CSR patients.[11,15] As discussed above, the presence of a Stiles-Crawford effect is expected to cause a combined effect, simulating both a filter and a decreased optical density. We have found the same type of change in other patients with central serous retinopathy. The data obtained during resolution of the serous detachment indicate that the optical density has returned to normal, but there is still a decreased sensitivity to light (I_0 = 4.7 log td). Von Meel et al[9] have shown that kinetics are abnormal only during the exudative phase of CSR, and returned to normal by this phase of the disease.

Discussion

Color matching can be used as a sensitive indicator of outer retinal abnormalities. Abnormalities in the color-match-illuminance effect follow well-specified patterns, predictable from theoretical considerations. An important factor in the clinical application of this technique is the consideration of the effect of decreased color discrimination in patients with retinal disease. Decreased color discrimination can lead to an increased match width, which in our technique would be seen as increased scatter of the individual settings. This increase in the match width could occur asymmetrically; that is, for example, discrimination could be worse towards green than towards red. By taking the mean setting as the color match, we could have a bias in the data. For most patients that we have tested, this is not a problem. The scatter of the individual settings is not large enough to skew the estimate of either the half-bleach illuminance or of the optical density. Figure 4 shows the individual color matches for Patient 1. The
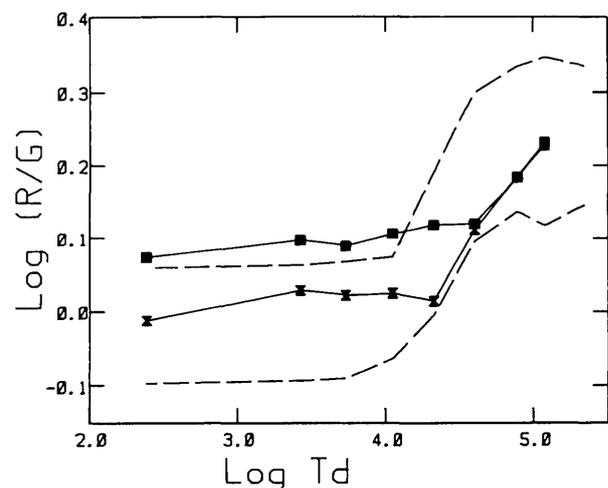

Fig. 7. Data from a patient with central serous retinopathy. Data were obtained during the active phase of the disease (squares), and 2 months later when the serous detachment had resolved (double triangles). I_0 for each run was 5.56 log td (during the active phase), and 4.72 log td (following resolution). Dashed lines as in Figure 5.

total matching range does not change appreciably with retinal illuminance, and thus the estimates obtained by using the match midpoint should provide a reasonable basis for estimating I_0 and the optical density. In patients with much worse color discrimination, care must be taken in interpreting the effect of increasing retinal illuminance. Similarly, if only the high illuminance data show a high variability, care must be taken in interpreting shifts in the color match with retinal illuminance.

In conclusion, we have developed a new, noninvasive technique for studying the function of the outer retina of the human eye. Patients with visual acuities of 20/50 or better and normal color vision can usually perform this task. An entire session takes approximately 1 hr. The technique is sensitive to factors that affect the kinetics and sensitivity of the foveal cone photopigments. Such factors produce well-specified changes in the data. Data obtained from actual patients can show several patterns of abnormalities, depending on the nature of the disease process.

Key words: retina, photopigments, bleaching, visual function, cone

Acknowledgments

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