Rhodopsin kinetics and rod adaptation in Oguchi’s disease

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The extra-foveal dark adaptation curve of the normal eye is bipartite; the early and late segments representing adaptation of the cone and rod mechanisms, respectively. In Oguchi’s disease (one of the congenital forms of night blindness) the cone branch may be extended for nearly two hours, after which time the rod branch appears, and thresholds descend slowly to the normal absolute level. It is usually assumed that this prolonged course of dark adaptation is due to an abnormality involving the synthesis of the rod pigment, rhodopsin. However, by means of fundus reflectometry, we have shown that the concentration of rhodopsin, its photosensitivity, and the rate at which it regenerates after photolysis are entirely normal. This lack of correspondence between visual pigment concentration and visual sensitivity could be demonstrated also by exposing the fully dark-adapted eye to light which bleached less than one per cent of the available rhodopsin. Whereas a normal subject quickly readapted to the dark-adapted level in about seven minutes, thresholds were raised nearly 3 log units in the case of a subject with Oguchi’s disease—and remained there for at least 30 minutes. Studies of the electroretinal responses (ERG and EOG) in this subject indicate that the visual disturbance results probably from defective neural processes within the retina. In this respect, Oguchi’s disease is similar to other forms of congenital stationary night blindness, although the transient nature of the defect suggests a different physiological basis.

Oguchi’s disease is a congenital disorder of night-blindness characterized clinically by a golden or gray-white discoloration of the fundus. Two additional features distinguish this condition from other forms of stationary nystagmus: (1) visual sensitivity, which is greatly reduced after more than 30 minutes of dark adaptation, becomes normal after several hours in complete darkness, and (2) the color of the fundus also reverts to normal after a prolonged stay in darkness. Although either of these features may be absent in variants of the disease, both were exhibited by the subject we have studied.

As a clinical entity, Oguchi’s disease ranks high among the rara aves of ophthalmic pathology; less than 100 cases have been reported, and the majority of these patients are Japanese. However, the study of this unusual disorder may contribute appreciably to our understanding of the visual process. Of particular interest in this regard is the remarkably slow rate at which the rod mechanism adapts to darkness. As Mann so aptly stated, “the
condition is not so much one of night blindness as of excessively slow dark adaptation." It is not surprising, therefore, that the disturbance of scotopic function is often attributed to a photochemical abnormality affecting the regeneration rate of rhodopsin, the light-sensitive pigment subserving rod vision. According to this hypothesis, it is assumed that the prolonged dark-adaptation process in Oguchi's disease is the result of retarded pigment kinetics, i.e., after exposure to light, rhodopsin is resynthesized at an abnormally slow rate. Indeed, this assumption appears even more likely in view of the causal relationship that has been postulated between long visual threshold and the concentration of bleached photopigment during dark adaptation of the normal eye. A

But, there is no evidence that photochemical and visual adaptation follow the same time course in Oguchi's disease—since the kinetics of rhodopsin have not been studied previously in this disorder. In the present experiment, therefore, the bleaching and regeneration properties of rhodopsin were objectively determined by means of fundus reflectometry, and the results were compared with the subjective dark-adaptation function measured in the same region of the parafoveal retina. In addition, correlative data have been obtained with the aid of some electrophysiological tests of retinal function.

Methods

The subject, an 18-year-old Caucasian woman, was one of several patients with typical Oguchi's disease who were examined by Carr and Gouras, and she was referred to in their paper as Case 3. Her only ocular complaint was poor night vision, first recognized at age 12. A 15-year-old sister had a similar disorder (Case 4 of Carr and Gouras), but otherwise the family history was negative, and there was no parenteral consanguinity.

Visual acuity was 20/20 bilaterally and, except for the fundus signs of Oguchi's disease, all ophthalmological findings were normal. When the fundus was first examined, a metallic silver-gray sheen that originated deep to the retinal vessels was seen in the midperiphery of both eyes. Following prolonged dark adaptation, the metallic reflex disappeared and fundus color was entirely normal (Mizuo's phenomenon). After about 15 minutes in moderate room illumination, patches of gray began to reappear in the midperiphery, and extended over this entire region after one hour. Noteworthy was the sparing of the posterior pole and the more peripheral retinal regions, which remained free of any visible discoloration, even after exposure to very high luminances. This fortuitous circumstance enabled us to perform fundus reflectometry in a normal area of retina, and thereby avoid having ambiguous color changes superimposed on measurements of the difference spectra.

Dark adaptometry. Testing was performed monocularly on a modified Goldmann-Weekers Adaptometer. After the subject's pupil had been dilated with 2 per cent cyclopentolate hydrochloride (Cyclogyl), she was light-adapted for 7 minutes to a luminance of 8,900 cd per square meter. Thresholds during dark adaptation were measured for a white, circular test field that subtended 4.5 degrees at the cornea, and was located 12 degrees in the nasal visual field (temporal retina). Test flashes were 0.7 second in duration.

Fundus reflectometry. The apparatus (Fig. 1), principles of measurement, and procedure used were similar to those previously described. A xenon arc lamp, operated at constant current from a stabilized DC power supply, provided the source S for both reflectivity measurements and bleaching. Light for the measuring beam passed through the heat filter C, was focused by lens L, upon a small circular aperture A, and then was collimated by lens Lc. The beam then traversed, in turn, a series of narrow-band interference filters which were mounted in spectral order on wheel W (driven at a rate of 2 rev. per second), and was directed to the subject's eye by prism P. The filters (half-band widths < 6 m\(\mu\)) covered the spectral range from about 400 to 640 m\(\mu\). With sliding mirror M removed from the optical path, lens Lc formed an image of aperture A, in the plane of the subject's dilated pupil. Aperture A, conjugate with the subject's retina, restricted the dimensions of the beam to a visual angle of 2 degrees. A small illuminated spot (not shown) was used for fixation, and placed the test region 12 degrees temporal to the fovea.

Light reflected at the fundus and emerging through the upper half of the pupil was reflected by prism P, to lens L which focused the rays onto the variable aperture A. The latter was made to correspond in area to the retinal image of the test field, and served to eliminate much of the stray light from other ocular surfaces. Accurate centration was ensured by observation through eyepiece E, which gave a magnified view of the subject's fundus and overlying test field. The light then fell on the cathode of a photo-
Fig. 1. Schematic diagram of the apparatus (not to scale). The collecting system has been turned through 90 degrees to show its components. For details please see text.

multiplier (EMI 9558 B*), the output of which was fed through an operational amplifier for oscilloscope display and computer processing.

The alternative optical path of Fig. 1 provided the bleaching beam. With shutter $H_t$ opened, and mirror $M$ in the position shown, the test field was occluded, and the bleaching light entered the eye after reflection at prism $P_t$. Throughout the bleaching period, the photomultiplier was occluded by shutter $H_t$. The intensity and spectral composition of the bleaching beam could be varied by filters at $F$; the angular subtense of the field was limited by aperture $A_t$ to 4 degrees 35 minutes in diameter. The remaining optical elements of the system performed in the manner described for the measuring beam.

The procedure was such as to obtain 8 to 10 complete spectral scans of the test region: first, with the eye fully dark adapted; again, after it had been exposed to an intense bleaching light for 30 seconds. For each measuring wavelength $\lambda$, the change in density $\Delta D(2)$ (i.e., the density change corresponding to light traversing the retina twice) was then computed. The rate at which the bleached photopigments regenerated was obtained from reflectivity measurements taken at various times after the bleaching light was extinguished. Plotting the values of $\Delta D(2)$ at $\lambda = 500 \text{ m}_{\mu}$ (near the maximum of the difference spectra) during the course of dark adaptation gave the time course of regeneration.

All data processing was performed on-line by a Control Data Corporation 160-A digital computer that was housed in a laboratory several blocks from our own, and was linked to the apparatus by Western Electric Data-Phones (Fig. 2, a). The output of the operational amplifier was a series of deflections, each of which corresponded to a given wavelength of the test beam (the oscilloscope trace of a portion of a single spectral scan is shown in Fig. 2, b). The amplitude of any point on the wave form is proportional to the light flux impinging on the photomultiplier after having twice traversed the eye. After transmission to the computer laboratory, the electrical signals enter an analogue to digital converter (which samples about 15 points $y_i$ on each wave form), and the digitized information is fed to the computer, where the values of $y_i$ are integrated to approximate the area under the curve (Fig. 2, c). This operation was performed for every wave form of 8 successive spectral scans taken during each test period. Thus, $\log_{10} A_t$ (used in the equation below) was the average of 8 values obtained for a given experimental condition. Density changes at each measuring
wavelength ($\Delta D_\lambda$) were computed from the relation:

$$\Delta D_\lambda = (\log A_b - \log A_t) \lambda,$$

where $A_b$ is the area of the deflection after bleaching, and $A_t$ is the area of the deflection following dark adaptation. Permanent records of the raw data, computed values of $\Delta D_\lambda$, and graphical plots of the density difference spectra were printed at the computer laboratory for detailed analysis; but graphs of the difference spectra were sent via the Data-Phones to an X-Y plotter in our laboratory for immediate appraisal of the results.

**Electro-oculography.** Changes in the corneo-fundal (standing) potential during light- and dark-adaptation were determined by means of the electro-oculographic technique described by Arden and Kelsey. The subject was required to fixate alternately two small neon lamps which were located in the horizontal plane, and separated by an angle of 20 degrees with respect to the eye. The lamps were electronically controlled to flash sequentially every 0.8 second. Eye movement potentials were picked up by chlorided silver electrodes near the inner and outer canthi of each eye, and recorded on an ink-writing oscillograph.

Recordings were obtained for periods of about 10 seconds, at intervals of one minute, under the following conditions: (1) during a 10 minute practice session at the ambient room illumination of 120 cd per square meter, (2) while in complete darkness for 15 minutes, and (3) during 15 minutes of light adaptation to 8,900 cd per square meter. The response amplitude resulting from a complete horizontal saccade was measured, and the average of five such readings at each one minute interval was taken as the value of the standing potential. The minimum potential recorded in darkness (dark trough), the maximum potential during light adaptation (light peak), and the ratio of light peak to dark trough (LP/DT) were determined from these data.
Results

Dark adaptometry. Fig. 3 shows visual threshold measurements in the paracentral retina as a function of time following a 7 minute exposure to 8,900 cd per square meter. The solid line drawn in Fig. 3, A illustrates the results obtained on a normal subject for the same test conditions. The initial branch of the normal bipartite curve, representing photopic (cone) adaptation, reaches a plateau within 6 minutes after extinguishing the light-adapting field. After 11 minutes in darkness, the scotopic (rod) branch appears and descends to a final threshold level, approximately at the 25 minute mark.

In the case of Oguchi's disease (open circles), cone adaptation proceeds normally, but rod function is not evident for the entire 35 minute test period covered by this graph. In Fig. 3, B, the time base has been reduced in order to chart threshold measurements taken over the next 3½ hours. The first sign of rod adaptation appears after nearly 2 hours of dark adaptation, and another 2 hours are required before the normal absolute threshold is attained. Thus, in regard to rod function, the rate of adaptation in the patient with Oguchi's disease is about 9 times slower than that in the normal.

Rhodopsin kinetics. Fig. 4 shows the density difference spectrum measurements of the same retinal region (12 degree temporal retina) examined previously by dark adaptometry. The density changes were computed from reflectivity measurements obtained: (1) after exposure to an intense white light, and (2) after 1 hour of dark adaptation; results are for a single experimental run. The retinal illuminance of the bleaching beam (1.05 x 10^8 trolands for 30 sec. = 7.5 log td sec.) was sufficient to bleach more than 90 per cent of the rhodopsin within the test area. The magnitudes of the density changes, the spectral location of the maximum (λ_max = 500 m), and the shape of the difference spectrum indicate that rhodopsin is the light-sensitive pigment undergoing photolysis, and that normal amounts are present in a paracentral region of this subject's retina.

The data of Fig. 5 provide evidence that rhodopsin has fully regenerated long before rod function is manifested subjectively. The curve shows the course of pigment regeneration as measured at λ = 500 m, from the density difference spectra obtained at various times during dark adaptation; the initial bleaching exposure was as before, 10^7.5 td sec. Values of ΔD_{500} were plotted relative to the maximum obtained after 4 hours in darkness (represented by the dashed line intersecting the scale of ordinates at unity). The results indicate that rhodopsin reached about 95 per cent of its maximum concentration after 30 minutes of dark adaptation; and was completely regenerated after 60 minutes, with...
a half-time of 3.5 minutes. No further change was detected over the next 3 hours. This time course is clearly within normal limits for rhodopsin kinetics.\textsuperscript{5,13}

**Adaptation following exposure to dim light.** The remarkable conclusion to be drawn from the results of Figs. 3 and 5 is that the changes in rhodopsin concentration and visual sensitivity during dark adaptation proceed independently of each other. Within the first 2 hours of dark adaptation, when thresholds were hovering about the cone plateau, rhodopsin was reforming at a normal rate. During the next 2 hours in darkness, as the visual thresholds slowly approached a normal level, rhodopsin concentration remained unaltered—having fully regenerated within the first hour of dark adaptation.

But before this conclusion is accepted, some consideration must be given to a possible source of error in the rhodopsin measurements. In a preliminary report, Rushton\textsuperscript{4} showed that, following a substantial bleaching exposure, the cone-rod transition ("kink") in the normal dark adaptation curve first appeared after more than 90 per cent of the available rhodopsin had regenerated. Prior to that time, rod thresholds were masked by the greater sensitivity of the cone mechanism. Thus, the entire scotopic segment of the dark-adaptation curve corresponded in time to the regeneration of the final 10 per cent rhodopsin. We must face the possibility, therefore, that fundus reflectometry fails to detect changes of the order of 0.015 density unit

\[(10 \text{ per cent of } \Delta D_{\text{max}} = 0.1 \times 0.15),\]

and it is the regeneration of this last fraction of rhodopsin that is retarded in our subject.

Fortunately, one can adopt a simple expedient to avoid the labor involved in demonstrating that Weale's technique is equal to the task; and provide, at the same time, independent evidence that rod thresholds do not await the regeneration of rhodopsin. First, our subject was dark-adapted for 4 hours, at which time her absolute threshold was normal. She was then light-adapted to a retinal illuminance of 1,250 scotopic trolands for a period of 1 minute

\[= 7.5 \times 10^4 \text{ td sec}.\]

**Fig. 4.** The difference spectrum measured at 12 degrees in the temporal retina of a subject with Oguchi's disease. A white bleaching light of 7.5 log troland seconds was used. The ordinates, \(\Delta D(2)\), give the density change for double transit through the retina; density losses due to bleaching are plotted as positive values.
Although this illuminance is capable of "saturating" the rod mechanism, it bleaches only about 0.8 per cent of the available rhodopsin, even if no regeneration takes place during the exposure (cf. Fig. 7). After extinction of the light-adapting source, thresholds were measured for almost 30 minutes with the results shown in Fig. 6; the findings for a subject with Oguchi's disease (open circles) are compared with those for a normal subject tested under the same conditions (continuous curve). Whereas, the normal observer recovered rapidly from this modest degree of light adaptation, our subject's thresholds (which were elevated initially to the same level) did not change for the entire test period. Thus, with more than 99 per cent of its rhodopsin intact, the rod mechanism remained markedly insensitive to light.

**Photosensitivity.** Carr and Gouras have suggested that an unusually high rhodopsin photosensitivity may account for the fact that, in Oguchi's disease, relatively feeble stimuli profoundly raise the visual threshold. In other words, a light which barely affects the concentration of rhodopsin in the normal eye would, on this view, bleach a considerable amount of rhodopsin in our subject. Were this the case, it might help to account not only for the results of Fig. 6, but also explain the extended dark-adaptation curve of Fig. 3 (e.g., each test flash given during dark-adaptation delays rhodopsin regeneration). Although rhodopsin photosensitivity is unlikely to exceed the normal on theoretical grounds, the problem is easily resolved experimentally.

In normal circumstances, rhodopsin bleaches according to equation 1:

\[
\frac{c_b}{c_0} = 1 - e^{-\alpha \gamma \tau}
\]

where \(\frac{c_b}{c_0}\) is the fraction bleached of the initial concentration \(c_0\); \(I\) is the intensity delivered for time \(\tau\), and \(\alpha \gamma\) is the photosensitivity (i.e., the product of the extinction coefficient \(\alpha\) and the quantum efficiency \(\gamma\)).

It is evident from Equation 1 that the photosensitivity \((\alpha \gamma)\) is given by the value \(1/(It)_r\), where \((It)_r\) is that exposure which bleaches \(1-(1/e)\) of the available rhodopsin. When \(It\) is expressed in td sec, \((It)_r\) is approximately \(10^7\) td sec, from measurements in the normal retina.\(^{10,11}\)
Accordingly, we determined (by means of fundus reflectometry) the effect on rhodopsin concentration of three bleaching exposures: \( I_t = 10^{5.5}, 10^{6.5}, \) and \( 10^{7.5} \) td sec., respectively. The results are shown in Fig. 7; the curve is a plot of Equation 1 for \( \gamma = 10^{-7} \) (td sec.)\(^{-1}\), and the dashed line indicates the expected value of \( C_b/c_o \) for a bleaching level of \( 10^7 \) td sec. The findings, obtained in a single experimental session, convincingly demonstrate a normal rhodopsin photosensitivity in our subject.

Electrophysiology. The amplitudes of the electro-oculograph (EOG) recorded at various times during periods of light- and dark-adaptation are shown in Fig. 8. Each point represents the average amplitude of five tracings obtained within several seconds of the time indicated. Parallel changes occurred in both eyes, although the left eye gave slightly larger potentials; these small differences, however, are attributed to differences in electrode placement, skin resistance, etc.

It should be noted that no special precautions were taken as regards preliminary dark-adaptation. For 10 minutes prior to the series of measurements obtained in darkness, recordings were made with the subject's eyes exposed to a luminance of 120 cd per square meter, a light level sufficient to raise her visual threshold more than 2 log units for the remainder of the test (cf. Fig. 6). This adverse effect on visual sensitivity, however, was not reflected in the electro-oculographic responses, all aspects of which were normal. The standing potential decreased to its lowest value after 7 minutes in darkness, increased sharply to a maximum after 7 minutes of light-adaptation, and decreased thereafter. The percentage change in amplitude between the light peak and dark trough (LP/DT \( \times 100 \)) is perhaps the most reliable index for assessing the light-induced change in the standing potential. For our subject, similar values were obtained in both eyes: 205 per cent, O.D.; 209 per cent, O.S. Both percentage increments are well within the normal range (185 to 322 per cent) reported by Arden and Barrada.\(^{16}\)

Electroretinographic (ERG) study of this subject was also performed using a conventional recording technique described previously.\(^{17}\) However, the ERG examination was less thorough than that of Carr and Gouras,\(^{6}\) and served merely to confirm their original observations (see Fig. 4 of their paper).

With regard to the cone mechanism, the photopic b-wave of the light-adapted eye, and the flicker response to intermittent stimulation (30 flashes per second) were normal. During dark-adaptation, on the other hand, the ERG responses were unlike those of the normal. Whereas, the amplitudes of the initial negative component (a-wave) of the ERG increased (in parallel with the normal response) throughout the course of dark-adaptation, only a small photopic-like positive potential was recorded. The large positive potential (b-wave) obtained from the normal retina could not be elicited, even after the eye had been dark-adapted for 4 hours, and both the visual threshold and pigment concentration had reached normal levels.
Fig. 7. The bleaching of rhodopsin in Oguchi's disease (data points). The continuous curve represents a plot of Eq. 1 (see text) for a rhodopsin sensitivity of $10^{-7}$ td sec$^{-1}$, the value derived from fundus reflection measurements in the paracentral retina of the normal eye.

Fig. 8. Amplitudes of the electro-oculographic tracings recorded from the right (filled circles) and left (open circles) eyes of our subject. Each point represents the average of five measurements obtained approximately at the times indicated. For details, please see text.

Discussion

An unexpected, but consistent finding appears to have emerged from the study of disorders involving congenital nightblindness. An earlier paper showed that the retinae of some congenital nyctalopes contained normal amounts of rhodopsin, and that this visual pigment bleached and regenerated at the normal rate. Similar results have now been obtained in a subject with Oguchi's disease. Although it would be unwise to generalize from the study of so few subjects, one fact is clear: impaired night vision cannot be attributed a priori to an abnormality affecting rhodopsin or its photochemical properties.

Having demonstrated that the visual anomaly in Oguchi's disease does not result from an aberration in rhodopsin kinetics, we may next inquire whether there is some other disturbance in retinal function, as distinct from perceptual. The electrophysiological findings indicate that there is. Although both the a-wave of the electroretinogram and the light rise in the standing potential of the eye are entirely normal, the scotopic b-wave of the ERG cannot be elicited, even in the fully dark-adapted eye. The results of recent studies suggest that the a- and b-wave components of the electroretinogram come from the vicinity of the receptor inner segment and bipolar cell layer, respectively; whereas, the light-induced change in the DC potential probably arises between these sites (e.g., near the outer plexiform layer). Thus, the region of the bipolar cells appears to be the earliest stage in the visual pathway exhibiting signs of defective function. It is interesting that electroretinal responses similar to those reported here were obtained from a subject with stationary nightblindness (Subject R. C. of Carr and associates), but the transitory visual abnormality in Oguchi's disease suggests that the nature of the defect differs in these conditions. Perhaps the most puzzling feature of the electrical response in Oguchi's disease is the lack of correspondence between visual sensitivity and the amplitude of the b-wave; despite a normal scotopic threshold after 4 hours in darkness, the b-wave remains severely depressed. A possible explanation for this disparity has been offered by Carr and Gouras.

We have no new observations concerning the relationship between the peculiar changes in fundus color and any aspect of visual physiology. The time course of the color phenomenon seen ophthalmoscopically and the dark-adaptation function
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seem quite different, e.g., brief exposure to dim light quickly raised thresholds without affecting the appearance of the fundus. Furthermore, the retinal region used for dark-adaptometry and fundus reflectometry appeared normal, even after exposure to intense bleaching lights. However, subtle color changes may have occurred and escaped detection by ophthalmoscopy. It is obvious that fundus reflectometry could be directed to this question, but our subject was available only for a short time. For this reason, other tests which might have contributed to our understanding of Oguchi’s disease (e.g., intensity discrimination, the area-intensity function during dark-adaptation) could not be performed.

Unlike other forms of congenital nightblindness where the scotopic system contributes little to vision, in Oguchi’s disease, the rod mechanism dark-adapts to a normal absolute threshold; but the rate at which it adapts is grossly abnormal. Oguchi’s disease thus provides a unique opportunity to compare, in the same retinal region, the time course of subjective adaptation with the regeneration rate of the rod photopigment, rhodopsin. The results of this comparison (Figs. 3 and 5) demonstrate the absence of any relation between the dark-adaptation function and the rate at which rhodopsin regenerates after bleaching.

This finding is difficult to reconcile with the view that log threshold is directly proportional to the concentration of "free opsin" (the protein moiety separated from rhodopsin on bleaching). According to Rushton’s latest hypothesis, however, these processes are not directly coupled; but are linked through the neural integrating mechanism of the rods—the rod “pool.” Thus, it is assumed that free opsin continually signals the pool as to its concentration, thereby affecting the organization of the pool (i.e., its ability to accept, integrate, and transmit to visual centers any additional signals resulting from rod stimulation). Now, it may be argued that the defect in Oguchi’s disease upsets the communication channels between free opsin and the pool, and, in so doing, interferes with the normal reorganization of the pool.

But the experiment of Fig. 6 is not readily compatible with an interpretation of this sort. Since, in this experiment, the amount of free opsin formed by the weak light-adapting field was negligible, its failure to properly signal the pool could be of little consequence; and yet, thresholds remained elevated for almost 30 minutes. Furthermore, scotopic thresholds underwent a change (decrease) of more than 2 logarithmic units (Fig. 3) during a period in which rhodopsin is fully regenerated (Fig. 5), i.e., in the absence of significant amounts of free opsin. Indeed, the ability of the eye to dark-adapt independently of changes in opsin concentration (in Oguchi’s disease) raises the question as to whether free opsin and the visual threshold are related in the normal retina. This point will not be belabored here. Suffice it to say that there is, as yet, no unequivocal experimental evidence to support this relationship.

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