Rhodopsin and the electrical activity of the retina in congenital night blindness

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The inability of night-blind persons to see at low levels of illumination is usually attributed to a defect involving the biosynthesis of the visual pigment, rhodopsin. Accordingly, it has been assumed that the retinal rods of the congenital nyctalope are deficient in rhodopsin or its precursors. To test this hypothesis, fundus reflectometry was performed on two subjects, each having a different form of congenital night blindness (dominant and recessive, respectively). In both subjects, the concentration of rhodopsin and the rate at which it regenerated after bleaching were normal. Clearly, a photochemical basis for this anomaly is untenable. However, the electrophysiological responses of these subjects showed distinct abnormalities: (a) In the case of recessive inheritance, there was a normal light rise in the eye's standing potential, normal a-wave potentials in the ERG, but a very much reduced scotopic b-wave. (b) In the dominant form, the light rise in the standing potential was abnormal, and all ERG responses were markedly depressed. These findings implicate the neural transmission pathways as the locus of disturbance in this anomaly. In view of a normal photochemistry, analysis of the results suggests that both the a-wave of the ERG and the light rise in the standing potential originate proximal to the outer segments of the receptors.

Nystagmus or night blindness occurs in a variety of circumstances: as an inherited defect, as a concomitant of some retinopathies, and as a sequela of vitamin A deficiency. Although we are concerned here only with the stationary, heritable forms of night blindness, it is important to mention some of the retinal changes caused by the lack of vitamin A, a precursor of the photosensitive pigments of rods and cones. Dowling and Wald\(^1\) showed that the vitamin A-starved rat became progressively less sensitive to light, i.e., night blind, as the rhodopsin content of the retina decreased—a consequence of having lost its prosthetic group, the aldehyde of vitamin A. With continued vitamin deprivation, opsin (the protein component of the visual pigment) also declined, the structural integrity of the retina was upset, and the retinal cells began to deteriorate. It is noteworthy that these changes could be reversed by the administration of vitamin A even after the onset of retinal degeneration.

There is little to suggest that the defect in hereditary nyctalopia corresponds to any stage of the pathological process observed in avitaminosis A. The fundi of congenital nyctalopes appear normal ophthalmoscopically,\(^2\) vitamin A therapy does not enhance visual sensitivity,\(^2\) and retinal structure is described as entirely normal.
in the sole histological report that has come to our attention.\(^4\)

Although Dowling and Wald also stress the need to distinguish the acquired defect from the various types of hereditary night blindness, they suggest that the heritable forms "may possibly involve the failure to synthesize the specific protein of the rods—rod opsin." However, there is compelling, albeit indirect, evidence to indicate that the rod elements of some hereditary nyctalopes are functionally normal despite the severe impairment of scotopic vision. In these cases, the a-wave of the electroretinogram, which appears to originate at the receptors,\(^5\) contains a normal scotopic component,\(^6\),\(^7\) and the standing potential of the eye reacts normally to light.\(^1\),\(^8\) It has been assumed that the light-induced change in the standing potential is generated by metabolic processes involved in rhodopsin synthesis.\(^9\) In view of these findings, Carr and Siegel\(^7\) are of the opinion that such heritable defects result not from the lack of rhodopsin or its precursors, but from an inability of the retina to utilize photochemical events, perhaps as a consequence of abnormalities in electrogensis or the transmission of electrical signals across the retina. But the electrophysiological findings cited do not provide unequivocal support for this hypothesis since the origins of the retinal potentials have not been resolved fully.

There is also evidence to suggest that other forms of congenital nyctalopia exist which have a different physiological abnormality affecting, perhaps, the photoreceptors. In some cases, for example, only a photopic (cone) response can be elicited by electroretinography,\(^10\),\(^11\) whereas in others, the light-induced change in standing potential is grossly abnormal.\(^12\),\(^13\)

Does the retina of the congenital nyctalope contain rhodopsin? It is apparent from the foregoing that this question is central to any hypothesis regarding the etiology of night-blinding disorders. Fortunately, an answer can be readily obtained by determining, in vivo, the nature and properties of the photosensitive substances in the retina. This we have done for two subjects with congenital, stationary night blindness. One of these gave evidence, electrophysiologically, of rod function; the other appeared to be completely devoid of scotopic activity. Irrespective of the electrical responses, fundus reflectometry in the peripheral retina of both subjects gave the normal difference spectrum of rhodopsin; in both, the magnitude of the density change produced by bleaching indicated normal amounts of photopigment; and in both, the pigment regenerated at the normal rate after photolysis. These data and the results of adaptometric and electrophysiological measurements are reported in the present paper. In the following paper\(^11\) we describe some experiments concerned with the relative contribution of the photopic and scotopic mechanisms to the visual system of these subjects.

**Methods**

**Subjects.** One of our subjects (P. M.), a 25-year-old woman, was from a family whose members inherit nyctalopia as a dominant trait. Those examined so far, namely, her father, two sisters, her two daughters, and two nieces, were night blind. The other subject (R. C.) was a 19-year-old man who has no familial history of nyctalopia; he was the offspring of a nonconsanguineous marriage. Neither of our subjects showed any pathological condition or complained of any symptom other than could be associated with the object of our study. That is, they have had difficulty with night vision since early childhood, and to the best of their knowledge, the condition has not altered since then. Both subjects had moderate degrees of myopia, but were corrected to 20/20 (1.0) Snellen acuity; perimetric fields and color vision were also normal.

**Dark adaptometry.** Testing was performed monocularly on a modified Goldmann-Weekers Adaptometer after the subject's pupil had been dilated with 10 per cent phenylephrine hydrochloride (Neosynephrine) and 1 per cent cyclopentolate hydrochloride (Cyclomol). Prior to an experimental run, the subject was light adapted for seven minutes to a luminance of 8,900 candela per square meter. Thresholds during dark adaptation were measured for a white, circular test-field, subtending 4.5 degrees at the subject's cornea and located 12 degrees in the nasal visual...
field (temporal retina). Test flashes were 0.7 second in duration.

**Electroretinography.** Electroretinal responses were elicited with 10 msec flashes of white light from the Xenon discharge lamp of a Grass photic stimulator. The open end of the lamp housing was covered with opal glass to give a uniformly illuminated field subtending a visual angle of 25 degrees. The control circuit of the stimulator permitted variation in flash luminance from about $4.3 \times 10^{6}$ to $6.8 \times 10^{7}$ cd per square meter in steps of approximately 0.3 log unit. Although the spectral characteristics of the flash vary somewhat between settings, this was not considered an important factor in the present experiment. Both flicker and single-flash stimulation were employed in an attempt to evoke photopic and scotopic responses, respectively. Electroretinograms are recorded as the potential change between a contact lens electrode (Burian-Allen) and a reference electrode adherent to the subject's forehead. The potentials were amplified, and recorded on an ink-writing polygraph (Grass Instrument Company).

The frequency response of the system was set for 3 db attenuation at 0.6 and 60 cycles per second.

**Standing potential measurements.** The electro-oculographic technique described by Arden and Kelsey was used to determine the effect of light on the corneofundal potential. Chlorided silver electrodes were affixed to the subject's skin near the lateral and medial canthi of each eye. The electrodes led to a differential amplifier (time constant = 0.5 second), the output of which was recorded on an ink-writing oscillograph. Throughout the bleaching period, the photopic field at the eye to an angle of 4 degrees, 35 minutes in diameter. The intensity and spectral composition of the beam could be varied by filters at $F$. The remaining optical elements of the system were shared with the measuring beam.

Light reflected at the fundus and emerging through the upper half of the pupil was reflected by prism $P_{s}$ to lens $L_{s}$ which focused the rays onto aperture $A_{s}$. The latter corresponded in area to the retinal image of the test field and served to eliminate much of the stray light from planes not conjugate with it. The light then fell on the cathode of a photomultiplier (EMI 9558B), the output of which was displayed on a direct-coupled oscilloscope and photographed.

For bleaching, the alternative optical path of Fig. 1 was used. With shutter $H_{s}$ opened, and mirror $M$ in the position shown, the measuring beam was occluded and the bleaching light entered the eye after reflection at prisms $P_{s}$ and $P_{t}$. Throughout the bleaching period, the photomultiplier was occluded by the shutter $H_{s}$. The aperture $A_{s}$ restricted the angular subtense of the bleaching field at the eye to an angle of 4 degrees, 35 minutes in diameter. The intensity and spectral composition of the beam could be varied by filters at $F$. The remaining optical elements of the system were shared with the measuring beam.

The procedure was such as to obtain a complete spectral scan of the test region first with the eye fully dark adapted, and again after it had been exposed to an intense bleaching light for 30 seconds. For each measuring wavelength, the changes in density $A_{k}$ (2), *i.e.*, the density changes corresponding to light traversing the retina twice, were then computed from measurements of the corresponding oscilloscope deflections. The rate at which the bleached photopigments regenerated was obtained from reflectivity measurements taken at various times after the bleaching light was extinguished. The change in density at $\lambda = 510 \text{ m}_{\mu}$, near the maximum...
Results

Dark adaptation. The results of dark adaptometry for nyctalopes P. M. and R. C. are shown in Fig. 2 where they are compared with the normal function obtained for the same test conditions. As is well known, the extrafoveal dark-adaptation curve of the normal eye is bipartite, the early and late parts representing the adaptation of the cone and rod mechanisms, respectively. For subject P. M., however, there is no discontinuity in the curve. Thresholds fall rapidly at first, but, after only three minutes in darkness, reach a minimum value that is retained throughout the subsequent test period. This final level is approximately 0.5 log unit above the normal cone plateau and nearly 3 log units above that of fully dark-adapted normal rods.

The results for R. C. give a somewhat different picture. Here the initial rate of adaptation is slower, but sensitivity continues to increase over a longer period of time. Furthermore, the thresholds do not follow a monophasic time course as closely as those of subject P. M.; a discontinuity
in the curve, suggestive of a transition from cone to rod vision, is detectable near the 15 minute mark. But despite this slight “kink,” thresholds after 30 minutes in darkness do not fall below the level of the cone branch of the normal dark-adaptation curve.

The absence of a discontinuity in the curve for subject P. M. suggests that thresholds over the entire adaptation period are due solely to the cone system. But even the cones are only one-third as sensitive as in the normal eye. And subject R. C. shows a similar reduction in cone sensitivity, if we assume that the portion of his dark-adaptation curve after the kink is due to rod function (cf. Carr, Ripps, Siegel, and Weale). This reduction in cone sensitivity is not a unique finding in congenital night blindness and as shown below is evidenced also in the electroretinogram. In many nyctalopes, therefore, photopic as well as scotopic vision may be affected.

Electrophysiology. Fig. 3 compares electroretinograms recorded from night-blind subjects P. M. and R. C. with those of a normal subject. In the dark-adapted state (top), the response of the normal eye to a brief flash of white light consists of an initial cornea-negative potential (a-wave) followed immediately by a larger cornea-positive potential (b-wave); these components increase in amplitude with the flash luminance. At the high luminances employed, both the a- and b-waves probably consist of contributions from photopic and scotopic mechanisms. The scotopic contribution, however, seems to be absent from the dark-adapted response of subject P. M. Only with the most intense flash (6.8 x 10⁷ cd per square meter) could an electroretinogram be recorded, and this has the features of a photopic response, i.e., a small a-wave with a sharply peaked positive potential. For subject R. C., on the other hand, a flash of 4.3 x 10⁶ cd per square meter evoked a recordable electrical response. At this luminance, the small a-wave is like that of

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**Fig. 3. Upper**, Electrical responses elicited in the dark-adapted eye at three levels of flash intensity. Note that although subject R. C. shows a-waves of normal amplitude, b-wave activity is much reduced. No measurable responses could be detected for subject P. M. at the two lowest intensities. **Lower**, Electrical responses elicited by flicker stimulation of the light-adapted eye.
the normal eye, but the b-wave is greatly reduced in amplitude. As the flash luminance is raised, only the a-wave increases in amplitude, whereas the positive potential remains small and pointed. The latter is the photopic b-wave which, in the normal eye, is masked by the rapidly ascending limb of the larger scotopic b-wave.

Fig. 3, bottom, shows the electrical responses elicited by flicker stimulation of the light-adapted eye. Under these conditions, the electoretinal response of the normal eye is essentially photopic. Photopic activity is evident also in the responses of both nyctalopes, although it is clearly depressed in the tracings from subject P. M.; for subject R. C., the responses are only slightly reduced as compared with the normal.

Fig. 4 shows the amplitudes of the electrooculogram at various times during the course of dark- and light-adaptation. Each point represents the average of five readings obtained over a period of several seconds. For ten minutes prior to the series of dark-adapted measurements, recordings were made with the eye moderately light-adapted to a luminance of 120 cd per square meter. Since the subjects had been exposed to this ambient illumination for several minutes while the electrodes were being fixed in position, the EOG responses show only small fluctuations in amplitude during this period. The data for subject R. C., whose EOG results were found to be normal, illustrate how the response amplitude changes when the eye is suddenly subjected to complete darkness. The potential declines slowly, reaching a minimum value after about seven minutes, and then rises during the final eight minutes of dark adaptation. The onset of a steady bright light (8,900 cd per square meter) causes the EOG to increase to a maximum after approximately nine minutes, but the amplitude falls thereafter and again approaches the base line. Taking as our measure of the light-induced change in stand-

Fig. 4. Electrooculograms of the nyctalopic subjects, recorded at three levels of retinal illumination. Each point represents the average amplitude of five pen swings in microvolts. The rise in potential found at the highest luminance level is expressed as the ratio (in percent) of the light peak to the dark trough. A light peak/dark trough ratio of about 180 percent is considered normal.
ing potential, the percentage increase as between the light maximum and dark minimum, i.e., light peak/dark trough × 100, we obtained for subject R. C. a value of 253 per cent. This is well within the range of 185 per cent to 322 per cent established by Arden and Barrada for a large sample of normal subjects.

The results for subject P. M. differ from those for R. C. in several respects. The initial amplitudes of the eye movement potential are about two and one-half times greater; for subject P. M. the response corresponds to a change of approximately 23 μV per degree, but it is only 9 μV per degree for subject R. C. This difference, however, is not inconsistent with the variation found among normal subjects, and is due in large part to differences in electrode placement, skin resistance, and so forth. During dark adaptation, the potentials decrease in amplitude, but do not reach the dark trough till eleven minutes have elapsed. With light adaptation, the potentials rise to a maximum in nine minutes, but the amplitude does not greatly exceed the initial value of the base line. The light rise of only 130 per cent is grossly abnormal.

**Fundus reflectometry.** Fig. 5 shows difference spectra measured in the parafoveal retina of the night-blind subjects; the retinal region was the same as that examined previously in the dark-adaptation study. In each case, the bleaching exposure was 7.5 log troland seconds, an intensity sufficient to bleach more than 90 per cent of the rhodopsin within the test area. For both subjects the maximum density change occurs at about 510 mμ, attesting to the fact that rhodopsin is present in the retinas of both nyctalopes. As regards magnitude and spectral position, the results are comparable with normal data published earlier.

Regeneration data obtained after exposure to the intense bleaching light are shown in Fig. 6. They represent the course of visual pigment regeneration as measured at λ = 510 mμ from the density difference spectra obtained at various times during

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![Fig. 5. Difference spectra measured at 12 degrees in the temporal retina of nyctalopic subjects R. C. and P. M. A white bleaching light of 7.5 log troland seconds was used. The ordinate ΔD(2) gives the density change for double transit through the retina; density losses due to bleaching are plotted as positive values. A line drawn by visual inspection through both sets of data represents the average curve of the two subjects.](image-url)
dark adaptation. The curves for both subjects show a relatively rapid rate of regeneration with half times of about 2.5 minutes; this, too, agrees with the results for normal subjects.\textsuperscript{14, 15}

Fig. 7 shows the results of two additional reflectometry experiments performed on subject R. C. In the first, the test area was located in the temporal retina, 30 degrees from the fovea. The bleaching exposure was, as before, 7.5 log troland seconds of white light, and again a rhodopsin difference spectrum was obtained. But for this retinal region the magnitude of the double density change at $\lambda_{\text{max}}$ was about 0.10, approximately 80 per cent of that measured at an eccentricity of 12 degrees. However, this decrease in $\Delta D (2)$ is in agreement with the findings of Campbell and Rushton\textsuperscript{20} for equivalent retinal loci in the normal eye, the lower value being due probably to the reduced population density of rods at the more peripheral site.

Also shown in Fig. 7 are results obtained in the fovea after bleaching with white light of 6.9 log troland seconds. The shift in the density difference spectrum to longer wavelengths is typical of the results obtained when cone pigments are bleached.\textsuperscript{17, 18} Clearly, then, this nyctalope's retina contains the same sort of photosensitive pigments as are found in the normal eye.

Discussion

The results of fundus reflectometry demonstrate conclusively that the retinas of both nyctalous contain rhodopsin. More significantly, the rhodopsin concentration, and the rate at which it regenerates after photolysis, are in no way abnormal. It is evident, therefore, that whatever else the
night-blindness may be due to, it does not arise from the absence of rhodopsin. Furthermore, any suggestion that the nyctalopes have fewer rods than the normal is rendered unlikely by the magnitude of the difference spectrum.\textsuperscript{21}

How are we to account for the above observations? Fundus reflectometry shows that our nyctalopes possess normal amounts of visual purple; the dark-adaptometry data indicate that this pigment subserves little, if any, visual function (but cf. Carr, Ripps, Siegel, and Weale\textsuperscript{11}). Where, then, is the message interrupted or so highly attenuated that it becomes virtually useless? Some tentative answers to these questions are suggested by the electrophysiological findings, but these are sufficiently different for our two subjects to warrant separate consideration.

For subject P. M., all of the electroretinal responses to light were markedly depressed. Consider first the light-induced change in the standing potential of the eye. The percentage increase in amplitude from the dark trough to the light peak was 130 per cent. But a comparable change can be obtained in normal subjects simply because the potential regains its base line value about 20 minutes after the beginning of dark adaptation.\textsuperscript{9} Thus, the bright-adapting light had little effect on the standing potential. This finding is difficult to explain if, as has been assumed, the light rise is due primarily to metabolic processes concerned with rhodopsin kinetics; for on our evidence, the rhodopsin cycle has not been affected in this subject. It seems unlikely, therefore, that the light rise in the DC potential of the eye is generated solely by the interaction of pigment epithelium and rod outer segments in the exchange of vitamin A.\textsuperscript{15} A more likely possibility, supported by the studies of Gouras and Carr,\textsuperscript{25} is that the source of the light rise is proximal to the receptors. These authors reported absence, or marked reduction, of the DC light rise after producing anoxia of the inner retinal layers (by occluding the central retinal artery). This procedure did not interfere with the functioning of the outer retinal layers as evidenced by the fact that the magnitude of the a-wave did not change significantly (see below).

Consider next the electrophoretographic data for subject P. M. The a-wave as well as the b-wave was severely depressed. Early studies on the origin of the a-wave suggested that it arose from the outer segments of the receptors,\textsuperscript{29} a region which in this nyctalope functions normally as regards the bleaching and regeneration of rhodopsin. However, further work shifted the origin to the "proximal receptor terminal."\textsuperscript{5, 26} Measures of the electroretinogram\textsuperscript{21} and early receptor potential\textsuperscript{6} from rats with congenital retinal dystrophy showed that the a-wave amplitude declined when the inner segments began to deteriorate, although at this stage of degeneration the outer segments appeared morphologically and functionally normal. Hence several lines of evidence favor the receptor inner limbs as the site of generation of the a-wave; and it is probably at this site that the lesion which produces this form of nyctalopia resides. We cannot, however, rule out the possibility of an abnormality in the functioning of the outer segment, despite the normal photochemistry. Little is known of the mechanism by which electrical activity is generated following the isomerization of visual pigment molecules. Conceivably, the initial stage of electrogenesis is faulty in this subject, and all subsequent neural activity is depressed. Furthermore, whatever the precise nature of the defect, the photopic system has also been affected; the cone segment of the dark-adaptation curve is elevated, and the photopic flicker response in the electroretinogram is reduced in amplitude.

The electrophysiological findings for subject R. C., however, suggest that here the defect occurs at a different level in the retina. In this case, both the a-wave of the electroretinogram and the light-induced rise in the standing potential of
the eye are normal. In view of the foregoing discussion, these findings indicate that the functional integrity of the outer retinal layers is maintained, i.e., from approximately the outer nuclear layer to the pigment epithelium.

The b-wave of the normal ERG, on the other hand, originates in the region of the bipolar cells. Thus, the absence of a recordable scotopic b-wave from subject R. C. suggests that the locus of his defect is at or just distal to the bipolar cells. Although the photopic components of his electroretinal response appear normal, or nearly so, it is doubtful whether the cone system is entirely spared, witness the abnormal cone segment of his dark-adaptation curve (Fig. 2).

The data for subject R. C. are of interest also in regard to the origin of the light rise in the DC potential of the eye. Although the scotopic b-wave of the electroretinogram was severely depressed, the percentage change in the DC potential was normal (Fig. 4). These findings suggest that the light rise originates distant to the origin of the b-wave potential. Furthermore, the study by Gouras and Carr demonstrated that the light rise occurred proximal to the site of a-wave generation. Thus, we infer that the light-induced change in the DC potential arises at a level between those responsible for the a- and b-waves of the electroretinogram.

Conclusion

Applying the techniques of electrophysiology and fundus reflectometry, we have been able to implicate the transmission lines proximal to the receptor outer segments as the site of the defect in congenital nyctalopia. However, it appears that the specific locus may differ in different individuals, a consequence perhaps of the mode of inheritance of their night blindness. Finally, our data help to explain why vitamin A therapy is of no avail, for, on our evidence, there is no vitamin A deficiency.

REFERENCES