

Action Spectra and Adaptation Properties of Carp Photoreceptors

P. WITKOVSKY, J. NELSON, and H. RIPPS

From the Departments of Physiology and Ophthalmology Research, Columbia University College of Physicians & Surgeons, New York 10032 and the Departments of Ophthalmology and Physiology, New York University School of Medicine, New York 10016

ABSTRACT The mass photoreceptor response of the isolated carp retina was studied after immersing the tissue in aspartate-Ringer solution. Two electroretinogram components were isolated by differential depth recording: a fast cornea-negative wave, arising in the receptor layer, and a slow, cornea-negative wave arising at some level proximal to the photoreceptors. Only the fast component was investigated further. In complete dark adaptation, its action spectrum peaked near 540 nm and indicated input from both porphyropsin-containing rods ($\lambda_{\max} \approx 525$ nm) and cones with longer wavelength sensitivity. Under photopic conditions a broad action spectrum, $\lambda_{\max} \approx 580$ nm was seen. In the presence of chromatic backgrounds, the photopic curve could be fractionated into three components whose action spectra agreed reasonably well with the spectral characteristics of blue, green, and red cone pigments of the goldfish. In parallel studies, the carp rod pigment was studied *in situ* by transmission densitometry. The reduction in optical density after a full bleach averaged 0.28 at its λ_{\max} 525 nm. In the isolated retina no regeneration of rod pigment occurred within 2 h after bleaching. The bleaching power of background fields used in adaptation experiments was determined directly. Both rods and cones generated increment threshold functions with slopes of +1 on log-log coordinates over a 3–4 log range of background intensities. Background fields which bleached less than 0.5% rod pigment nevertheless diminished photoreceptor sensitivity. The degree and rate of recovery of receptor sensitivity after exposure to a background field was a function of the total flux ($I \times t$) of the field. Rod saturation, i.e. the abolition of rod voltages, occurred after $\approx 12\%$ of rod pigment was bleached. In light-adapted retinas bathed in normal Ringer solution, a small test flash elicited a larger response in the presence of an annular background field than when it fell upon a dark retina. The enhancement was not observed in aspartate-treated retinas.

A comprehensive account of the visual process must necessarily include the functional properties of its sensory elements. Until recently, however, the difficulty in penetrating photoreceptors with microelectrodes precluded direct

measurement of their electrical activity. But with refinements in technique, intracellular recordings have been obtained from the visual cells of many vertebrate species, and there is now abundant evidence that rods and cones respond to illumination with a slow hyperpolarizing potential whose amplitude is intensity dependent (Bortoff, 1964; Tomita, 1965; Werblin and Dowling, 1969; Baylor and Fuortes, 1970; Toyoda et al., 1969; Toyoda et al., 1970). Moreover, it is becoming increasingly apparent that photoreceptors serve not only as transducers, but are involved also in the adaptive and information processing functions of the visual system. For example, the results obtained by Werblin (1971) in mudpuppy and by Dowling and Ripps (1971) in the skate suggest that the receptors govern to a large extent the sensitivity changes observed in more proximal neurons during visual adaptation, and Baylor et al. (1971) have demonstrated excitatory and inhibitory interactions between cones in the turtle retina.

Although a great deal of important information has been culled from intracellular measurements on photoreceptors, the technique is not yet generally applicable to studies requiring long periods of observation because of the ease with which the recording electrode is dislodged (Tomita et al., 1967). There are, however, a number of procedures by which it is possible to isolate and to record with extracellular electrodes the mass electrical response of the photoreceptors (cf. Brown et al., 1965; Fatechand et al., 1962; Furukawa and Hanawa, 1955; Tomita, 1957). One of these, the application of sodium aspartate (Furukawa and Hanawa, 1955) has been found effective in selectively suppressing responses that originate proximal to the receptors, leaving, in the isolated retina, a light-evoked potential that is similar in sign and waveform to the component (P III) of the electroretinogram to which receptor responses contribute (Sillman et al., 1969; Penn and Hagins, 1969). In the present work, we employed this method to record rod and cone responses in the carp retina and to study their behavior and spectral properties in light and dark adaptation. In addition, we have made some preliminary experiments with center-surround illumination to determine whether receptor interaction can be demonstrated in normal and aspartate-treated carp retina.

METHODS

Freshwater carp (*Cyprinus carpio*) were dark-adapted for at least 2 h before the start of an experiment, and subsequent preparative procedures carried out under dim red light. The excised eye was sectioned equatorially, the lens extracted, and most of the vitreous was removed with absorbent paper. After separation from the eyecup, the retina was placed receptor side up on filter paper soaked with Ringer solution (Witkovsky, 1965) in which 100 mM sodium aspartate was substituted for the equivalent concentration of NaCl. The preparation was quickly transferred to a small lucite chamber, flooded with aspartate-Ringer solution for 5 min, and drained of excess fluid before recording. A continuous stream of moist gas, either 100% O₂ or a mixture

of O₂ and CO₂, was passed over the preparation; there was no detectable difference in response characteristics as a function of the gas used. Although aspartate treatment was used routinely to isolate receptor potentials, a number of control experiments were performed in the standard Ringer solution (see Results).

Transretinal potentials were recorded between a Ringer-filled glass pipette that was lowered with the aid of a micromanipulator to the distal edge of the receptors, and a chlorided silver coil in contact with the moist filter paper on which the retina had been placed. The pipette was drawn from capillary tubing, flame polished to a tip bore of 10–20 μm , and connected via another Ag-AgCl wire to a cathode follower (W-P Instruments, Inc., Hamden, Conn.) having an input impedance of 1000 M Ω . The output of the cathode follower was amplified further, displayed on an oscilloscope, and recorded on one channel of a Grass penwriter (Grass Instrument Co., Quincy, Mass.) (bandpass = 0–35 Hz). For differential depth recording, a Ringer-filled micropipette with a tip diameter of 1–5 μm and 5–20 M Ω resistance was either advanced through the outer pipette, or positioned independently on the retina to within a few micrometers of the larger electrode. Signals were led from the micropipette to a second cathode follower and penwriter channel.

Light stimuli were projected into the retina by a dual-beam optical system that utilized a DC-operated 150 W xenon arc lamp to produce both test and adapting fields. The spectral distribution of the xenon arc, which varies little between 400 and 700 nm was obtained from manufacturer specifications (Osram, Berlin). Relatively monochromatic test fields were obtained by passing one beam through a grating monochromator (Canalco, Inc., Rockville, Md.); a series of interchangeable apertures permitted variation in stimulus diameter from 80 μm to 1.9 mm. The duration of the test flash (usually 200 ms) was controlled by an electromagnetic shutter, and intensity variation over an 8 log unit range was by means of a pair of calibrated variable-density circular wedges (Eastman Kodak Co., Rochester, N. Y., deposited metal film type). The adapting (background) field originated from a second port in the arc housing, and its intensity and spectral composition were controlled by Kodak Wratten neutral density and color filters (No. 47, blue; No. 58, green; No. 15, yellow; or No. 25, red). Three 1 mm KG-1 heat filters (Schott and Gen., Jena) were interposed in the optical paths of both test and adapting fields so that about 83% of the energy of the "white" light was restricted to wavelengths between 400 and 700 nm. The two beams were combined in a half-silvered mirror and directed to the retina at approximately 30° to the normal by a prism. The energy densities in the plane of the retina were measured with a photomultiplier that had been calibrated against a standardized thermopile.

In order to determine the effects of the various adapting conditions on visual pigment concentration, the changes in retinal transmissivity induced by bleaching exposures were measured over a wavelength range of 380–720 nm. Except for a few modifications, the apparatus and procedures have been described previously in detail (Dowling and Ripps, 1970), and only the essential features of the method are given below. The isolated retina was placed in a transparent chamber and positioned under infrared illumination on the stage of a microspectrophotometer. Light from a DC-operated xenon arc was interrupted by a series of 30 interference filters that were mounted in spectral order on a rotating wheel driven at the rate of 5 rps. This pro-

duced the sequence of nearly monochromatic test beams that were passed through a 3 mm² area of the retinal section and on to the cathode of a photomultiplier. The photocell signals were fed into an operational amplifier, the output of which was converted into digital form for computer processing. Light measurements, at each test wavelength, were obtained first on the dark-adapted retina, and again after a specific light adapting exposure. From such data we determined the changes induced by photolysis, i.e., the difference between the density spectrum of the visual pigment and that of the products into which the parent pigment is transformed by light. In addition, recordings taken at various times after the adapting light was extinguished permitted a determination of the degree to which the parent pigment had regenerated during dark adaptation.

RESULTS

Photochemical Changes in the Isolated Retina

Although the carp retina contains rod and cone receptors (in the ratio of about 7:1), only one photosensitive substance has been detected in solution (Wald, 1939; Crescitelli and Dartnall, 1954). The density spectrum of this substance indicates that it is a porphyropsin, $\lambda_{\max} = 523$ nm (Crescitelli and Dartnall, 1954), derived from the scotopic elements. Transmissivity measurements on the isolated retina (Fig. 1) yielded results comparable to those obtained in solution. The bleaching difference spectrum (continuous curve)

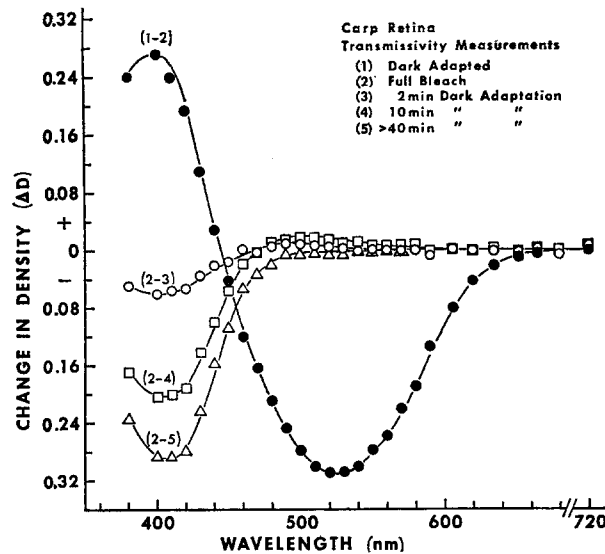


FIGURE 1. Difference spectra of carp porphyropsin obtained at various times after a full bleach. Curve 1-2 shows the loss of optical density due to bleaching the parent pigment ($\lambda_{\max} \approx 525$ nm) and an increase in density due to the formation of a photoproduct ($\lambda_{\max} \approx 400$ nm). Curves 2-3, 2-4, and 2-5 illustrate the thermal decay of the 400 nm photoproduct and a transient increase in optical density near 490 nm. Note that there is no increase in optical density in the region of 525 nm after bleaching, indicating that no regeneration of porphyropsin has occurred.

shows that exposing the dark-adapted retina to strong illumination produced a density loss maximal at 525 nm, and the formation of a photoproduct that absorbs maximally in the region of 400 nm. Particularly noteworthy was the lack of density changes attributable to cone photopigments; i.e., both the λ_{\max} and shape of the difference spectrum closely approximate those of relatively pure porphyropsin solutions.

The absence of a cone contribution in the density difference spectrum is surprising in view of their numbers, but the short length of the cone outer segment may result in a low axial density of pigment. On the other hand, the measured density D_m of porphyropsin is quite high, and represents a lower limit as regards its *in situ* density D_λ —the axial density within the receptor. Light scatter, interreceptor spaces, and the presence of cones contribute to a reduction in D_m , which on average was 0.28 at 525 nm. We estimate that rod outer segments occupy a fractional area f of about 0.8, whereas cones and interreceptor spaces make up $(1 - f)$ of the total area exposed to the measuring beam of the spectrophotometer. Since $D_m = \log_{10}[(1 - f) + f \cdot 10^{-D}]$ (Ripps and Weale, 1965), consideration of this factor alone would bring the mean *in situ* density at 525 nm to 0.39. This value takes on special significance with regard to spectral sensitivity measurements, for the absorption spectrum of a visual pigment corresponds to its density spectrum only when $D < 0.1$ (Dartnall, 1957). However, we may calculate the appropriate absorption spectrum from the relation $A_\lambda = 1 - 10^{-D_\lambda}$. Fig. 2 shows the computed results

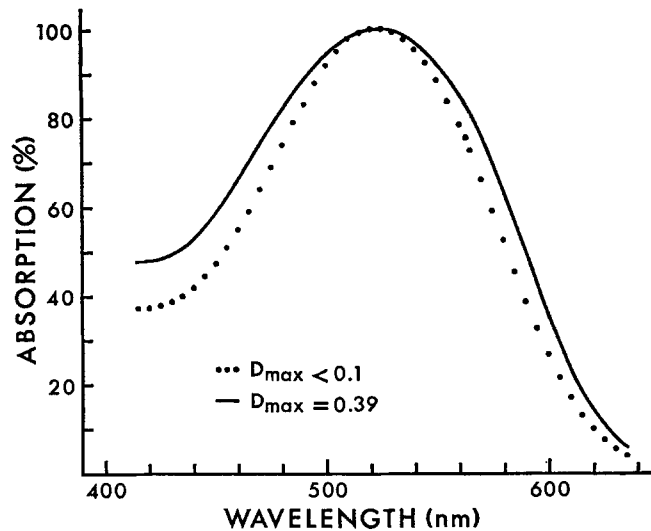


FIGURE 2. The absorption spectrum of an infinitely dilute concentration of a porphyropsin with $\lambda_{\max} \approx 523$ nm is shown by the dotted line (Bridges, 1967). The solid line indicates the absorption spectrum of carp porphyropsin corrected for the estimated *in situ* optical density of 0.39. The scale of ordinates represents absorption expressed as percent of maximum.

for $D_{\max} = 0.39$ (continuous curve) together with the absorption spectrum for a dilute solution of carp porphyropsin (Bridges, 1967).

Also shown in Fig. 1 is the sequence of thermal events that occur after extinguishing the bleaching light (dashed-line curves). With time in the dark, there is a gradual loss of the yellow photoproduct, and some evidence for the formation of a transient intermediary absorbing at $\lambda \approx 490$ nm (curves 2 and 3). The latter is no longer present in the data obtained after 40 min of dark adaptation, nor is there any evidence that porphyropsin regenerates in the isolated retina after 2 h in darkness. Whereas it appears unlikely that porphyropsin regenerates in the isolated carp retina, we cannot be certain that longer periods of dark adaptation would not yield evidence of pigment formation. This possibility should be considered since the regeneration of carp porphyropsin *in vivo* first begins after a lag of nearly 1 h, and requires an additional 4 h of dark adaptation for completion (Baumann, 1971).

In later sections, we examine the effects of light adaptation on receptor potentials in the carp retina. The extent to which the various adapting fields altered the concentration of visual pigment was determined by comparing transmissivity measurements on the dark-adapted retina with those obtained after a wide range of bleaching conditions (cf. Ripps and Weale, 1969). After each partial bleach, the retina was exposed to a period of intense illumination that virtually denuded it of visual pigment. Since the density difference ΔD ,

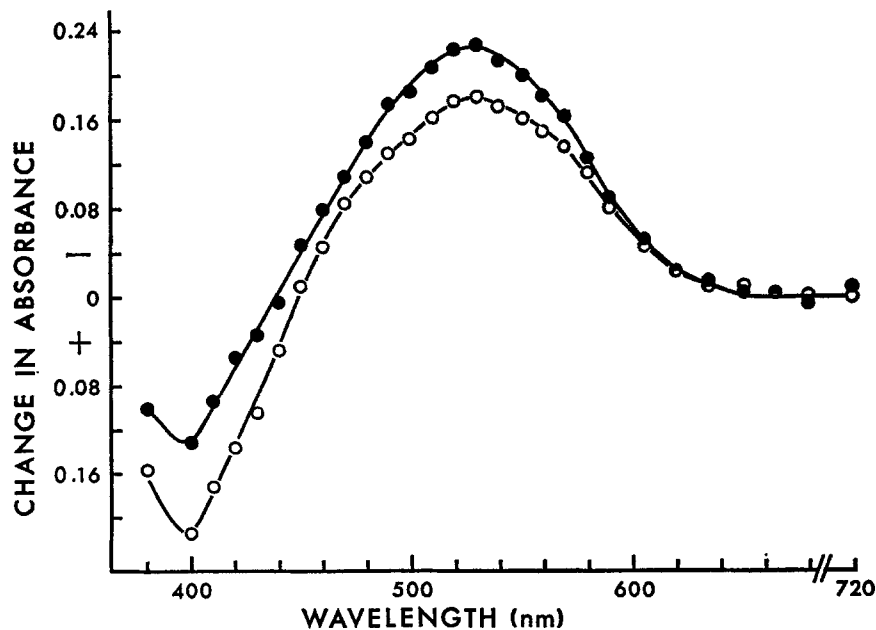


FIGURE 3. The difference spectrum of carp porphyropsin produced by a brief (≈ 1 ms) flash from a xenon discharge lamp (open circles). After a total bleach (filled circles) there is an increase in the magnitude of the difference spectrum. See text for details.

due to the final bleach is proportional to the maximum concentration C_o of porphyropsin in the dark-adapted retina, then the fraction bleached C_b/C_o by exposure to an irradiance I for time t is given by: $C_b/C_o = \Delta D_b/\Delta D_o = 1 - \exp(-\alpha\gamma It)$, where ΔD_b is the density change produced by the partial bleach, and $\alpha\gamma$ (the product of extinction coefficient and quantum efficiency), the photosensitivity of the pigment. The results of this procedure are illustrated in Figs. 3 and 4. Fig. 3 shows difference spectra produced by flash photolysis (open symbols) and after completely bleaching the same retina with an intense beam from a heat-filtered tungsten source (filled symbols). Comparing the ΔD values at $\lambda = 525$ nm indicates that the xenon flash bleached 80% of the available pigment. A similar experimental protocol was followed to determine the photolytic effects of exposure to yellow (Wratten 15) adapting fields. Fig. 4 plots the fraction of porphyropsin bleached as a function of the retinal radiant exposure in millijoules-square centimeters. In this form, the data can be compared directly with the conditions of light adaptation that were used in subsequent experiments.

DIFFERENTIAL DEPTH RECORDING As shown schematically in Fig. 5, coaxial electrodes were applied to the retina such that the outer pipette (A)

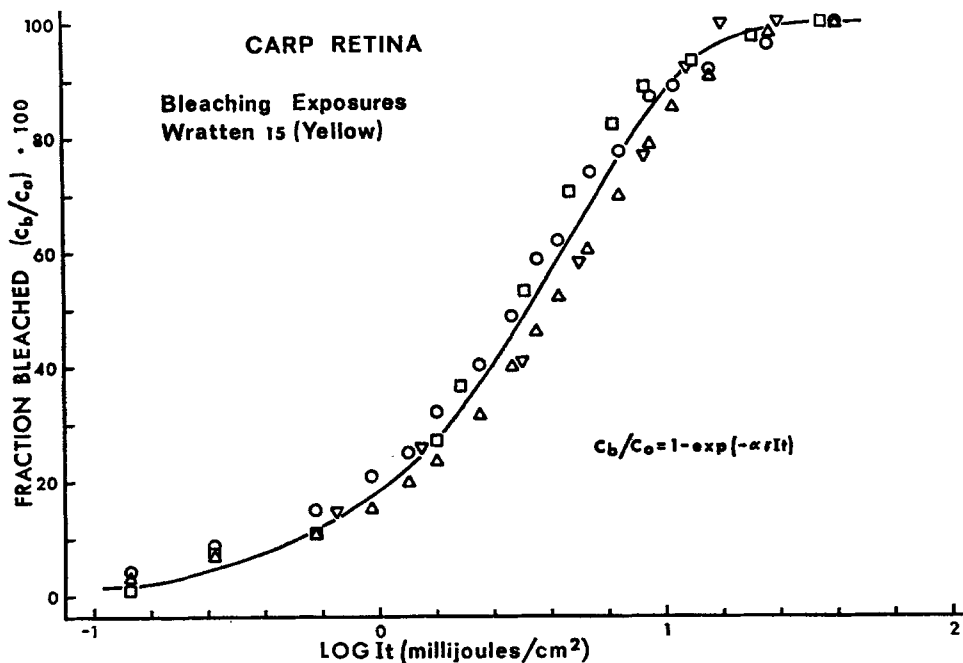


FIGURE 4. The percent ($C_b/C_o \times 100$) of carp porphyropsin bleached by exposures to different energies of yellow (Wratten 15) light is shown by the symbols. The solid line is the theoretical bleaching curve given by the equation $C_b/C_o = 1 - \exp(-\alpha\gamma It)$, where α is the extinction coefficient of the pigment, γ the quantum efficiency of the reaction, I , the light intensity, t , the duration of exposure.

contacted the distal border of the photoreceptor layer, whereas the inner pipette (*B*) could be adjusted in depth with respect to (*A*); gain settings for the two channels were set initially so as to register zero potential when both electrodes were at the retinal surface. The tracings on the left side of Fig. 5 show a series of DC recordings obtained with the retina in normal Ringer solution. The conventional electroretinogram (ERG)—with polarity reversed due to upward orientation of the receptor layer—was recorded across the

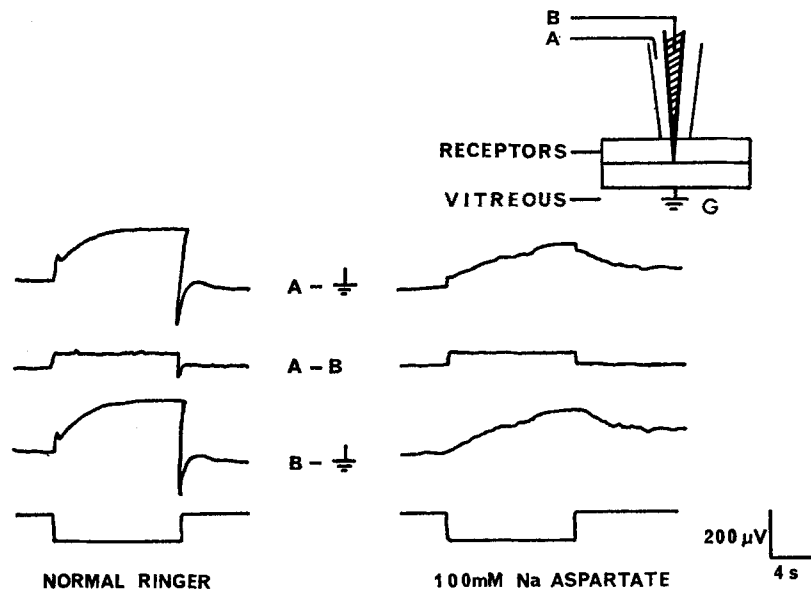


FIGURE 5. Sample recordings of mass photoreceptor potentials in normal Ringer (left column) and aspartate-Ringer (right column) treated retinas. The insert at upper right shows that electrode *A* rests on the distal tips of the receptors, electrode *B* is advanced to a point 100–125 μm from the distal surface. The reference is a silver-silver chloride coil in contact with the vitreous. The lower trace in each column indicates the time-course of a 10 s stimulus. Three recording channels register the responses of electrode *A* to ground, electrode *B* to ground, and the difference between the potentials recorded by electrodes *A* and *B*. Note that the penwriter traces are on curvilinear coordinates. See text for discussion.

retina (*A-G*). In response to a 10 s light stimulus, the ERG consisted of three or four components: a rapid positive deflection representing the leading edge of the *a*-wave, a small negative *b*-wave (not always present), a slow positive wave, and a pronounced off-response that overshoot the base line. A similar sequence was observed when the inner pipette, advanced to a depth of 100–125 μm from the retinal surface, served as the active electrode (*B-G*), but the initial positive deflection was significantly reduced in amplitude. This reduction can be attributed partially to the fact that P III of the fish retina is comprised of distal and proximal components (Murakami and Kaneko, 1966), of

which primarily the latter is registered when the recording electrodes bypass the receptor layer (Sillman et al., 1969). On the other hand, the voltage generated across the receptors (*A-B*) consisted principally of a sustained positive potential, together with a small off-response. It should be noted that alignment of the electrode in the center of the test spot was essential for recording the nearly rectangular waveform of the receptor potential, since central and surround illumination evoked responses of opposite polarity (cf. Motokawa et al., 1959).

With similar recording conditions and the aspartate-treated retina, the light-evoked potentials were less complex (Fig. 5, right column), since aspartate eliminated both the *b*-wave and the off-response of the ERG. Consequently, only a positive potential, of rapid onset, was recorded across the photoreceptor layer. Furthermore, the transretinal recording reveals that the slowly rising positive wave noted previously was also retained after aspartate treatment. It appears that this slow potential arises proximal to the receptors, for its magnitude was virtually the same when recorded transretinally or between the inner pipette and the vitreal reference electrode. Since the slow wave was often the larger of the two positive potentials (particularly under scotopic conditions), the response amplitudes referred to in subsequent sections were measured from the base line to the peak of the initial rapid deflection (distal P III).

In the carp retina, S-units (presumably large horizontal cells) are readily impaled by a microelectrode (Witkovsky, 1967). We recorded S-potentials in two preparations before the addition of aspartate. After immersion in aspartate, we were unable to again locate an S-unit, although the retina was probed repeatedly with the micropipette and the same pipette was still capable of registering S-potentials when tested on a fresh retina. We did not pursue this observation further, but Cervetto and MacNichol (1971) and Dowling and Ripps (1971) have demonstrated the loss of horizontal cell activity in the skate retina *during* the application of sodium aspartate.

The Dark-Adapted Threshold

Preparation of the retina for electrophysiological study inevitably produced a small degree of light adaptation. Therefore, at the start of each experimental session, responses to weak test flashes ($\lambda = 500$ nm) were recorded periodically until threshold reached a stable, minimum value. In the fully dark-adapted retina, whether rinsed in normal or aspartate Ringer solutions, the "absolute" threshold, i.e. the energy required to elicit a $10 \mu\text{V}$ receptor potential, was approximately $5.8 \times 10^{-4} \mu\text{W}\cdot\text{cm}^{-2}$, and the difference in sensitivity between preparations was never more than 0.25 log unit. Since $1 \mu\text{W}\cdot\text{cm}^{-2} = 2.5 \times 10^{13}$ quanta (500 nm) $\cdot\text{s}^{-1}\cdot\text{cm}^{-2}$, the threshold for a 200 ms exposure corresponds to 2.9×10^8 quanta $\cdot\text{cm}^{-2}$ incident upon the retina. The cross-sectional area of a carp rod is about $3 \mu\text{m}^2$, and the optical density

of porphyropsin at 500 nm ≈ 0.36 . Assuming a quantum efficiency of one, and neglecting stray light effects, a threshold response was obtained when each rod within the area of illumination absorbed about 4–5 quanta. Although this threshold value is clearly dependent upon such factors as stimulus area, the criterion chosen for threshold, and the absorption properties of the visual pigment, the experimental parameters that were employed tended in fact to maximize the quantum requirements. Thus, the small number of quantal absorptions suggests that the responses to a test wavelength of 500 nm were derived from the scotopic system. In the rod-dominated rat retina, for example, Cone (1963) reported that threshold for the isolated *a*-wave required the absorption of 2–4 quanta per rod.

The Scotopic Action Spectrum

Several experiments were performed in which response amplitudes were determined as a function of stimulus intensity for test wavelengths from 420–660 nm (in steps of 40 nm). Voltage versus intensity (*V*-log *I*) curves were drawn, and the intensity *I*₀ required to elicit a criterion voltage read from the graphs. The reciprocal of *I*₀, corrected for quantum flux and expressed in logarithmic units, gave the relative sensitivity values plotted in Fig. 6. The data agree reasonably well with the absorption spectrum of carp porphyropsin

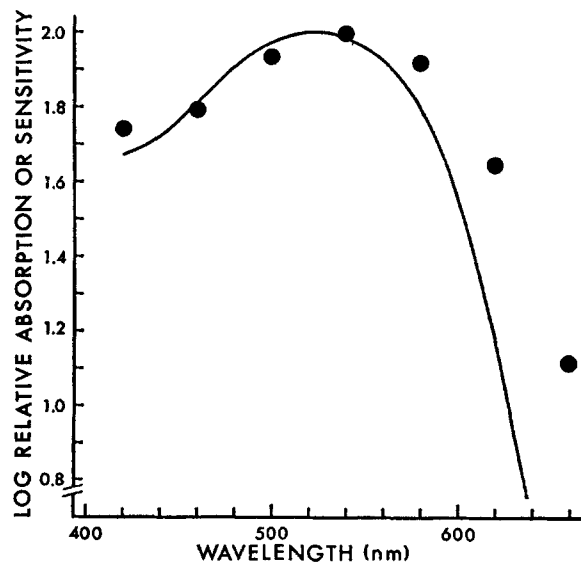


FIGURE 6. The action spectrum of the aspartate-treated, scotopic photoreceptor response is compared to the absorption spectrum of the rod pigment corrected for optical density = 0.39 (solid line). Note that the action spectrum is more sensitive at $\lambda \geq 540$ nm than would be predicted by the rod photopigment alone. Data points represent the average of eight experimental runs ($N = 8$).

(continuous curve) for wavelengths ≤ 540 nm, but are consistently higher at longer wavelengths. The findings suggest that a red-sensitive (cone) mechanism contributes to the electrical responses of the dark-adapted retina when the stimulus wavelength > 540 nm. This possibility receives support from experiments based on Stiles's two-color increment threshold technique (Stiles, 1949). The procedure involved determining the efficacy of various chromatic backgrounds in suppressing near threshold responses to test flashes of 500 and 620 nm, respectively. Obviously, if only one spectral mechanism generates the responses to these test wavelengths, then the inhibitory effects of the chromatic fields will be independent of test wavelength. However, the results showed that the 620 nm stimulus was more sensitive to long-wavelength backgrounds, whereas the reverse held for 500 nm test flashes. Thus, at least two spectrally distinct types of receptor subserve the sensitivity function of Fig. 6.

The Increment Threshold

Recent experiments have shown that the sensitivities of vertebrate photoreceptors can be markedly decreased by ambient illumination too weak to bleach a significant fraction of their visual pigments (Boynton and Whitten, 1970; Frank, 1971; Dowling and Ripps, 1971). Carp photoreceptors are no exception. Fig. 7 shows the change in threshold ΔI (above dark-adapted

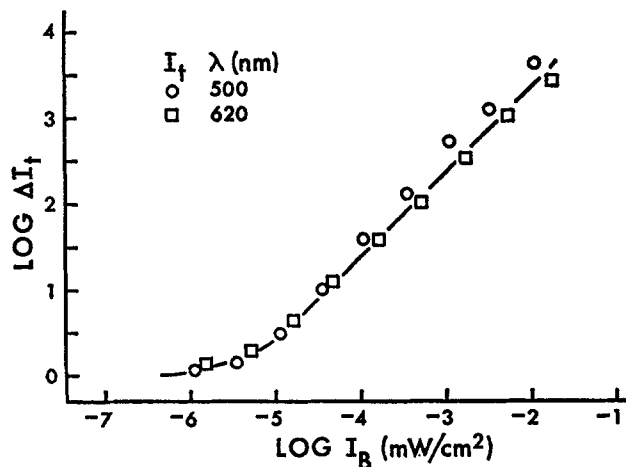


FIGURE 7. Increment threshold functions of the aspartate-treated, scotopic photoreceptor response. The log difference (ΔI_t) between absolute dark thresholds and that measured upon a Wratten 15 field is plotted against intensity of the background field ($\log I_b$). Test wavelengths of I_t were 500 nm, circles ($N = 5$) or 620 nm, squares ($N = 3$). The slope of the function on a log-log plot is +1 over most of its course. At field strengths greater than $\log I_b = -4$, relative thresholds for 500 nm are higher than for 620 nm test flashes. The adapting field uniformly illuminated the entire retina; the superimposed test spot was 1.9 mm in diameter.

levels) induced by a yellow (Wratten 15) background field, as the intensity I_B of the latter was increased in 0.5 log unit steps over an effective range of 4 log units. The test stimuli were delivered 45–60 s after the onset of the adapting field, since we had found that about 30 s was required to reach stable increment thresholds with backgrounds that did not bleach significant fractions of porphyropsin. Thresholds were tested at two wavelengths ($\lambda = 500$ and 620 nm) with nearly equivalent results; both sets of data show that ΔI increases in direct proportion to I_B , and neither function exhibits any sign of saturation, i.e., $\Delta I \rightarrow \infty$. The cumulative bleaching produced by I_B was exceedingly small at most background levels. For example, $I_B = 3.5 \log \text{ mW} \cdot \text{cm}^{-2}$, which raised threshold nearly 100-fold, bleached less than 0.5% of the porphyropsin in carp rods. Indeed, a 1 min exposure to the brightest background used in these experiments reduced the porphyropsin concentration only by about 11%. It is likely that the photosensitivities of the cone pigments are not appreciably different from that of porphyropsin (Marks, 1963). Thus, over most of its course, the increment threshold function for carp receptor potentials is not associated with a significant reduction in the concentration of visual pigments.

An interesting feature of Fig. 7 is the similarity between results obtained for the two chromatic test fields. The spectral sensitivity curve (Fig. 6) indicates that in the dark-adapted retina, 500- and 620-nm stimuli elicit rod and cone responses, respectively. Although the evidence is hardly compelling, there is the suggestion that a rod-cone transition occurs at $I_B \approx -4.0 \log \text{ mW} \cdot \text{cm}^{-2}$, since with brighter backgrounds, thresholds for $\lambda = 500$ nm fall consistently above those at 620 nm. We compute that the intensity of the background field at which a rod-cone transition is seen is equivalent to a monochromatic flux at $\lambda = 525$ nm of 5×10^{10} quanta/cm² per s. In rat and human retinæ, rod saturation becomes evident when $I_B \approx -4.2 \log \text{ mW} \cdot \text{cm}^{-2}$ (Aguilar and Stiles, 1954; Green, 1971), equivalent to a flux at 500 nm (the λ_{max} of rat rods) of 3×10^{11} quanta/cm² per s.

The Spectral Properties of Carp Cones

It was not obvious from the increment threshold data of Fig. 7 as to whether light adaptation of the carp retina alters the spectral characteristics of the receptor potentials, i.e., induces a Purkinje shift. A more direct approach to this question was taken by determining the spectral sensitivity function upon a "white" background that bleached about 18% of the rod pigment. The results, shown in Fig. 8, indicate that under strongly photopic conditions the λ_{max} is indeed displaced to longer wavelengths, maximum sensitivity occurring in the region of 580 nm. Of particular interest are the relative sensitivities at 500 and 620 nm, the test wavelengths of Fig. 7. Note that the sensitivity at 620 nm is only about 0.2 log unit greater than at 500 nm, whereas in the dark-adapted

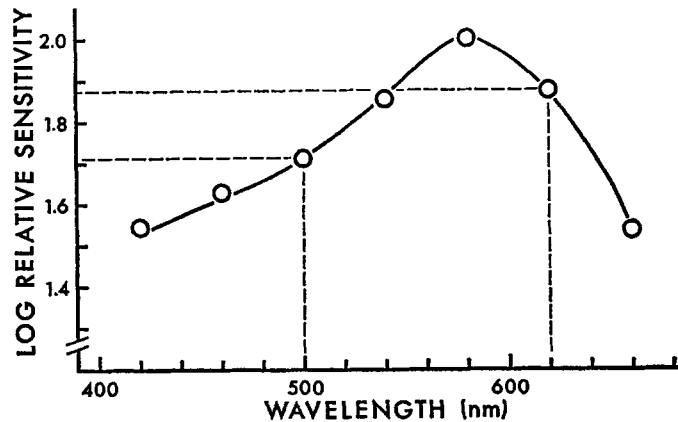


FIGURE 8. The action spectrum of the aspartate-treated photopic photoreceptor response with $\lambda_{\max} \approx 580$ nm. Dotted lines indicate that relative sensitivity at 620 nm is almost 0.2 log units greater than at 500 nm. See text for details.

retina (Fig. 6) the latter was more effective by approximately 0.3 log unit. Thus, if the high intensity portion of the increment threshold function was determined solely by the photopic system, we would expect the data at 500 nm to fall 0.5 log unit above those at 600 nm. Since the disparity is on the order of 0.2 log unit, it appears likely that these levels of light adaptation did not fully suppress the scotopic receptors.

The breadth of the photopic spectral sensitivity function suggests that it represents the envelope of more than one cone mechanism. We attempted to isolate the individual components using a modification of the chromatic adaptation technique employed successfully by Stiles (1959) and Wald (1964) in their analyses of human color vision. In the latter studies, increment thresholds for test fields spanning the visible spectrum were measured in the presence of bright, colored backgrounds that selectively suppressed responses in a given spectral region. We followed a similar procedure except that the retina was exposed first to a brief, intense field that bleached $\sim 20\%$ of the rod photopigment. The preparation was then kept in darkness until thresholds attained a stable level before introducing the chromatic field upon which spectral sensitivity was tested. This maneuver effectively eliminated the rod contribution to the mass receptor potential (see following section) and ensured that the electrical responses were derived from the photopic receptors.

Fig. 9 shows the averaged results obtained with each of the chromatic adapting fields, adjusted on the basis of preliminary trials to the brightest intensity that still permitted threshold measurements across a sufficient range of test wavelengths. A blue (Wratten 47 or 47B) adapting field that transmitted strongly in the region of 460 nm, gave rise to a spectral function with $\lambda_{\max} \approx 610$ nm. On the other hand, a purple (Wratten 35) field, having trans-

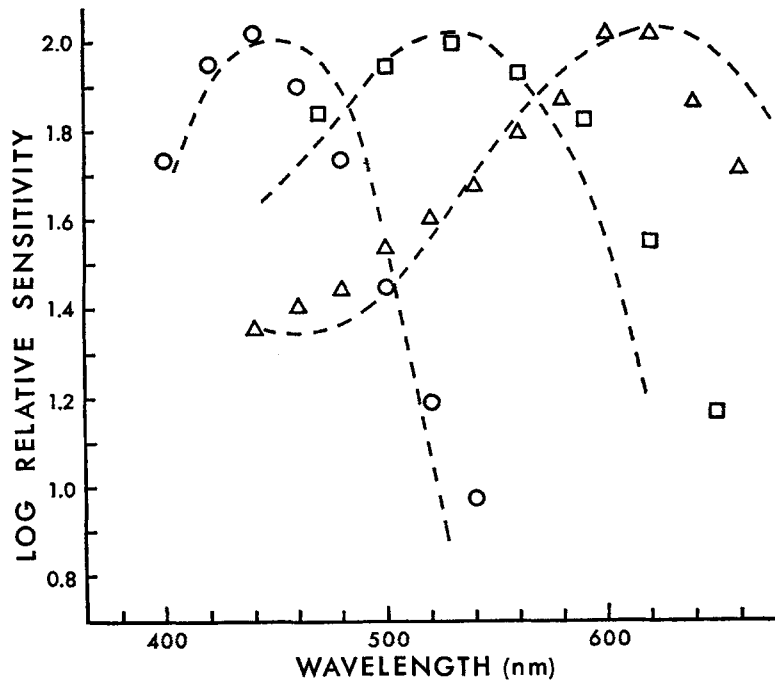


FIGURE 9. Action spectra of the aspartate-treated, photopic photoreceptor response, determined in the presence of steady chromatic fields. Circles: blue cone sensitivity ($N = 3$) upon a yellow field (Wratten 15); squares: green cone sensitivity ($N = 3$) upon a magenta field (Wratten 35); triangles: red cone sensitivity ($N = 5$) upon a blue field (Wratten 47 or 47B). The dashed lines show the difference spectra of blue, green, and red goldfish cone pigments (Marks, 1965).

mission bands in the red ($\lambda > 660$ nm) and blue (330–450 nm) parts of the spectrum, isolated primarily the green-sensitive receptors with $\lambda_{\max} \approx 530$ nm. The blue-sensitive receptors were more difficult to detect in spite of reports that in the carp they may be present in greater numbers than the green-sensitive elements (Tomita, 1965; Tomita et al., 1967). With a bright yellow (Wratten 15) adapting field, short-wavelength responses adequate for measurement were obtained only in two fish, but the results were consistent in showing a narrow spectral function with $\lambda_{\max} \approx 440$ nm. Also shown in Fig. 9 are the mean difference spectra of the three cone pigments detected by microspectrophotometry in goldfish (Marks, 1965). There is reasonable agreement between the two sets of spectral measurements, but differences in λ_{\max} and shape are apparent.

Dark Adaptation in the Isolated Retina

Recording the electroretinographic *b*-wave from the isolated, perfused rat retina, Weinstein et al. (1967) have demonstrated the early phase of dark

adaptation that occurs independently of photopigment regeneration (cf. Dowling, 1963). Recently, Frank (1971) has shown that this so-called "neural" adaptation is evident also in the P III component of the frog ERG. We have observed a similar phenomenon in the responses of carp receptors. This is illustrated in Figs. 10 and 11 where the change in threshold (with respect to absolute threshold) is plotted as a function of time after varying degrees of light adaptation.

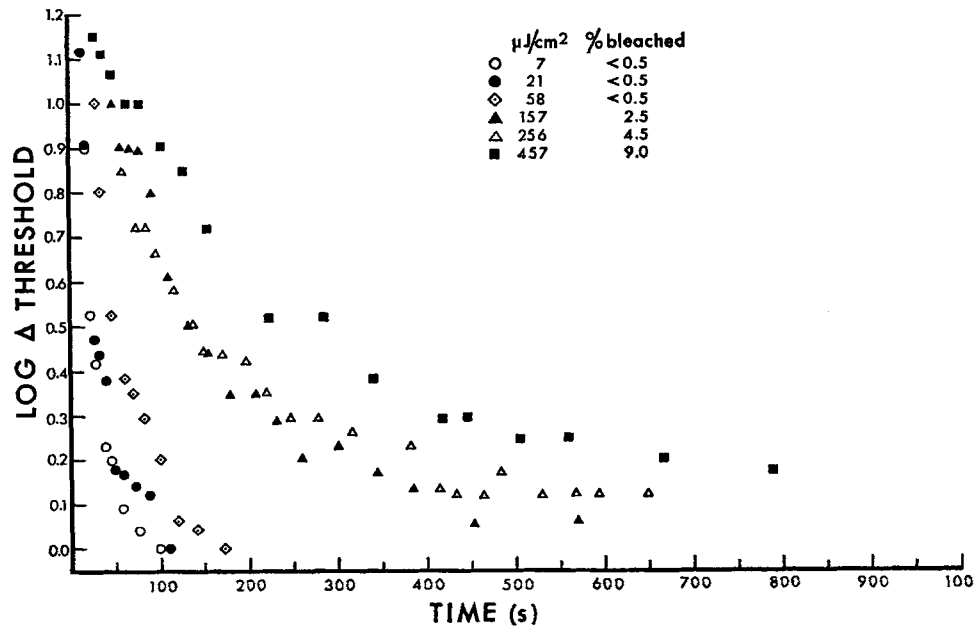


FIGURE 10. Recovery of sensitivity by the aspartate-treated, scotopic, photoreceptor response after cessation of controlled energy exposures to a yellow field (Wratten 15). Log Δ threshold is the difference between absolute dark threshold and that determined after exposure to the adapting field. The total flux ($\mu\text{J}/\text{cm}^2$) and cumulative bleaching power of the field lights are given at upper right. After weak exposures, full recovery of sensitivity occurs within 3 min. Stronger exposures which bleach a measurable fraction of rod pigment both prolong the return of, and effect a permanent loss in, sensitivity.

The results of Fig. 10 were obtained from one retina and represent a succession of dark adaptation runs, each preceded by exposure to a given intensity I_B for time t ; the cumulative bleaching due to increments in $I_B \cdot t$ (see Table in Fig. 10) was read from the curve describing the data of Fig. 4. Lights too feeble to bleach a significant fraction of the rod pigment produced only a transient rise in threshold (of about 1 log unit), followed by a rapid recovery (< 3 min) to the dark-adapted value. However, after stronger illumination (bleaching 2.5–9% of the porphyropsin), the rate of dark adaptation was appreciably slower and final thresholds were elevated above the absolute level.

Although the final threshold plateau after the 9% bleach was only about 0.2 log unit higher than at the start of the experiment, spectral sensitivity measurements taken at the end of dark adaptation revealed a small shift in λ_{\max} to longer wavelengths, i.e., a greater contribution from photopic elements. And when the fraction bleached exceeded 15%, the spectral functions were similar to the photopic sensitivity curve of Fig. 8. In addition, bleaching large fractions of the visual pigment greatly retards the rate of dark-adaptation. This is illustrated in Fig. 11 where threshold measurements at 500 and

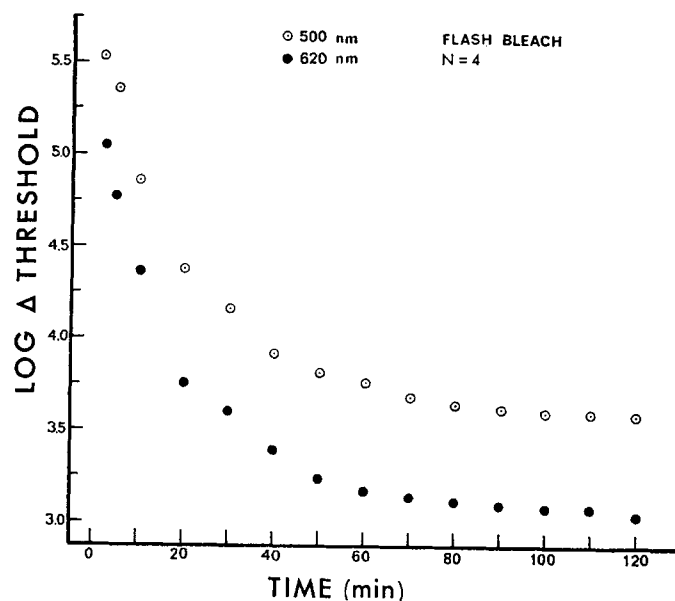


FIGURE 11. Recovery of sensitivity by the aspartate-treated photoreceptor response after a flash bleach which removed 80% of the rod pigment. Thresholds at 500 and 620 nm are plotted with respect to their absolute dark values. The two functions run in parallel, but separated by 0.5 log units, due to the permanent loss of the rod contribution to receptor sensitivity. See text for details.

620 nm are plotted as a function of time after an intense xenon flash that bleached about 80% of the rod photopigment (cf. Fig. 3). The flash exposure raised thresholds to both test wavelengths by more than 5 log units, and subsequent recovery to stable levels took almost 90 min. Also of interest is the 0.5 log unit difference that separates the two curves throughout the course of dark adaptation. It will be recalled that between dark- and light-adapted preparations, the sensitivity at 620 nm increases by 0.5 log unit relative to that at 500 nm due presumably to the loss of rod function. Thus, the slow rate of dark adaptation usually associated with the scotopic system is exhibited by carp cones after intense light adaptation.

Interaction in the Normal Retina

Several experiments were performed under photopic conditions (i.e. after having bleached away at least 20% of the rod pigment) to determine the effect on cone receptor potentials of illumination falling outside the area of the test field. In the aspartate-treated retina, background illumination, irrespective of its distribution, tended to suppress the responses elicited by a small test spot. Thus, whether I_B flooded the retina, or was restricted to the test area, or illuminated only the region surrounding the test field, response amplitudes decreased and ΔI_i increased as the level of I_B was raised. However, a striking difference was observed when some of these patterns were shone upon the "normal" isolated retina, i.e., one bathed in Ringer's without sodium aspartate. With test and background fields superposed and of equal dimensions, the background again depressed sensitivity in the test area. But when the steady background consisted of an annular field surrounding the test area, the responses to test flashes were greatly enhanced. In 14 retinas tested, the maximal enhancement produced by the annular field varied from 1.3- to 12.0-fold.

Fig. 12 shows two experiments in which ERG recordings were taken before, during, and after the onset of a chromatic adapting field. The latter completely covered the retina save for a 1 mm central zone within which a slightly smaller test field was flashed at a constant intensity. The first two traces in each sequence are the responses obtained before introducing the surround illumina-

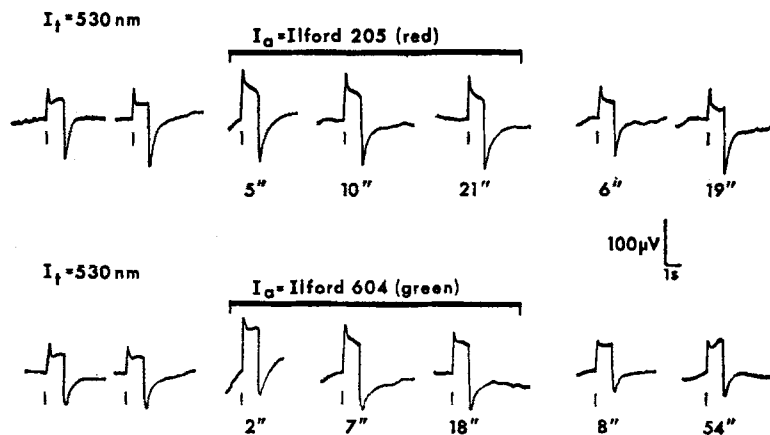


FIGURE 12. Electoretinograms from the isolated retina in response to spot stimuli ($\lambda = 530$ nm) before, during, and after exposure to red and green annular adapting fields. The retina was bathed in normal Ringer solution. The initial, rapid positive potential is derived primarily from the photoreceptors (a -wave), whereas the large negative transient represents the off-response to the 1 s test flash. Note the marked enhancement of the a -wave during light adaptation, and the rapid return to control levels when the annulus was extinguished. For details see text.

tion. In the absence of aspartate, the ERG comprises the *a*-wave (initial positive deflection), a small amplitude *b*-wave, and a large negative potential at the termination of the stimulus. Records taken only a few seconds after the onset of I_b show the marked increase in response amplitude induced by the surround. The change in the *a*-wave is quite pronounced, and the magnitude of the off-response is also larger. The degree of enhancement can be seen to diminish with time, but it is not clear whether this is due to a reduction in the effectiveness of the surround, or to the frequency at which bright test flashes were delivered. When the background was extinguished, the *a*-wave returned quickly to its original amplitude. The records of Fig. 12 illustrate the effects of red (Ilford 205) and green (Ilford 604) annuli on the responses to a 530 nm test flash. Other color combinations gave qualitatively similar results, but the degree of enhancement was quite variable from one preparation to the next. In addition, the relative intensities of test and surround fields influenced the results; in general, the brighter the surround, the more it enhanced the response evoked by a constant test flash. This is shown in the V -log I curves of Fig. 13, for which a yellow (Wratten 15) annulus and red ($\lambda = 620$ nm) test spot were employed. Note that with the most intense background field used, a 3.8-fold enhancement occurred in response to a near saturating test flash.

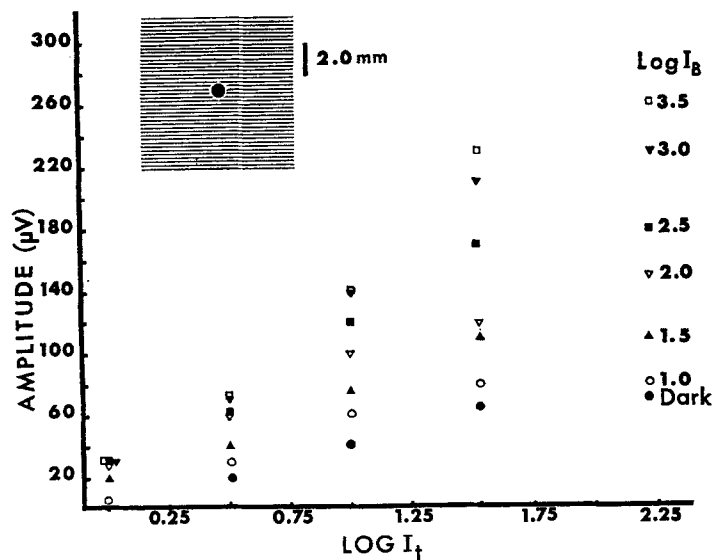


FIGURE 13. Increment threshold functions of a photopic retina not treated with aspartate. A 0.9 mm diameter test spot, $\lambda = 620$ nm, was centered in a large annular Wratten 15 field with a 1.0 mm dark center. The log intensity of the field I_b is given at right. Each set of points represents the responses to test flashes of several intensities (I_t) indicated on the abscissa. The amplitude of the *a*-wave in microvolts is indicated on the left-hand ordinate. Note that the absolute magnitude of the response to a given value of I_t increases in proportion to the strength of I_b .

DISCUSSION

Interpretation of the present results is based on the assumption that the initial (fast) component of the extracellularly recorded ERG of the aspartate-treated retina derives from the photoreceptors. The available evidence strongly supports this view. Several recent studies, including our own, have shown that sodium aspartate abolishes the *b*-wave, the S-potential, and the P III response originating proximal to the receptors (Sillman et al., 1969; Cervetto and MacNichol, 1971; Dowling and Ripps, 1971). The intracellular records from skate indicate that aspartate depolarizes the horizontal cells, rendering them completely unresponsive to photic stimuli (Cervetto and MacNichol, 1971; Dowling and Ripps, 1971). Although their mode of action on other retinal neurons has not yet been examined, the anions of aspartic and glutamic acid are known to cause depolarization of neuronal elements elsewhere in the nervous system (Curtis and Crawford, 1969), and relatively low concentrations of these substances suppress the spike discharge of ganglion cells (Kishida and Naka, 1967, 1968) as well as the compound action potential recorded from the optic nerve (Ames et al., 1967).

It is not at all clear at present why the visual receptors are less susceptible to aspartate and glutamate, but it is likely that the receptor membrane lacks some component with which these agents react (Ames et al., 1967). However, in addition to photoreceptor activity, a relatively slow potential of the same polarity is retained after aspartate treatment (Fig. 5). Faber (1969) has shown that the source of this potential lies near the receptor terminals and the sink extends throughout the rest of the retina, suggesting that it is probably glial in origin. Because of its slow rise-time, this response does not distort either the amplitude or latency of the fast receptor component. Thus, it appears that the application of sodium aspartate provides a useful method for isolating the receptor potential in the vertebrate retina.

An often perplexing problem in the analysis of electrical signals from mixed retinæ is that of distinguishing between rod and cone contributions to the response. The distinction is facilitated when the sensitivity of one mechanism is grossly different from the other, or when it is possible to exploit the properties of a given cell type, e.g., the directional sensitivity of the cones. In the carp, however, the comparatively low rod:cone ratio (and probably other factors) results in a large cone contribution to the mass receptor potential even in the dark-adapted preparation, and despite the fact that the test beam was projected obliquely onto the retina. Thus, the scotopic spectral sensitivity curve rises above the porphyropsin absorption spectrum at wavelengths >540 nm (Fig. 6). It is noteworthy that in the dark-adapted intact fish, the spectral function for the ERG *b*-wave also departs from the photopigment spectrum at long wavelengths (Witkovsky, 1968). Indeed, there is no assurance that the

criterion voltage for wavelengths ≤ 540 nm is generated solely by the rods, although its spectral characteristics and quantal requirements make it highly probable that the response is rod dominant. Moreover, the marked Purkinje shift that occurs when a significant fraction of the rod pigment is bleached is further evidence that the rods subserve dark-adapted thresholds when the test wave-length ≤ 540 nm.

In view of the foregoing, it seems reasonable to suppose that the increment thresholds measured at 500 nm (Fig. 7) reflect rod function—at least over the first 2 log units of effective background intensities. With brighter backgrounds, the cones provide a larger proportion of the incremental voltage, but the small spectral change (e.g. as between thresholds at 500 and 620 nm) suggests that the rod response is not suppressed completely. Nevertheless, both segments of the incremental threshold function show a linear relation between I_t and I_B ; i.e., the slope of the log-log plot is unity. Since almost the entire curve of Fig. 7 is a consequence of background fields too weak to alter significantly the concentration of light-sensitive pigments, it is clear that the signals from carp photoreceptors do not depend solely on quantum catch (Rushton, 1965). Furthermore, carp rods, like those of the frog (Frank, 1971) and skate (Dowling and Ripps, 1972) exhibit “neural” adaptation, during which time they undergo marked threshold changes while the concentration of porphyropsin remains unchanged (Fig. 10).

Whereas moderate levels of light adaptation do not seem to saturate the rods, bleaching a large fraction of their photopigment is an effective means of suppressing the rod contribution to the mass receptor potential. In Fig. 11, for example, where the xenon flash bleached 80% of the porphyropsin, the 0.5 log unit difference between thresholds at 500 nm and 620 nm is precisely the value predicted by the Purkinje shift (compare Figs. 6 and 8). Moreover, the difference is maintained throughout the course of dark adaptation; i.e., rod potentials did not make an appearance after 2 h in darkness. Since we were unable to detect cone pigments by spectrophotometry, it is uncertain whether this slow dark adaptation process is related to the regeneration of cone pigments, or is the consequence of “neural” adaptation. The latter usually refers to a rapid phase of adaptation, but Baumann (1967) and Frank (1971) have reported that the isolated, perfused frog retina dark adapts at an extremely slow rate after exposure to a brief, intense light that bleaches a large fraction of the available rhodopsin. In both of these investigations, the experimental conditions precluded the regeneration of rhodopsin, but neither study provides information on cone pigment kinetics. Whether or not cone pigments regenerate in the isolated carp retina, it is apparent that the final (“dark-adapted”) threshold is at least 100-fold higher than would be expected if the rise were due simply to a lowering of quantal absorptivity; i.e. even if 90% of the cone pigments were bleached (and remained so), the threshold at $\lambda = 620$ nm would,

on this account, be elevated only 1 log unit. Thus, the electrical responses of cones as well as rods are dependent upon factors other than their ability to absorb quanta.

The suppression of rod activity by bleaching, combined with selective chromatic adaptation, enabled us to identify three classes of cone receptor with λ_{\max} at 440, 530, and 610 nm, respectively (Fig. 9). Considering the difference in the nature of the measurements these results agree remarkably well with the microspectrophotometric data from single goldfish cones (Marks, 1963, 1965; Liebman and Entine, 1964), and the estimates obtained by Tomita et al. (1967) from intracellular voltage measurements on carp cones. There is agreement also in that none of the foregoing studies provide evidence for a fourth cone type, maximally sensitive in the region of 665–680 nm. Although no more than three cone pigments have been found by microspectrophotometry in any retina studied thus far (Liebman, 1972), Witkovsky (1967, 1968) reported photopic spectral sensitivity curves with $\lambda_{\max} \approx 665$ nm for *b*-wave and S-potential responses in the carp retina. And in the tench, another relative of the carp, both luminosity and chromatic S-units are said to receive signals from a cone with $\lambda_{\max} = 680$ nm (Naka and Rushton, 1966). In these experiments the 665 or 680 nm maxima were revealed in the presence of short-wavelength adapting fields. However, in goldfish retina, Daw and Beauchamp (1972) recorded from two ganglion cells whose spectral sensitivity peaked at 670 nm irrespective of the color of the adapting field. On the other hand, three distinctly different experimental methods, all of which examine directly the spectral properties of the photoreceptors, have failed to detect this long-wavelength receptor. It remains for future research to reconcile these divergent results.

Another phenomenon for which a satisfactory explanation is not immediately available is the *a*-wave enhancement induced by surround illumination. The fact that the effect could not be produced in the aspartate-treated retina suggests that it is mediated by feedback from cells proximal to the receptors. This sort of circuitry is revealed by the work of Baylor et al. (1971), who have shown that interaction between turtle cones can be carried by the horizontal cells. However, their studies did not utilize steady, luminous annuli, and it would be premature to speculate on the mechanism of the observed effects based on the few experiments that we have conducted. Nevertheless, this phenomenon should be considered when relating photopic action spectra of retinal cells to the absorption spectra of cone pigments.

This study was supported by National Institutes of Health grant EY 00280 and a Fight-for-Sight grant to Dr. Witkovsky and EY 00285, EY 18766 to Dr. Ripps.

We thank Jane Zakevicius for preparing the drawings.

Received for publication 28 September 1972.

REFERENCES

- AGUILAR, M., and W. S. STILES. 1954. Saturation of the rod mechanism of retina at high levels of stimulation. *Opt. Acta.* 1:59.
- AMES, A., III, Y. TSUKADA, and F. B. NESBETT. 1967. Intracellular Cl^- , Na^+ , Ca^{2+} , Mg^{2+} , and P in nervous tissue; response to glutamate and to changes in extracellular calcium. *J. Neurochem.* 14:145.
- BAUMANN, C. 1967. Sehpurpurbleichung und Stäbchenfunktion in der isolierten Froschnetzhaut. III. Die Dunkeladaptation des skotopischen Systems nach partieller Sehpurpurbleichung. *Pflugers Arch. Gesamte Physiol. Menschen Tiere.* 298:70.
- BAUMANN, C. 1971. Regeneration of porphyropsin *in vivo*. *Nature (Lond.)* 233:484.
- BAYLOR, D. A., and M. G. F. FUORTES. 1970. Electrical responses of single cones in the retina of the turtle. *J. Physiol. (Lond.)* 207:77.
- BAYLOR, D. A., M. G. F. FUORTES, and P. M. O'BRYAN. 1971. Receptive fields of cones in the retina of the turtle. *J. Physiol. (Lond.)* 214:265.
- BORTOFF, A. 1964. Localization of slow potential responses in the *Necturus* retina. *Vision Res.* 4:627.
- BOYNTON, R. M., and D. N. WHITTEN. 1970. Visual adaptation in monkey cones: recordings of late receptor potentials. *Science (Wash. D. C.)* 170:1423.
- BRIDGES, C. D. B. 1967. Spectroscopic properties of porphyropsins. *Vision Res.* 7:349.
- BROWN, K. T., K. WATANABE, and M. MURAKAMI. 1965. Early and late receptor potentials of monkey cones and rods. *Cold Spring Harbor Symp. Quant. Biol.* 30:457.
- CERVETTO, L., and E. F. MACNICHOL. 1971. Pharmacology of horizontal cells in the isolated perfused skate retina. *Biol. Bull. (Woods Hole)* 141:381.
- CONE, R. A. 1963. Quantum relations of the rat electroretinogram. *J. Gen. Physiol.* 46:1267.
- CRESCITELLI, F., and H. J. A. DARTNALL. 1954. A photosensitive pigment of the carp retina. *J. Physiol. (Lond.)* 125:607.
- CURTIS, D. R., and J. N. CRAWFORD. 1969. Central synaptic transmission-microelectrophoretic studies. *Annu. Rev. Pharmacol.* 9:209.
- DARTNALL, H. J. A. 1957. *The Visual Pigments*. Methuen and Co. Ltd., London.
- DAW, N. W., and R. D. BEAUCHAMP. 1972. Unusual units in the goldfish optic nerve. *Vision Res.* 12:1849.
- DOWLING, J. E. 1963. Neural and photochemical mechanisms of visual adaptation in the rat. *J. Gen. Physiol.* 46:1287.
- DOWLING, J. E., and H. RIPPS. 1970. Visual adaptation in the retina of the skate. *J. Gen. Physiol.* 56:491.
- DOWLING, J. E., and H. RIPPS. 1971. Aspartate isolation of receptor potentials in the skate retina. *Biol. Bull. (Woods Hole)* 141:384.
- DOWLING, J. E., and H. RIPPS. 1972. Adaptation in skate photoreceptors. *J. Gen. Physiol.* 60:698.
- FABER, D. S. 1969. Analysis of the slow transretinal potentials in response to light. Ph.D. Thesis. State University of New York, Buffalo, N. Y.
- FATECHAND, R., M. LAUFER, and G. SVAETICHIN. 1962. Retinal receptor potentials and their linear relationship to light intensity. *Science (Wash. D. C.)* 137:666.
- FRANK, R. N. 1971. Properties of "neural" adaptation in components of the frog electroretinogram. *Vision Res.* 11:1113.
- FURUKAWA, T., and I. HANAWA. 1955. Effects of some common cations on electroretinogram of the toad. *Jap. J. Physiol.* 5:289.
- GREEN, D. G. 1971. Light adaptation in the rat retina: evidence for two receptor mechanisms. *Science (Wash. D. C.)* 174:598.
- KISHIDA, K., and K. I. NAKA. 1967. Amino acids and the spikes from the retinal ganglion cells. *Science (Wash. D. C.)* 156:648.
- KISHIDA, K., and K. I. NAKA. 1968. Interaction of excitatory and depressant amino acids in the frog retina. *J. Neurochem.* 15:833.

- LIEBMAN, P. 1972. Microspectrophotometry of photoreceptors. In *Handbook of Sensory Physiology*. H. J. A. Dartnall, editor. Springer-Verlag, Berlin. vol. VII/I. 481.
- LIEBMAN, P. A., and G. ENTINE. 1964. Sensitive low light-level microspectrophotometer: detection of photosensitive pigments of retinal cones. *J. Opt. Soc. Am.* **54**:12.
- MARKS, W. B. 1963. Difference spectra of the visual pigments in single goldfish cones. Ph.D. Thesis. Johns Hopkins University, Baltimore, Md.
- MARKS, W. B. 1965. Visual pigments of single goldfish cones. *J. Physiol. (Lond.)*. **178**:14.
- MOTOKAWA, K., T. OIKAWA, K. TASAKI, and T. OGAWA. 1959. The spatial distribution of electrical responses to focal illumination of the carp's retina. *Tohoku J. Exp. Med.* **70**:151.
- MURAKAMI, M., and A. KANEKO. 1966. Differentiation of PIII subcomponents in cold-blooded vertebrate retinas. *Vision Res.* **6**:627.
- NAKA, K. I., and W. A. H. RUSHTON. 1966. S-potentials from colour units in the retina of fish (Cyprinidae). *J. Physiol. (Lond.)*. **185**:536.
- PENN, R. D., and W. A. HAGINS. 1969. Signal transmission along retinal rods and the origin of the electroretinographic a-wave. *Nature (Lond.)*. **223**:201.
- RIPPS, H., and R. A. WEALE. 1965. Analysis of foveal densitometry. *Nature (Lond.)*. **205**:52.
- RIPPS, H., and R. A. WEALE. 1969. Flash bleaching of rhodopsin in the human retina. *J. Physiol. (Lond.)*. **200**:151.
- RUSHTON, W. A. H. 1965. The sensitivity of rods under illumination. *J. Physiol. (Lond.)*. **178**:141.
- SILLMAN, A. J., H. ITO, and T. TOMITA. 1969. Studies on the mass receptor potential of the isolated frog retina. I. General properties of the response. *Vision Res.* **9**:1435.
- STILES, W. S. 1949. Increment thresholds and the mechanisms of colour vision. *Doc. Ophthalmol.* **3**:138.
- STILES, W. S. 1959. Color vision: the approach through increment-threshold sensitivity. *Proc. Natl. Acad. Sci. U. S. A.* **45**:100.
- TOMITA, T. 1957. A study on the origin of intraretinal action potential of the cyprinid fish by means of pencil-type microelectrode. *Jap. J. Physiol.* **7**:80.
- TOMITA, T. 1965. Electrophysiological study of the mechanisms subserving color coding in the fish retina. *Cold Spring Harbor Symp. Quant. Biol.* **30**:559.
- TOMITA, T., A. KANEKO, M. MURAKAMI, and E. L. PAUTLER. 1967. Spectral response curves of single cones in the carp. *Vision Res.* **7**:519.
- TOYODA, J., H. HASHIMOTO, H. ANNO, and T. TOMITA. 1970. The rod response in the frog as studied by intracellular recording. *Vision Res.* **10**:1093.
- TOYODA, J., H. NOSAKI, and T. TOMITA. 1969. Light-induced resistance changes in single photoreceptors of *Necturus* and *Gekko*. *Vision Res.* **9**:453.
- WALD, G. 1939. The porphyropsin visual system. *J. Gen. Physiol.* **22**:775.
- WALD, G. 1964. The receptors of human color vision. *Science (Wash. D. C.)*. **145**:1007.
- WEINSTEIN, G. W., R. R. HOBSON, and J. E. DOWLING. 1967. Light and dark adaptation in the isolated rat retina. *Nature (Lond.)*. **215**:134.
- WERBLIN, F. S. 1971. Adaptation in a vertebrate retina: intracellular recording in *Necturus*. *J. Neurophysiol.* **34**:228.
- WERBLIN, F. S., and J. E. DOWLING. 1969. Organization of the retina of the mudpuppy, *Necturus maculosus*. II. Intracellular recording. *J. Neurophysiol.* **32**:339.
- WITKOVSKY, P. 1965. The spectral sensitivity of retinal ganglion cells in the carp. *Vision Res.* **5**:603.
- WITKOVSKY, P. 1967. A comparison of ganglion cell and S-potential response properties in carp retina. *J. Neurophysiol.* **30**:546.
- WITKOVSKY, P. 1968. The effect of chromatic adaptation on color sensitivity of the carp electroretinogram. *Vision Res.* **8**:823.