Visual Adaptation in
the Retina of the Skate

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ABSTRACT The electroretinogram (ERG) and single-unit ganglion cell activity were recorded from the eyecup of the skate (*Raja erinacea* and R. *oscellata*), and the adaptation properties of both types of response compared with *in situ* rhodopsin measurements obtained by fundus reflectometry. Under all conditions tested, the b-wave of the ERG and the ganglion cell discharge showed identical adaptation properties. For example, after flash adaptation that bleached 80 % of the rhodopsin, neither ganglion cell nor b-wave activity could be elicited for 10–15 min. Following this unresponsive period, thresholds fell rapidly; by 20 min after the flash, sensitivity was within 3 log units of the dark-adapted level. Further recovery of threshold was slow, requiring an additional 70–90 min to reach absolute threshold. Measurements of rhodopsin levels showed a close correlation with the slow recovery of threshold that occurred between 20 and 120 min of dark adaptation; there is a linear relation between rhodopsin concentration and log threshold. Other experiments dealt with the initial unresponsive period induced by light adaptation. The duration of this unresponsive period depended on the brightness of the adapting field; with bright backgrounds, suppression of retinal activity lasted 20–25 min, but sensitivity subsequently returned and thresholds fell to a steady-state value. At all background levels tested, increment thresholds were linearly related to background luminance.

INTRODUCTION

The decrease in visual sensitivity during light adaptation and its subsequent recovery in darkness are phenomena which depend primarily on retinal processes, both neural and photochemical. Studies to elucidate the photochemical basis for sensitivity changes in the rod mechanism of the rat eye have demonstrated that the relation between electroretinographic (ERG) b-wave threshold and rhodopsin concentration during dark adaptation is given by the equation:

$$\log (I_t/I_0) = k (R_0 - R_t)/R_0$$

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where R_t and I_t are, respectively, the rhodopsin concentration and threshold intensity at time t during the course of dark adaptation. R_0 and I_0 represent these parameters in the fully dark-adapted eye, and k is a constant of proportionality (Dowling, 1960, 1963).

Although this expression adequately describes the experimental findings over a sensitivity range of about 5 logarithmic units, and a similar relation is obtained for subjective measurements in the human eye (Rushton, 1961 a), there is some question as to whether the ERG b-wave provides a valid index of *visual* sensitivity. For example, recent experiments on frog retinas suggest that adaptation measured at the ganglion cell may be significantly different from that determined electroretinographically (Donner and Reuter, 1968; Baumann and Scheibner, 1968). According to Donner and Reuter (1965), threshold responses of frog ganglion cells obtained during the slow (presumably photochemical) phase of dark adaptation appear to be related not to the *level* of rhodopsin in the retina, but rather to the *rate* at which rhodopsin is being regenerated.

The work of Hamasaki and Bridges (1965) also suggests that under certain conditions the ERG b-wave may not be a good indicator of retinal excitability. These authors reported that after a bright flash is delivered to the dark-adapted elasmobranch eye, the ERG response to a second flash may be completely suppressed. But during the period of ERG desensitization, the signaling of higher centers appeared relatively unaffected, for the tectal response was of only slightly lower amplitude than that elicited by the initial flash.

The foregoing observations, coupled with recent evidence indicating that the b-wave of the ERG probably arises mainly in the Müller (glial) cells of the retina (Miller and Dowling, 1970), stress the importance of examining the electrical and photochemical correlates of adaptation in the retina of a single species—preferably one in which the visual receptors are predominantly of one type. The elasmobranch eye seems appropriate for such experiments, because it has been reported that many species of elasmobranchs have all-rod retinas (Rochon-Duvigneaud, 1943) a feature which greatly facilitates the interpretation of photochemical measurements.

Some preliminary experiments were performed on the dogfish (*Mustelus canis*), but we found that the retina in the isolated eyecup of this species survived poorly. Many of the preparations gave no b-wave or ganglion cell activity; only the negative PIII component of the ERG was routinely recorded. As a substitute, the eye of the skate was tried and found to be ideal. The retina is very hardy, and in the eyecup it gave large b-waves and ganglion cell responses for up to 8 hr. The receptor cells appear to be only rods (see below), and the recordings, both photometric and electrical, were extraordinarily consistent from one eye to the next.

MATERIALS AND METHODS

Preparation of the eyecup was similar for both the electrophysiological and photo-chemical measurements. Eyes were enucleated in dim red light from skates (*Raja erinacea and R. oscellata*) which had been dark-adapted for 3–18 hr. After hemisecting the globe, the posterior segment was trimmed to a rectangular piece of tissue approximately 1 × 1½ cm in size. A convenient feature of the skate eye is that the retina adheres firmly to the pigment epithelium and seldom detaches during any of the preparative procedures.

A portion of the superior half of the skate retina is backed by a silvery tapetal layer; all the spectral reflection measurements and most of the electrophysiological recordings were made in this region of the eye. No differences were observed between electrical recordings from tapetal and nontapetal areas, but the former greatly enhanced fundus reflection for the spectrophotometric determinations.

As much of the vitreous humor as possible was teased away, and the piece of eyecup was placed on a wad of cotton soaked in elasmobranch Ringer solution. Moist, cold oxygen was blown gently over the tissue. When prepared in this manner, the retina yielded stable electrical responses for periods of 6–8 hr; and after bleaching, approximately 90 % of the visual pigment was regenerated.

Histology Small pieces of eyecup, excised in the manner described above, were immersed for 1 hr in 2 % OsO_4 buffered with 0.14 M veronal acetate; after dehydration, the tissue was imbedded in Araldite epoxy resin and hardened. Sections 1–3 μ thick were cut for light microscopy, and either stained with the Richardson (1964) method or left unstained for phase-contrast examination. Thin sections were stained successively in uranyl acetate and lead citrate and studied in an RCA EMU-3G microscope.

Fundus Reflectometry Visual pigment kinetics were studied *in situ* with a rapid fundus reflectometer (Weale, 1959), modified for microscopic observation (Fig. 1). Collimated light from a 150 w xenon arc lamp (operated from a current-stabilized DC supply) passed sequentially through a series of 29 narrow-band interference filters mounted in spectral order on a wheel rotating at 5 rev/sec. The beam was turned through 90 degrees by an Ultropak incident light system (E. Leitz, N. Y.), and focused to a 2 mm diameter spot on the surface of the eyecup. The latter, placed vitreous side uppermost on Ringer-soaked cotton, rested in a Plexiglas chamber carried on the stage of an Ortholux microscope (E. Leitz, N. Y.).

Light reflected from the eye was collected by a 3.8× objective which imaged the test area in the aperture plane of a Leitz MPV microphotometer. An infrared image converter, attached to the reflex viewing section of the MPV, enabled the experimenter to align the preparation with infrared light, and to adjust the aperture size so as to accept only light reflected from the test region. When the viewing mirror was moved out of position, the spectral test lights fell on the cathode of a photomultiplier (EMI 9558 Q), the output of which was fed through an operational amplifier to an FM tape recorder and monitor oscilloscope.

The procedure was such as to obtain reflection measurements for at least eight com-

plete spectral scans of the test region, first with the eye fully dark-adapted, and again after it had been exposed to an intense bleaching light. Flash bleaching was performed with a xenon discharge lamp (Honeywell Strobonar 65D, Honeywell, Inc., Fort Washington, Pa.) which dissipated more than 95 % of its total light output within 0.8 msec. Calibration with an integrating thermopile (Ripps and Weale, 1969 b) indicated that the flux density delivered on the retina was 0.02 joule/cm². At this level, flash irradiation bleached 75–82 % of the available rhodopsin in the test region.
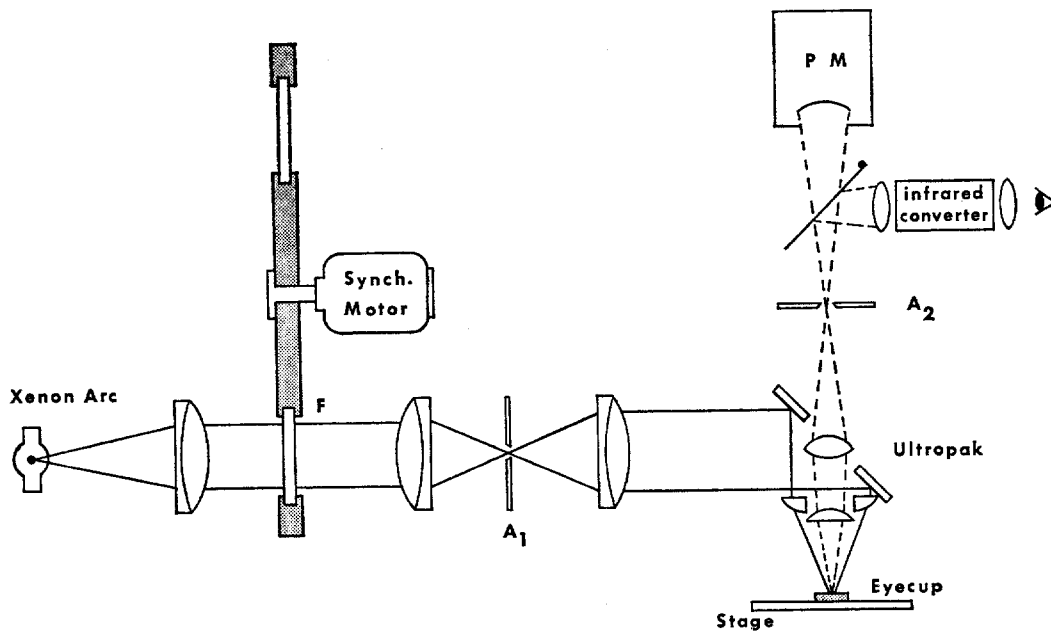

FIGURE 1.   The essential features of the microscope reflectometer. The interference filter wheel is driven by a synchronous motor at 5 rev/sec. The incident light illuminator of the Ultropak images aperture A_1 on the retinal surface of the eyecup; this area is then imaged at aperture A_2 by the transmission section (microscope objective) of the Ultropak. The position of a hinged mirror determines whether light from the test field is reflected to the infrared converter for viewing, or allowed to fall on the photomultiplier.

Prolonged bleaching exposures were obtained from a heat-filtered microscope lamp which, unattenuated, gave a retinal irradiance of 9.7 mw/cm². For each test wavelength (from 360–660 nm, in steps of approximately 10 nm), the changes in density produced by the bleaching exposure were computed from measurements of the photocell signals under dark- and light-adapted conditions. The rate at which the bleached photo-pigment regenerated was obtained from reflectivity measurements taken at various times during the course of dark adaptation.

The taped analogue signals were subsequently digitized and processed on a PDP-81 computer (Fig. 2 a). The output of the photomultiplier as displayed on the oscilloscope is a series of deflections, each of which corresponds to a given wavelength of the test beam (Fig. 2 b). The amplitude of any point on the waveform is propor-

tional to the light flux impinging on the photocell after having twice traversed the retina. These signals enter an analogue-to-digital converter which samples 35–40 points (y_i) on each waveform; the values of y_i are then integrated to approximate the area under the curve (Fig. 2 c). This operation was performed for every waveform of eight successive spectral scans taken during each recording period. The computer averaged the eight integrated values representing a given wavelength, and derived
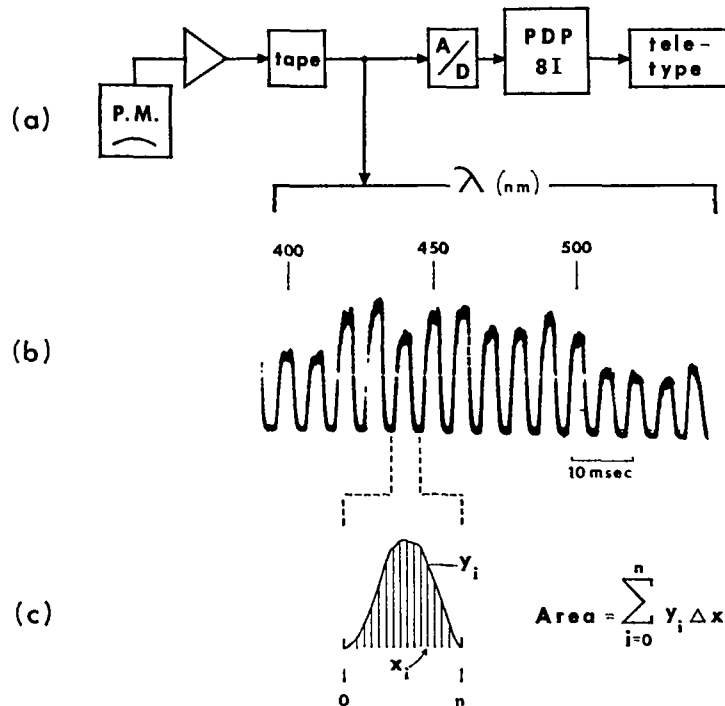

Figure 2. Signal processing of the fundus reflection measurements. (a) Block diagram of the procedure for recording and converting analogue signals to digital form for computer analysis. (b) A portion of a single spectral scan photographed from the face of a cathode-ray oscilloscope. (c) The area of each waveform is calculated for use in determining difference spectra. Details in text.

the density changes from the relation: $\Delta D_\lambda = \log (A_b/A_a)_\lambda$ where A_b and A_a represent the areas of the deflections for wavelength λ before and after bleaching, respectively (Ripps and Snapper, 1970).

Electrophysiology The ERG was recorded with a Ringer-moistened wick electrode in contact with the retinal surface of the eyecup; the moist cotton on which the piece of tissue rested served as the reference electrode. Both electrodes were connected via chlorided silver wires to the input stage of a capacitance-coupled preamplifier (Tektronix type 122) set for a time constant of 1 sec. The responses were displayed on a Tektronix type 564 storage oscilloscope and photographed on Polaroid film.

A few ERG experiments were performed on intact skates. On these occasions, the

animals were anesthetized by adding tricaine methanesulfonate to their seawater tank. They were then removed from the tank, pinned to a wooden board, and maintained by allowing seawater to flow through a rubber tube inserted into one spiracle. The operculum overlying the pupil was cut away to allow the maximum amount of light to enter the eye.

Ganglion cell responses were recorded by a platinum-iridium microelectrode placed on the inner retinal surface by means of a micromanipulator. The electrode was sharpened electrolytically to a tip diameter of 5–15 μ and insulated with Insl-X (Insl-X Products Corp., Yonkers, N. Y.). Tip insulation was broken by a current pulse and the exposed portion was plated with a platinum chloride solution. The responses were amplified and photographed as described above, except that the time constant of the preamplifier was reduced to 0.002 sec.

Test and adapting fields were provided by a dual beam optical system. Both beams were derived from tungsten filament microscope lamps and could be independently controlled with respect to size, intensity, position, and spectral composition. Test flashes of 0.2 or 0.1 sec duration were used routinely for threshold studies. Full field illumination was employed to elicit the ERG, but the optical system was modified for ganglion cell recording so as to provide circular stimulus spots 0.25–2.5 mm in diameter. The adapting field, which uniformly illuminated the entire piece of tissue, was similar for both ERG and ganglion cell experiments. The retinal irradiance of the adapting field was measured with a calibrated Eppley thermopile and Keithley microammeter. In order to study the course of dark adaptation after flash irradiation, the Strobonar unit was employed, with the lamp mounted at the same distance from the preparation as in the photochemical experiments.

RESULTS

Some General Properties of the Skate Retina

STRUCTURE   Fig. 3 is a montage of light micrographs illustrating the histological appearance of the skate retina. In several respects, the retina of this species resembles that of other fish; the horizontal cells are enormous and there is a paucity of ganglion cells (Fig. 3 a). However, an important feature of the skate retina is that the receptor layer appears to contain only rods (Fig. 3 a). Careful examination in the light microscope of both stained and unstained preparations, and preliminary electron microscopic study, have failed so far to reveal any receptoral detail which could be considered characteristic of cones. For example, the bimembranous discs of the outer segments appear pinched off from the plasma membrane, and only the spherule type of synaptic base has been observed at the proximal ends of the receptors (Fig. 3 b).

Within the inner nuclear layer there are, in addition to the very large horizontal cells, a number of amacrine cells which have particularly large cell bodies (Fig. 3 b). A marked degree of neuronal convergence is indicated by the relatively high receptor:bipolar cell ratio (Fig. 3 c). Also shown in Fig. 3 c is

the course taken by the dendritic processes of the bipolar cells which run distally between the horizontal cells in pairs or in small groups

Another interesting feature of the skate retina concerns the distribution of ganglion cells. Fig. 3 d is a flat section of a retina, stained with methylene blue, which was kindly prepared for us by Professor F. Vrabec. The ganglion cells, although few in number, tend to lie in rows parallel to the bundles of optic nerve fibers. This observation may explain why in some vertical sections no ganglion cells are evident for considerable distances, whereas in other sections several may be seen.

A final point of interest is that there are no pigment granules in the pigment epithelium overlying the tapetal area of the skate retina (Fig. 3 a); only the choroid is pigmented. In the tapetal portion of the skate eye, we found no difference in reflection at 660 nm between light- and dark-adapted retinas, indicating that pigment migration (if present) is not a factor in determining sensitivity changes during adaptation.

FUNDUS REFLECTOMETRY In order to determine the visual pigment of the retina and to measure the fraction of pigment bleached by the various light-adapting conditions used in the electrophysiological studies, reflectivity measurements of the dark-adapted eye were compared with those obtained after xenon flash irradiation and prolonged tungsten exposures. Density-difference spectra resulting from flash and time exposures are illustrated in Fig. 4; both sources were heat filtered and used at maximum intensity. The 1 min exposure bleached nearly all the available rhodopsin and produced, in this retina, a maximum density change (for light traversing the retina twice) of about 0.22. In other preparations, maximum values after a full bleach ranged from 0.22 to 0.33 density units. On the other hand, the xenon flash (which delivered fewer quanta to the retina) produced a density change of 0.17; i.e., 80% of the maximum obtained with the 1 min exposure. The results of other flash photolysis experiments demonstrated that, irrespective of total pigment density, flash irradiation bleached 75–82% of the rhodopsin within the test area. A series of control experiments, in which density changes were measured as a function of retinal irradiance, showed that the bleaching efficacy of flash and time exposures was similar—a situation that holds also in the periphery of the human retina (Ripps and Weale, 1969 *b*).

It is also apparent from Fig. 4 that aside from differences in magnitude, the two curves exhibit somewhat different spectral characteristics: after flash irradiation, the λ_{max} of the density difference spectrum was at 510 nm; after the 1 min bleach, the λ_{max} was at 500 nm. Comparable results have been obtained in the human retina where the long wavelength shift produced by flash photolysis was attributed to the formation of photoproducts absorbing in the blue region of the spectrum (Ripps and Weale, 1969 *b*). The large gain in density at
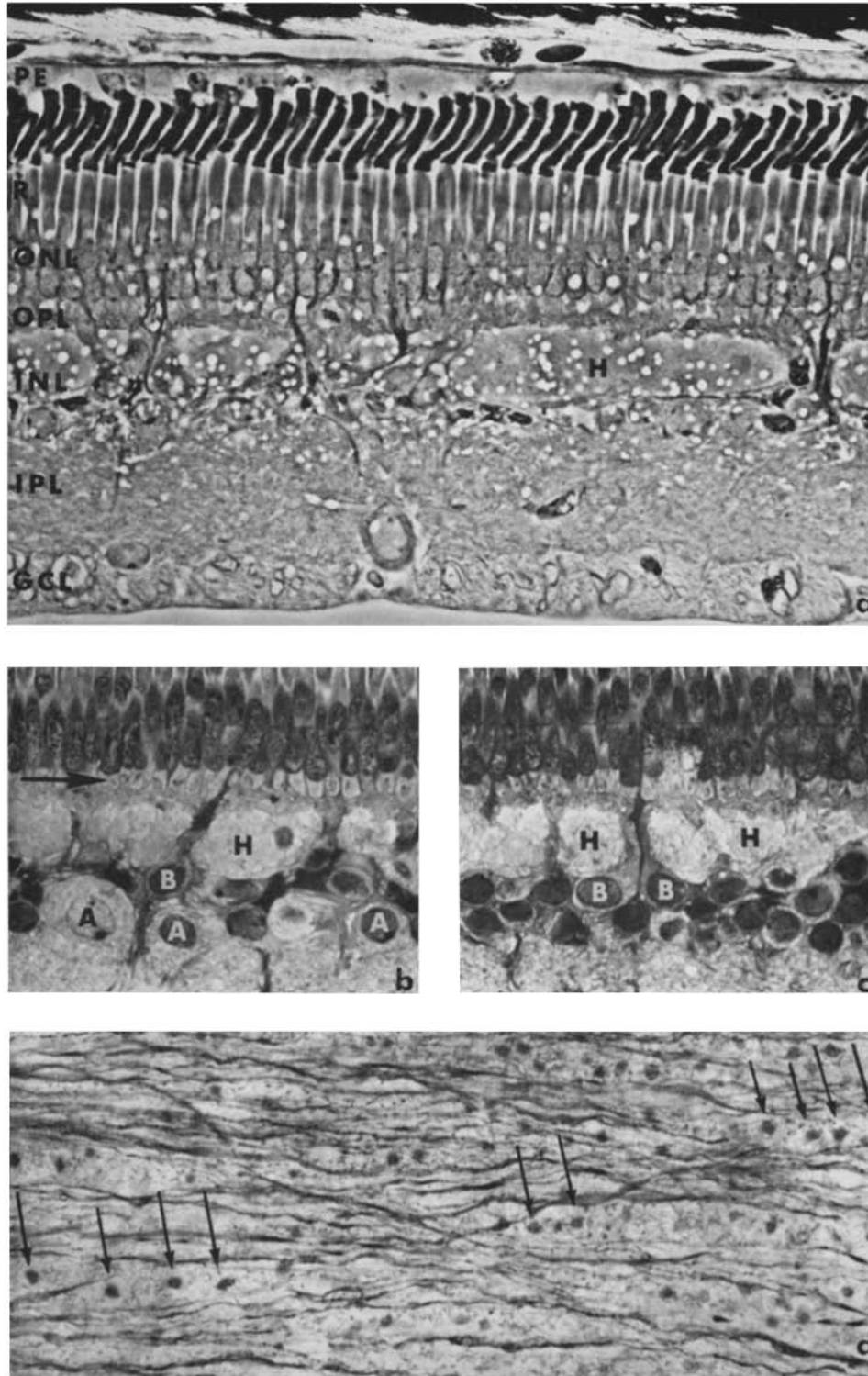
Figure 3

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380 nm indicates the formation of such photoproducts in the skate retina. With longer exposures (e.g., 1 min), some of these photoproducts have decayed to relatively transparent substances, and the difference spectrum begins to approximate the absorption spectrum of the parent pigment. In fact, the 500 nm peak obtained after the 1 min bleaching exposure is compatible with the absorption maximum at 497 nm determined by Beatty (1969) from retinal extracts of another species of skate (*Raja binoculata*).
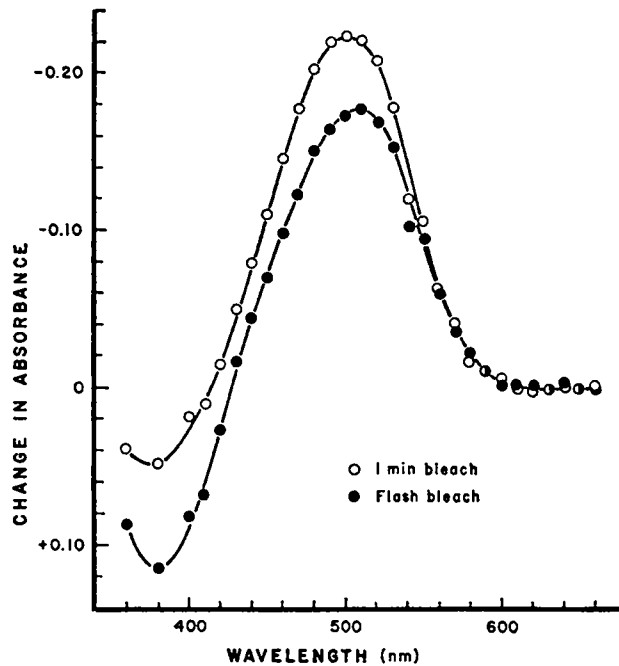

FIGURE 4.   Density-difference spectra recorded after flash and 1 min bleaching exposures of the dark-adapted skate retina. Expressed in equivalent units, the retinal radiant dose delivered by the flash was 0.02 joule/cm^2; that produced by 1 min of continuous illumination was about 0.55 joule/cm^2. The changes in absorbance are for double transit through the retina, negative values indicating a loss and positive values a gain in optical density.

FIGURE 3.   Histology of the skate retina. (*a*) Phase-contrast micrograph of a transverse section through the retina. The receptors (*R*) appear to be all rods. Note the large horizontal cells (*H*) and the absence of screening pigment in the pigment epithelium (*PE*). *ONL*, outer nuclear layer; *OPL*, outer plexiform layer; *INL*, inner nuclear layer; *IPL*, inner plexiform layer; *GCL*, ganglion cell layer. × 500. (*b* and *c*) High-power light micrographs of stained skate retina showing receptor terminals (arrow), and cells of inner nuclear layer (*H*, horizontal cells; *A*, amacrine cells; *B*, bipolar cells). Note in *c* the apical dendrites of two bipolar cells extending distally between the horizontal cells. × 650. (*d*) Low-power micrograph focused on the ganglion cell layer of flat-mounted retina stained with methylene blue. The ganglion cells (arrows) tend to lie in rows parallel with the bundles of optic nerve fibers. × 175.

Rhodopsin regeneration after flash photolysis was derived from measurements of difference spectra recorded during the course of dark adaptation. The essentials of the procedure are illustrated in Fig. 5. The dashed-line curve represents a bleaching difference spectrum; i.e., the density differences produced by flash bleaching of the dark-adapted retina. Immediately after the
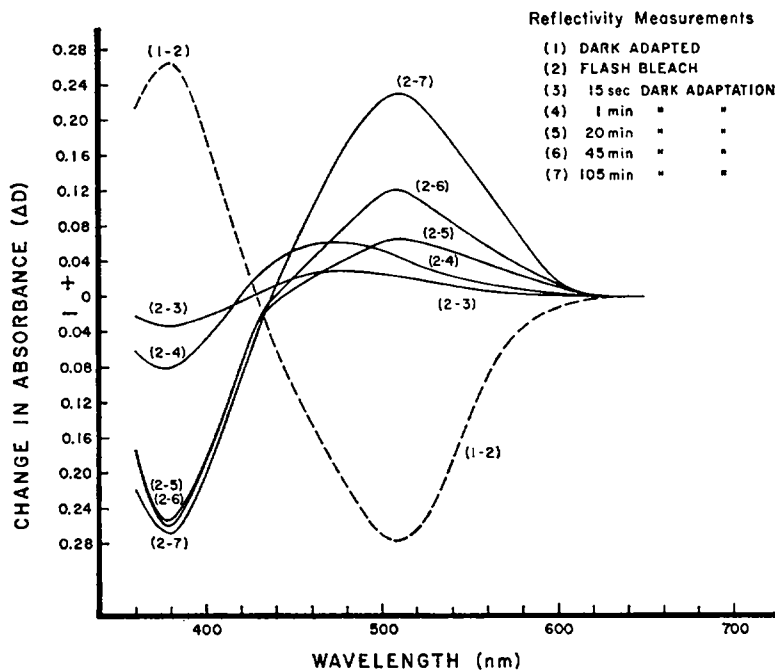

FIGURE 5. Density-difference spectra obtained from reflectivity measurements of the skate retina taken at the times indicated by the numbers in parentheses. The dashed-line curve (1-2) shows the bleaching difference spectrum produced by a full Strobonar flash. Subsequent changes in absorbance (continuous curves) represent the regeneration spectra. See text.

flash exposure, the retina has been depleted of most of its rhodopsin (ΔD_{510} = −0.26), but almost an equivalent gain in density is evident at λ = 380 nm. The remaining curves of Fig. 5 represent "regeneration" spectra; i.e., the density differences between recordings taken at specified times after the flash exposure and those obtained within the first few seconds after the flash. The earliest dark reactions clearly involve transient photoproducts rather than the replenishment of bleached rhodopsin. During the first minute of dark adaptation (curves 2–3 and 2–4), there is a loss of density in the region of 380 nm, together with the formation of a substance absorbing maximally in the region of 460–480 nm. After 15–20 min in darkness, most of the substance absorbing

at 380 nm has decayed, and the regeneration of rhodopsin is indicated by a gain in density at 510 nm, which increases to more than 80% of its initial value after 105 min of dark adaptation.

The main long-lived photoproducts found in rods after flash bleaching are metarhodopsin II, which absorbs maximally at about 380 nm, and pararhodopsin (or meta III) which absorbs at about 465–470 nm (Matthews et al., 1963; Weale, 1967; Frank and Dowling, 1968; Cone and Cobbs, 1969). Fig. 6
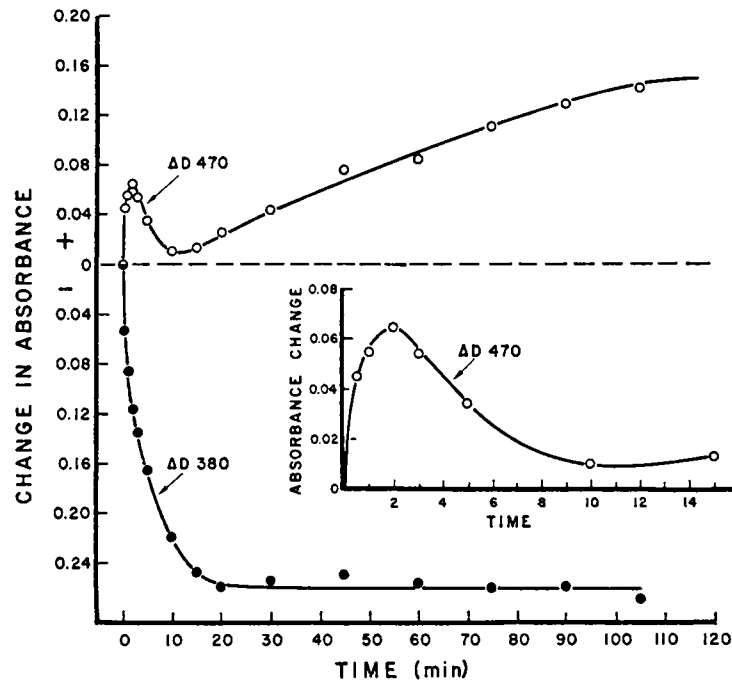

FIGURE 6. Changes in absorbance at 380 and 470 nm after flash bleaching the skate retina. Measurements at 380 nm show only a loss in density lasting about 20 min. At 470 nm, density increases for the first 2 min, then declines over the next 8–10 min; the increase in 470 nm absorption occurring thereafter is due to rhodopsin regeneration.

shows the change of absorbance at these two wavelengths after a flash bleach of the skate retina. Absorbance at 380 continuously falls, reaching a plateau after about 15–20 min. Absorption at 470 increases for the first 2 min after the flash, then declines over the next 8 min. Thereafter the density at 470 slowly increases, due to the regeneration of rhodopsin. The changes in absorbance at 470 and 380 nm observed in the skate retina after a flash bleach are similar to those described in other vertebrate retinas, although the time course of formation and decay of the photoproducts is somewhat different (Frank and Dowling, 1968; Ripps and Weale, 1969 *a*).

ELECTRICAL ACTIVITY   A few sample tracings of ERG and ganglion cell responses from the dark-adapted skate retina are reproduced in Fig. 7. The principal components of the ERG were a large, positive potential (b-wave) preceded by a smaller, negative deflection (a-wave). At low stimulus intensities, only the b-wave was discernible; as the intensity was raised, the a-wave became evident, the b-wave amplitude increased, and the early segment of another positive potential (the c-wave) could be seen following the b-wave.
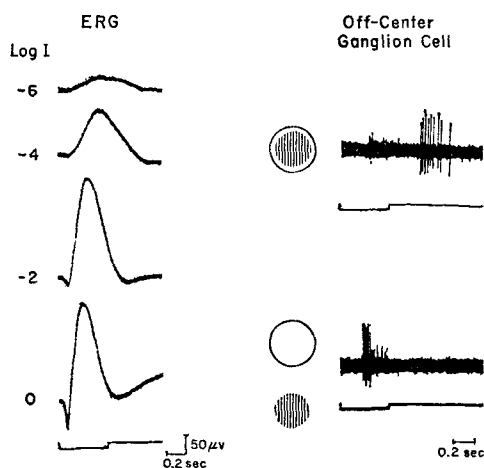

FIGURE 7.   Sample ERG and ganglion cell responses from the dark-adapted skate retina. At low intensities, only the b-wave is evident; at higher intensities, an a-wave precedes the b-wave. The ganglion cells respond to light with bursts of impulses, and their receptive fields are organized in an antagonistic center-surround fashion. Here, an off-center ganglion cell responds at the cessation of illumination when the center of the field is illuminated. Surround illumination elicits a burst of impulses during illumination. In this and in subsequent figures the numerical values for log I refer to the density of the neutral filters placed in the test or background beams (log $I \sim -D$).

The ganglion cells responded with bursts of impulses at either the onset or cessation of illumination, or both. Receptive fields were mapped for several units, all of which exhibited antagonistic center-surround organization. This feature is illustrated in Fig. 7 for an "off"-center cell. With the stimulus spot centered on the recording electrode, no response was elicited during illumination, but the unit discharged vigorously when the light was turned off. Moving the stimulus spot to a peripheral location produced opposite reactions to the onset and cessation of illumination. The centers of the receptive fields were quite large (1.5 mm in diameter), and approximately equal numbers of "on"- and "off"-center units were encountered. Although the extent of the surround region was more difficult to ascertain, it appeared to comprise an annular region extending at least 3 mm from the borders of the central zone.

A striking characteristic of the skate retina is the slowness with which it

adapts to light and darkness. Although this property will be considered in greater detail in the following sections, some of its consequences are illustrated in Figs. 8 and 9. Fig. 8 shows ERG responses obtained from the eye of an intact animal to double flashes spaced 2.5 sec apart. With relatively dim stimuli, the second flash elicited a b-wave of almost the same amplitude as that evoked by the first flash. With brighter stimuli, however, the b-wave elicited by the second flash was reduced in amplitude to about 15% of the initial response. Fig. 9 shows graphically the relation between the amplitude of the b-wave to a second flash and the interflash interval; the results were obtained from an eye-
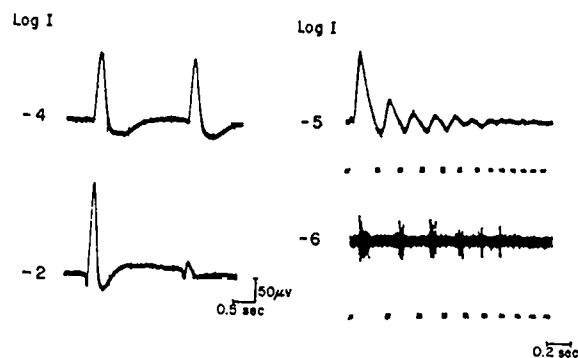
Figure 8. The response of the skate retina to double flashes (left) and flickering light of increasing rate (right). At lower stimulus intensities (log I = −4), flashes spaced 2.5 sec apart elicit ERG's of comparable magnitude; with brighter stimulus flashes (log I = −2), the second flash elicits an ERG only 10–15% the amplitude of the first response. Neither the b-wave nor the ganglion cell discharge will follow flickering light at a rate faster than 5/sec. The flickering stimulus used here was of optimal intensity for eliciting flicker responses; the retina responds less well to either brighter or dimmer flickering light.

cup preparation at two different stimulus levels. Note that when bright stimuli were used, the eye was unresponsive to the second flash for more than 5 sec, and an interval of about 11 sec was required between flashes to elicit a b-wave half the amplitude of that evoked by the first flash. Hamasaki and Bridges (1965) report similar effects with double flash stimulation in several other species of elasmobranch.

The foregoing observations suggest that the skate retina is ill-equipped to resolve intermittent stimuli. That this is indeed the case is shown also in Fig. 8 for both the b-wave and ganglion cell discharge. The electrical responses to increasing stimulus frequencies gradually decrease, and disappear completely when the flash rate exceeds 5/sec. This value represents the highest flicker frequency we have observed either the b-wave or ganglion cell response to follow, and it is achieved only with relatively low intensity stimuli; at both higher and lower intensities, flicker fusion occurs at lower frequencies.

Light and Dark Adaptation

A principal objective of this study was to compare the adaptation properties of the ERG and ganglion cell discharge. In order to test thresholds, criterion responses were established for each; a 10 μv b-wave in the ERG, and a burst of two or three impulses at the ganglion cell provided just detectable responses. Almost all the adaptation data on the ganglion cells were obtained from "on"-center cells, which seemed easier to work with for threshold measurements.
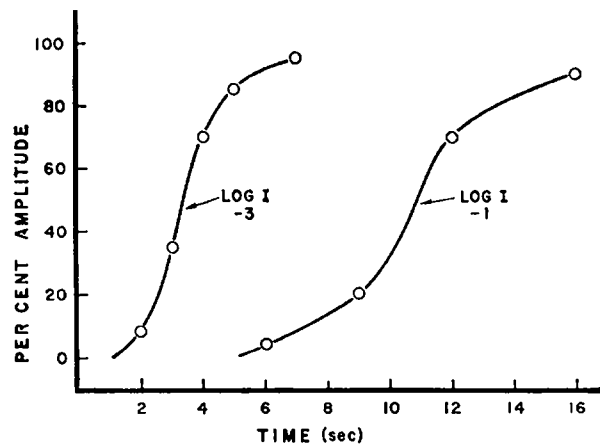

FIGURE 9. The relation between b-wave amplitude elicited by a second flash and inter-flash interval. With lower flash intensity (log I = −3), a b-wave of 50% the amplitude of the dark-adapted response can be elicited after 4 sec; with the brighter flash (log I = −1), 11 sec between flashes is required to elicit a b-wave of 50% the amplitude of the first response.

The dark-adapted thresholds, determined both electroretinographically and at the ganglion cell, were remarkably constant from one preparation to the next. However, full field illumination was used to elicit the ERG, whereas ganglion cell sensitivity was tested with a spot of light slightly smaller than the center of the receptive field (∼1 mm in diameter). In these circumstances, ganglion cell sensitivity was about 1 log unit greater than ERG sensitivity. Since we are concerned here with changes in *relative* sensitivity, the comparison of ERG and ganglion cell data was facilitated by adjusting the former along the scale of ordinates by 1 log unit so as to equate dark-adapted thresholds.

INCREMENT THRESHOLDS   A comparison of increment thresholds for the ERG and ganglion cell response over a range of 6 log units of adapting luminance is shown in Fig. 10. For both types of response, the relation between log threshold and log background is linear with a slope of almost exactly 1.

The linearity of these functions suggests that only one receptor type contributes to the responses over this wide range of adaptation. Further evidence that the electrical responses are subserved by a single mechanism was obtained by testing spectral sensitivity to red (Wratten filter No. 29) and blue (Wratten No. 49) stimuli. Thresholds were measured first in the dark-adapted preparation, and again after it had been light-adapted to various background levels. Although the reduced intensity of the colored test fields precluded measure-
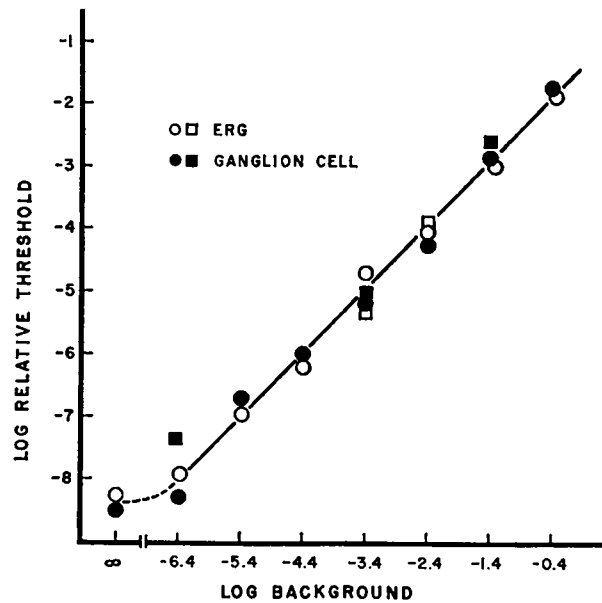

Figure 10. Increment thresholds for the ERG and ganglion cell responses. For both types of response the relation between log relative threshold and log background intensity is linear with a slope of nearly 1 over a range of 6 log units of adapting luminance. In order to facilitate comparison of the ERG and ganglion cell responses, the ERG data were shifted down on the scale of ordinates by 1 log unit. Log I = 0 on the scale of abscissas corresponds to a retinal irradiance of 0.48 mw/cm^2.

ments when the background exceeded − 1.4 log unit, no differences in relative spectral sensitivity were observed for backgrounds up to and including this level.

We learned very early in these experiments that a considerable length of time (up to 40 min) may be required to establish a stable increment threshold level in the skate retina, especially during exposure to bright adapting fields (cf. Figs. 13 and 15). This very slow light adaptation process will be discussed in a later section. For the present, it is sufficient to note that the increment thresholds of Fig. 10 were obtained after light adaptation was complete, and retinal sensitivity had reached a stable level.

DARK ADAPTATION   Fig. 11 shows the changes in ERG and ganglion cell thresholds as a function of time following flash irradiation of the skate retina. After the Strobonar flash, which bleached approximately 80% of the rhodopsin in the retina (cf. Fig. 4), electrical responses could not be elicited with our brightest stimuli for about 10 min; this period of insensitivity varied from one preparation to the next, and probably accounts for the scatter seen in the data during the early part of dark adaptation. Over the next 10 min,
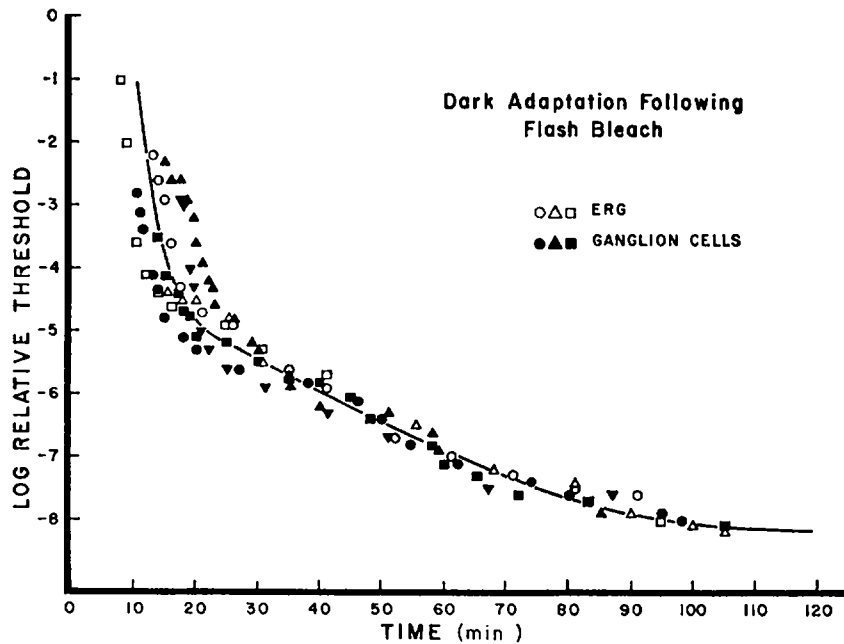

FIGURE 11.   Dark adaptation of ERG (b-wave) and ganglion cell responses after flash bleaching. Electrical responses could not be elicited for the first 10 min of dark adaptation; over the next 10 min thresholds fell rapidly to within about 3 log units of absolute sensitivity. Thereafter recovery of sensitivity was slow, and complete dark adaptation required about 2 hr. As in Fig. 10, the ERG data have been shifted down on the scale of ordinates by 1 log unit.

thresholds for both types of response fell rapidly to within 3 log units of dark-adapted threshold. Thereafter, recovery of sensitivity was considerably slower, the thresholds falling in nearly linear fashion for the next 60–80 min and then even more gradually until, after approximately 2 hr, the dark-adapted threshold level was reached. A single curve satisfactorily represents the data of both ERG and ganglion cell responses, indicating that there is no significant difference between these electrical measures of dark adaptation.

Data showing the course of pigment regeneration during dark adaptation are presented in Fig. 12; the curve, taken from Fig. 11, refers to the right-hand scale of ordinates and provides a direct comparison between rhodopsin levels

and the fall of ERG and ganglion cell threshold after equivalent bleaching maneuvers. After a full flash bleach, the retina is left with about 20–22% of its full complement of rhodopsin. For the first 10–15 min after the flash, rhodopsin regenerates rather slowly (cf. Dowling and Hubbard, 1963), but by 20 min after the flash rhodopsin has begun to regenerate more rapidly and almost linearly with time. This linear recovery of rhodopsin continues for 60–80 min and rhodopsin regeneration is complete after about 100–120 min.
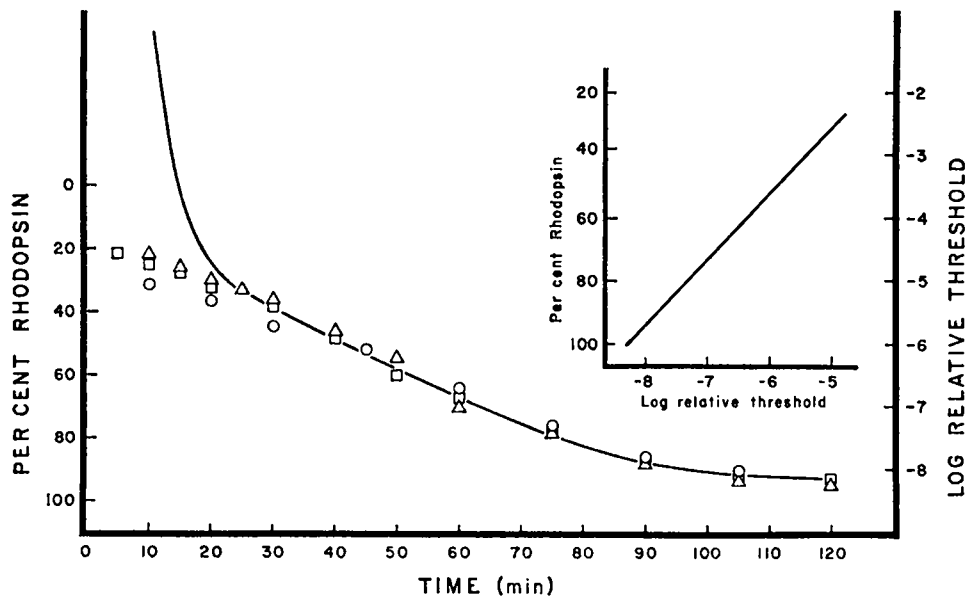

FIGURE 12. Rhodopsin regeneration (symbols) compared to recovery of sensitivity o ERG and ganglion cell responses during dark adaptation. The curve of dark adaptation is from Fig. 11. There is no relation between visual sensitivity and rhodopsin for the first 20 min, but thereafter there is a close correspondence between recovery of rhodopsin and threshold. The linear relation between log threshold and rhodopsin concentration is shown in the insert.

There is clearly a large disparity between electrical thresholds and rhodopsin levels during the first 20 min of dark adaptation. An initial period of insensitivity and the early fast component of dark adaptation are familiar properties of the vertebrate visual system (Blanchard, 1918; Baker, 1963; Rushton, 1961 *b*; Dowling, 1963) and do not correlate well with any of the *in situ* photochemical changes that have been studied (Frank and Dowling, 1968). These early components of dark adaptation appear to be mediated by neural rather than photochemical mechanisms (see Discussion).

On the other hand, the slow recovery of log threshold that occurs during the final 100 min of dark adaptation corresponds almost exactly with the time course of rhodopsin regeneration during this period. Thus, plotting log thresh-

old vs. percent rhodopsin (insert to Fig. 12) yields a linear relation similar to that which describes the relation between rhodopsin concentration and log ERG threshold in the rat eye (Dowling, 1963; Weinstein, et al., 1967). Furthermore, it is important to emphasize that the present results apply also to measurements of ganglion cell sensitivity.

EXTENDED LIGHT ADAPTATION   We noted earlier that when the skate retina is exposed to a bright adapting light, an unusually long period of time is required before responses to incremental stimuli can be elicited, and even longer periods to reach a stable threshold level. The following experiments examine the details of this slow light adaptation process. The open symbols of Fig. 13 show ERG thresholds during the course of light adaptation to three different intensities of background illumination; in each case, the adapting light was turned on at time zero. Using a dim adapting field (log I = −5.4), ERG responses could be elicited within 30 sec of its onset. The threshold was initially very high (nearly 6 log units above dark-adapted threshold), but
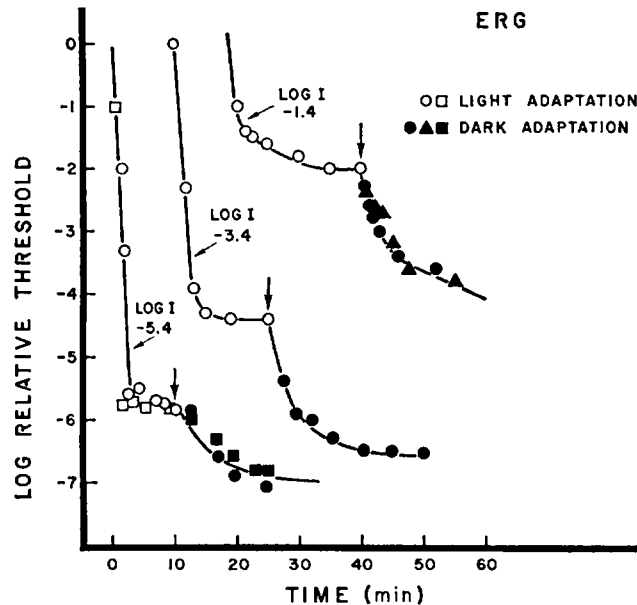

FIGURE 13.   The course of light (open symbols) and dark (solid symbols) adaptation of the ERG to three different intensities of background illumination. In each case the adapting light was turned on at time zero; initially the b-wave could not be elicited with any stimulus intensity. The duration of the initial unresponsive period depended on the intensity of the adapting luminance and lasted 10–20 min with the brighter adapting lights. After b-wave activity reappeared, thresholds fell rapidly to incremental threshold levels (Fig. 10). When the adapting lights were extinguished, dark adaptation was rapid after nonbleaching light adaptation (log I = −5 and −3.4), and slow after bright (bleaching) light adaptation (log I = −1.4).

dropped precipitously to 1 log unit above absolute threshold where it sta-bilized. When the adapting light was extinguished (arrow), thresholds fell rapidly (solid symbols) to the dark-adapted level.

With backgrounds of intermediate intensity (log I = −3.4), the early stages of light adaptation were marked by a prolonged period during which the ERG could not be elicited by our brightest test flashes. After 10 min, however, the b-wave could be recorded, and thresholds fell quickly to a stable level, ap-proximately 3 log units above the dark-adapted value. The background light was extinguished after 25 min of light adaptation; subsequent dark adaptation was fairly rapid, and was essentially complete about 15 min later.

With very bright adapting lights (log I = −1.4), the skate retina is entirely unresponsive for 15 min or so (Fig. 13); b-wave threshold could be determined only after about 20 min of light adaptation but an additional 10–15 min was required for the final increment threshold level to be reached. Dark adapta-tion after such a prolonged exposure shows an initial, rapid fall of threshold followed by a slower phase of recovery. Complete dark adaptation requires about 2 hr (Fig. 16).

Fig. 14 illustrates the sequence of events associated with light adaptation of the ERG. The dark-adapted records show typical responses (b-wave–domi-nated) near threshold. After the adapting light (log I = −3.4) was turned on, no electrical response could be evoked from the retina for about 5 min. Be-
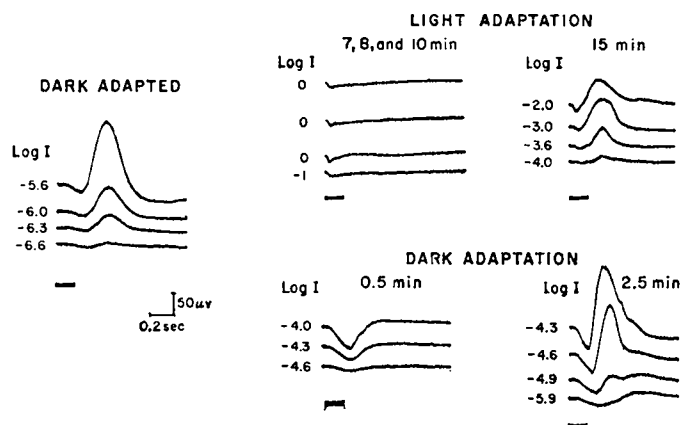

FIGURE 14.   Selected ERG's recorded during a light and dark adaptation experiment. The adapting luminance was log I = −3.4. The dark-adapted records are b-wave–dominated near threshold. After the adapting light was turned on, the ERG response could not be recorded for 5 min. At 7 and 8 min a small a-wave was elicited with the brightest flash. At 10 min, a b-wave appeared; and by 15 min the ERG threshold was near its final increment level. Immediately after the adapting light was extinguished, the ERG showed an initial large negative wave (a-wave) near threshold, but by 2½ min into dark adaptation the ERG was again b-wave–dominated.

tween 5 and 10 min after the beginning of light adaptation, a small isolated a-wave could be elicited, but only with the brightest test stimuli available. The first sign of a b-wave occurred at the 10 min mark, and after 15 min the threshold was near its final increment level and the responses were again b-wave–dominated. ERG responses obtained during the early stages of dark adaptation also show interesting changes in waveform. 30 sec after the adapting light was extinguished, the responses near threshold were a-wave–dominated. By 2½ min after the beginning of dark adaptation the responses near threshold continued to show initial large negative waves, but the positive b-wave was more evident. After 5 min of dark adaptation, the ERG responses were b-wave dominated as in the dark-adapted preparation.

The ganglion cells light adapt in very much the same manner as the ERG. Fig. 15 shows the course of light and dark adaptation of a ganglion cell when the retina was exposed to two different intensities of adapting light. The initial silent period has about the same duration for the ganglion cell response as with the b-wave of the ERG. After the cell begins to respond to stimuli, thresholds fall rapidly to the incremental threshold level and remain there. The course
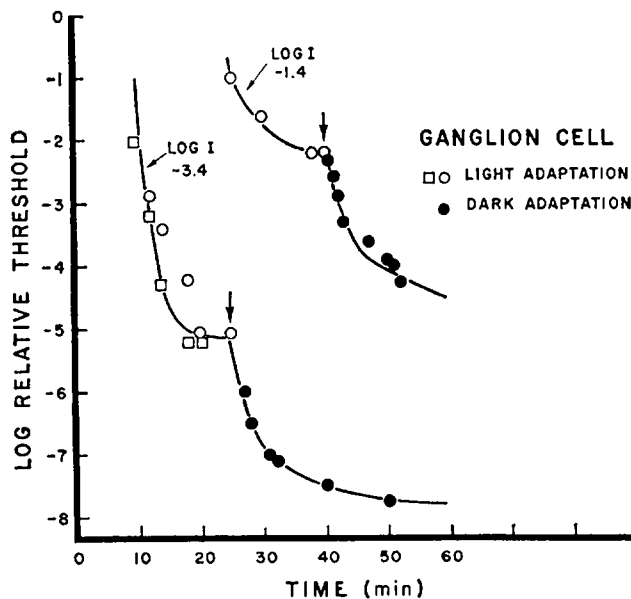

FIGURE 15. Light and dark adaptation of a ganglion cell to two adapting luminances. Immediately after the adapting light was turned on, the cell was unresponsive to flashes of any intensity for 10–20 min. The duration of the unresponsive period depended on the adapting luminance. After activity resumed, thresholds quickly fell to incremental sensitivity levels and remained there. Dark adaptation was fast after dim light adaptation, slow after bright light adaptation. Note the similarity of light adaptation for the ganglion cell and ERG b-wave responses (Fig. 13).

of subsequent dark adaptation of the ganglion cell is also very similar to that observed with the ERG. With the lower adapting luminance (log I = -3.4), recovery is completed in about 15 min. With the brighter adapting light, dark adaptation shows an initial rapid phase followed by a slow phase, and complete dark adaptation requires over 2 hr. Fig. 16 shows that the slow phase of dark adaptation after prolonged bright light adaptation appears to be similar for the ERG and ganglion cell response.

Fig. 17 shows ganglion cell records during light adaptation. When the background field (log I = -3.4) was first turned on, there was an initial burst of
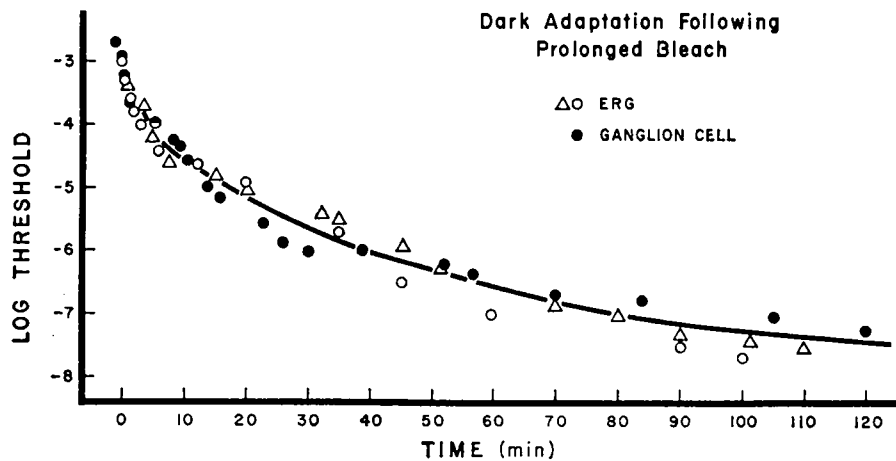

Figure 16. The course of dark adaptation of the skate retina following a prolonged (40 min) light adaptation to log I = -1.4. Dark adaptation is slow and follows a similar time course for both the ERG and ganglion cell response. The ERG threshold data have been shifted down the scale of ordinates by 1 log unit.

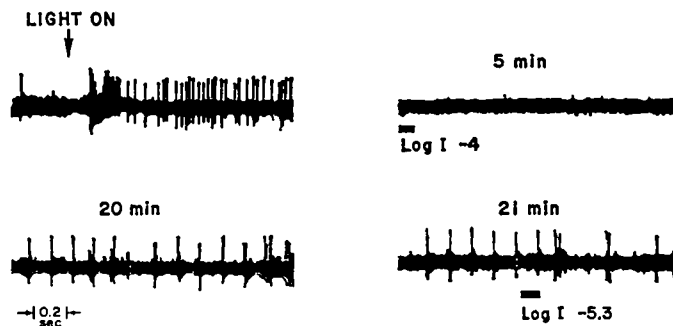Figure 17. Ganglion cell records during light adaptation. When the background light was first turned on, there was a vigorous burst of impulses lasting 20–30 sec. For the next 8–10 min, the cell was silent; flashes of any intensity were ineffective. After 10 min, the cell began to fire spikes, eventually quite rhythmically. At this time, flashes above increment threshold evoked spikes and interrupted the rhythmic firing pattern.

impulses followed by a sustained discharge that lasted for about 20–30 sec. For the next 8–10 min of light adaptation the cell was electrically silent and unresponsive to the brightest incremental flashes. Note the lack of background activity in the record taken 5 min after the adapting light was turned on (Fig. 17) indicating that nearby ganglion cells were also quiet during this period.

After 8–10 min, the cell began to fire spikes spontaneously—first erratically, but gradually the discharge became very regular and rhythmic. At the time the cell began again to exhibit activity, it was also possible to drive the cell with light flashes. The rhythmic firing of the cell during light adaptation and the response to an incremental flash are shown also in Fig. 17. The rhythmic firing lasted as long as the light remained on. Flashes above increment threshold evoked spikes and interrupted the rhythmic discharge for a short period. Very near threshold, it was sometimes easier to detect the interruption in rhythmic bursting rather than the extra spikes.

The rhythmic firing that occurs while a background light is on was observed at most adapting intensities, but no relation between adapting luminance and rate of rhythmic activity was noted. The cell then remained relatively silent during dark adaptation unless stimulated by light. On several occasions, however, a marked increase in spontaneous activity occurred during final stages of the dark adaptation process following intense light adaptation. This activity would subside as absolute threshold was approached.

DISCUSSION

Electrical Measures of Retinal Sensitivity

The results of the present study show that in the skate retina the b-wave of the ERG and the spike activity of ganglion cells display remarkably similar adaptation properties. Thresholds follow the same temporal course during dark adaptation, the increment threshold functions run parallel, and both electrical responses are readily extinguished by intermittent stimuli at low flicker frequencies. In addition, the onset of a moderately bright adapting light induces an unusually long refractory period for both types of response. It appears, therefore, that b-wave sensitivity provides a reliable measure of retinal excitability in the skate. Recent studies have shown that a direct correlation between b-wave and ganglion cell responses also applies to threshold measurements in the isolated rat retina (Weinstein, 1969).

In view of the close correspondence between b-wave and ganglion cell sensitivity in skate and rat, it is difficult to account for the discrepancy between ERG and tectal response reported by Hamasaki and Bridges (1965). In their experiments on the intact shark, these authors found that an intense stimulus flash could suppress for several seconds the ERG to a second flash of equal strength, whereas the tectal response to the second flash was only reduced in amplitude. However, the ERG is a mass potential requiring the activation of a

large number of retinal elements to produce a detectable response. Thus, suppression of the ERG does not preclude the possibility that some areas of the retina remain responsive to either direct or stray light stimulation, and perhaps stimulation of these regions is sufficient to elicit a substantial response at the optic tectum.

Early Dark Adaptation

For nearly 15 min after flash bleaching the skate eye, retinal sensitivity is so markedly depressed that neither ERG nor ganglion cell thresholds are measurable; but within the next 5 min or so, there is nearly a 10,000-fold increase in sensitivity (Fig. 11). Thus, during the first 20 min of dark adaptation, thresholds fall from some indeterminate (perhaps infinite) value to about 3 log units above final threshold. In other visual systems, a similar, very rapid fall of threshold has been observed to occur during the early stages of dark adaptation (Blanchard, 1918; Rushton, 1961 *b*; Baker, 1963; Dowling 1963).

Recently Donner and Reuter (1967) have suggested that the early, rapid phase of adaptation may be determined by the fading of one of the photoproducts formed on irradiating rhodopsin, namely metarhodopsin II. They calculated that low concentrations of meta II could have a profound effect on ganglion cell sensitivity in the frog. Frank and Dowling (1968), however, compared directly b-wave thresholds with spectral transmission measurements of the isolated, living, rat retina, and found no correlation between sensitivity and the kinetics of any of the long-lived photoproducts detectable after flash photolysis. They suggest that these early sensitivity changes are governed by processes that are intrinsically neural in origin (see also Dowling, 1963).

Our results on the skate eyecup contribute little toward resolving this problem. Although we have obtained measurements on the temporal course of photoproduct decay (Fig. 6), a quantitative comparison with threshold changes is precluded by our inability to elicit any electrical responses for about 10–15 min after the flash exposure. By that time, 90–100% of the photoproduct absorption at both 470 and 380 nm has already decayed. However, it is noteworthy that during this early period of dark adaptation, not even the a-wave of the electroretinogram could be elicited by our brightest stimuli. Since the a-wave is probably generated distally in the retina (Brown et al., 1965; Tomita, 1963), the results indicate that the desensitizing mechanism acts at a very early stage in signal transmission, probably in the receptors themselves.

Rhodopsin and the Slow Course of Dark Adaptation

In the skate retina, threshold measurements obtained during the final 100 min of dark adaptation correlate rather precisely with the *in situ* concentration of rhodopsin. As we have shown (Fig. 12), log threshold intensity can be expressed as a linear function of concentration for this range of adaptation; that

is

$$\log I_t = -kc_t + b \tag{1}$$

where I_t and c_t are the threshold intensity and rhodopsin concentration, respectively, at time t in dark adaptation, and k and b are constants.

This expression applies equally well when thresholds are determined electroretinographically in the frog (Baumann, 1967) and rat (Dowling, 1960, 1963), or by subjective methods in man (Rushton, 1961 a). However, dark adaptation measurements on frog ganglion cells (Donner and Reuter, 1965; Baumann and Scheibner, 1968) appear to be at variance with equation (1), and it may be argued on theoretical grounds that the linear relation is an approximation that describes a limited (although extensive) portion of the dark adaptation curve (Wald et al., 1963; Weale, 1964). It seems appropriate, therefore, to make explicit some reservations concerning equation (1), and to consider in greater detail the experimental findings purported to be at odds with the linear function relating log threshold and rhodopsin concentration.

A LIMITING CONDITION   It is obvious that if the initial light-adapting exposure completely depletes the rods of their rhodopsin, the threshold must rise to infinity, whereas equation (1) predicts a moderately high, but finite, value (Wald et al., 1963). But if c must assume values > 0, it is necessary to provide some estimate of the smallest value of c to which equation (1) can be applied.

The fact that the threshold intensity approaches infinity when $c \rightarrow 0$ is merely another way of stating that light must be absorbed to be biologically effective. Consequently, the probability of quantal absorption sets *minimal* threshold levels for all values of c. For example, if the rhodopsin concentration is halved, the threshold must be raised *at least* by a factor of two ($\Delta \log I_t = 0.3$). And when 99.9% of the rhodopsin has been bleached ($c = 0.1\%$), the threshold must be *at least* 3 log units above the fully dark-adapted level. Thus, if the only factor influencing threshold were the reduced absorptivity associated with the loss of photopigment, the log I_t vs. c function would follow the dashed-line curve of Fig. 18.

Now by plotting on the same graph some of the experimental findings cited previously, it is apparent that none of these results is consistent with the notion that log threshold is a function solely of pigment absorptivity. The data points invariably lie well above the lower boundary set by such probability considerations, and within each experiment there is a linear relation between c and log I_t over the range of concentration tested. When these linear functions are extrapolated to lower values of c (dotted lines), we find that they would all intercept the dashed curve at concentrations $< 0.01\%$ (i.e., log $I_t > 4.0$). Therefore, this value may be taken to represent an approximate lower limit for which equation (1) is still compatible with the restrictions imposed by the nature of quantal absorption. From a practical standpoint, however, the lower

limit is rarely approached, since in all likelihood the preadapting exposure does not remove the full complement of rhodopsin from the receptors. Even in the isolated retina (where regeneration is not a significant factor) a small fraction of the bleached photopigment is probably restored by photic isomerization of photoproducts (Hubbard and Kropf, 1958), as well as by thermal processes (Cone and Brown, 1969).
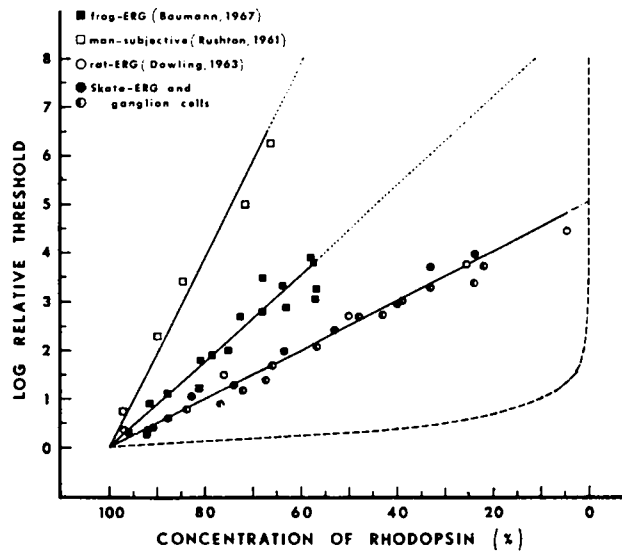

FIGURE 18. Log threshold as a function of rhodopsin concentration. Data points are from the experimental studies indicated, and have been adjusted along the scale of ordinates so as to equate the absolute threshold at log I = 0. The dashed-line curve represents the expected relation if threshold intensity varied inversely with the *in situ* concentration of rhodopsin.

THE SPECTRAL SENSITIVITY FUNCTION   Weale (1964) has correctly noted that equation (1) is incompatible with the equation linking the absorption spectrum of rhodopsin with the scotopic spectral sensitivity curve. The latter states that, at any wavelength, λ, the threshold intensity I_λ requires the absorption A_λ of a constant number of quanta reaching the retina:

$$1/I_\lambda = k_1 A_\lambda \tag{2}$$

And if the *in situ* density D_λ of visual pigment is small (<0.2), then:

$$A_\lambda \simeq 2.303 D_\lambda \tag{3}$$

But

$$D_\lambda = \alpha_\lambda c \delta \tag{4}$$

where α_λ is the extinction coefficient and c is the concentration of rhodopsin, and δ is the path length of absorbing material within the receptor. Substituting (3) and (4) in equation (2) we obtain:

$$1/I_\lambda = k_2 \alpha_\lambda c$$

or

$$\log I_\lambda = -\log c - \log \alpha_\lambda + k \qquad (5)$$

It is immediately apparent that equations (1) and (5) are fundamentally different, although both claim to predict the relation between threshold and pigment concentration. Nevertheless, each equation can be said to apply under the appropriate experimental conditions. Equation (5) describes the spectral variation in threshold, I_λ, at any *fixed* level of adaptation (or specific time in the course of dark adaptation), whereupon the concentration c is a *constant* and the variation in I_λ is a function of the extinction coefficient α_λ of the photopigment.

Equation (1), on the other hand, represents the changes in threshold at *different* levels of adaptation. Under rigorous experimental conditions, the wavelength of the test stimulus is fixed, and hence α_λ is a constant determined by the absorption spectrum of the pigment. Consequently, the variation in $\log I_t$ during dark adaptation is a function not of α_λ, but of the pigment concentration (or its optical density, since $D \sim c$). We do not know the mechanism by which the loss of photopigment causes a rise in threshold, but there is abundant evidence that a threshold response does not depend, in these circumstances, upon the absorption of a constant amount of incident light (cf. Fig. 18, and Barlow, 1964). Thus, bleached photopigment effectively sets a new threshold level in accordance with equation (1); and at any given level of sensitivity, the spectral variation in threshold can be obtained from equation (5). Moreover, the fact that the slope constant of equation (1) varies among different species (Fig. 18) suggests that differences in neural organization play an important role in determining the influence of c on $\log I_t$.

DARK ADAPTATION AND THE RATE OF RHODOPSIN REGENERATION   The results of recent studies (Donner and Reuter, 1965) on rhodopsin regeneration and ganglion cell activity in the excised eye of the frog have been interpreted to suggest that log threshold is related to the *rate* at which rhodopsin regenerates rather than to its concentration. In these experiments, Donner and Reuter showed that the dark adaptation curve of single units in frog consists of at least three branches: (*a*) an initial cone segment, (*b*) a mesopic portion during which both rods and cones presumably contribute to threshold (in accordance with their relative sensitivities to the test lights), and (*c*) a final rod branch. The latter begins after 100 min of dark adaptation, when more than 75% of the bleached rhodopsin has regenerated, and continues for another

hour and a half to complete dark adaptation. It is noteworthy that during the rod branch of dark adaptation there is clearly a linear relation between log threshold and the *in situ* concentration of rhodopsin (see Fig. 8 of Donner and Reuter, 1965).

But the rod branch represents less than half of the time required for dark adaptation, and the corresponding threshold change is only a little more than 1 log unit. However, when visual sensitivity is subserved by both rods and cones, it is sometimes possible to isolate the rod contribution by measuring thresholds with a test wavelength that stimulates selectively the scotopic mechanism. This is essentially what Donner and Reuter have attempted to do for the mesopic range of dark adaptation. They assumed that frog cones are maximally sensitive in the region of 560 nm (after Granit's [1942] photopic dominator function), whereas the red (rhodopsin) rods of the frog absorb maximally at 502 nm. On the basis of these spectral differences, analysis of their results suggested that by using a test stimulus of 503 nm, only the rhodopsin rods would contribute to thresholds measured after 40 min of dark adaptation. When these data were then corrected for sensitivity changes induced by the migration of melanin granules from the pigment epithelium (Bäck et al., 1965), a surprising result emerged: rod thresholds remained practically unchanged between 40 and 90 min into dark adaptation—a period during which frog rhodopsin regenerates most rapidly and at a nearly constant rate—to about 75% of its maximum concentration. Thus, if the threshold data represent rod "adaptation," it is necessary to conclude that thresholds are linearly related to the rate of rhodopsin regeneration; i.e., a constant rate (no matter how slow or fast) maintains threshold at a constant level.

We are aware of no other instance in which large changes in the concentration of photopigment fail to produce significant changes in visual sensitivity. In the skate, for example, when rhodopsin is regenerating as a linear function of time, log threshold is falling at the same rate (Fig. 12), and similar observations have been made on the rat retina (Dowling, 1960). Even when pigment is bleached under experimental conditions which preclude regeneration (i.e., the isolated, perfused retina), rhodopsin concentration and threshold are related in accordance with equation (1) (Weinstein et al., 1967).

There is, however, a more serious problem concerning the interpretation of Donner and Reuter's results. At the time Donner and Reuter put forward their hypothesis, they could not have known that the frog retina contains two different cone pigments, one of which (λ_{max} = 502 nm) is indistinguishable from the pigment of the red rods (Liebman and Entine, 1968). Thus, separating rod and cone activity by spectral selectivity is unlikely to be effective in the frog. It would seem, therefore, that the only period during which valid comparisons can be made between rhodopsin kinetics and rod thresholds in the frog is from 100–200 min of dark adaptation—the range over which Donner and Reuter's data show a linear relation between log I_t and c. In this con-

nection, it is important to note that Baumann and Scheibner (1968) have obtained results on frog ganglion cells which suggest that sensitivity rather than its logarithm is proportional to rhodopsin concentration (or to its absorptivity when the *in situ* density is high). While this conclusion is not seriously undermined by the scatter of their data, it is becoming increasingly evident, as indicated above, that distinguishing rod from cone signals in mixed retinas is more difficult than was previously thought.

The difficulty of extricating the electrical responses of the rod mechanism does not arise in connection with the results on the skate—which appears, both anatomically and functionally, to be an all-rod animal. Spectral sensitivity measurements on the dark- and light-adapted eye are equivalent, the retina is unresponsive to fast flicker, there is no evidence for a rod-cone kink in either dark adaptation or increment threshold functions, and cone-like receptors have not been detected histologically.

Light Adaptation and Increment Thresholds

Light adaptation of the scotopic mechanism is a prolonged process in the skate; after an initial period of complete insensitivity, it still required up to 20 min (depending on the intensity of the adapting field) for sensitivity to reach a stable level. Slow light adaptation may be a rather general characteristic of the rod system, for it is known to occur in human rods as well (Smith and Dimmick, 1957).

However, after the skate eye is adapted to various background luminances, the increment threshold measurements are unlike those obtained subjectively in man. For example, Aguilar and Stiles (1954), employing an ingenious method for measuring rod thresholds at photopic background levels, showed that the human rod mechanism saturates, i.e. $\log I_t \to \infty$, at background luminances which bleach less than 1% of the available rhodopsin (Weale, 1962). In the skate, on the other hand, a measurable response could eventually be obtained in the presence of a background which bleached more than 95% of the rhodopsin content (log background $= -0.4$ in Fig. 10), and similar increment threshold data have been reported for the rat ERG (Dowling, 1967). Moreover, results obtained on the hooded rat by psychophysical methods (Muntz et al., 1969), and on the *Aotes* monkey from recordings of the evoked cortical potential (Kaplan and Zaret, personal communication) also show that rod saturation does not occur at photopic (bleaching) luminances in these nocturnal species. These findings suggest that rod saturation may be unique to animals with mixed retinas. At first sight, it would appear that this view is not supported by the results obtained in rod monochromacy in human subjects (Fuortes et al., 1961), but there is evidence that these subjects are not entirely devoid of cone function (Siegel et al., 1966).

We have not observed the complete loss of the b-wave response during light

adaptation as described in other species of elasmobranch (Hamasaki and Bridges, 1965), although, as already mentioned, there was transient insensitivity during which the b-wave could not be elicited. However, it is important to emphasize that whenever the b-wave response was suppressed, so too was the ganglion cell discharge. This suppression phenomenon may be peculiar to fish, since it has been reported to occur in goldfish (Reynaud, 1969) but not in the retinas of terrestrial vertebrates.

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