S-Potentials in the Skate Retina

Intracellular recordings during light and dark adaptation

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ABSTRACT   The S-potentials recorded intracellularly from the all-rod retina of the skate probably arise from the large horizontal cells situated directly below the layer of receptors. These cells hyperpolarize in response to light, irrespective of stimulus wavelength, and the responses in photopic as well as scotopic conditions were found to be subserved by a single photopigment with $\lambda_{max} = 500$ nm. The process of adaptation was studied by recording simultaneously the threshold responses and membrane potentials of S-units during both light and dark adaptation. The findings indicate that the sensitivity of S-units, whether measured upon steady background fields or in the course of dark adaptation, exhibits changes similar to those demonstrated previously for the ERG b-wave and ganglion cell discharge. However, the membrane potential level of the S-unit and its sensitivity to photic stimulation varied independently for all the adapting conditions tested. It appears, therefore, that visual adaptation in the skate retina occurs before the S-unit is reached, i.e., at the receptors themselves.

INTRODUCTION

The S-potential is a slow, graded response to light, first recorded by Svaetichin (1953) from distal regions of the fish retina, and observed since in every class of vertebrate. Two types of S-potential have been described: luminosity or L-potentials are hyperpolarizing to light of any wavelength; chromatic or C-potentials may be either hyperpolarizing or depolarizing depending upon stimulus wavelength (MacNichol and Svaetichin, 1958). Although the mechanisms responsible for generating S-potentials have yet to be defined, there is now good evidence that the S-potentials arise from horizontal cells (Werblin and Dowling, 1969; Kaneko, 1970; Steinberg and Schmidt, 1970), and serve to mediate lateral interactions in the outer plexiform layer of the vertebrate retina (Werblin and Dowling, 1969; Kaneko, 1971; Naka, 1971; Baylor, Fuortes, and O'Bryan, 1971).

Some of the characteristics of the S-potentials, and their localization to elements of the distal retina, have prompted the suggestion that the S-units control adaptational changes in the retina (Mitarai et al., 1961; Rushton, 1962) and might function as an "adaptation pool"—a hypothetical regulatory station that receives its input from the receptors and establishes the signal size transmitted across the retina to the ganglion cells (Rushton, 1963). Although an attractive concept, there is little by way of experimental evidence to substantiate this view. For example, the behavior of L-units in the carp retina during the early stages of light and dark adaptation has been reported to bear no relation to the sensitivity changes characteristic of ganglion cell responses (Witkovsky, 1967). In addition, S-units appear to saturate when the retina is exposed to moderately bright adapting fields, i.e. superimposed flashes of any intensity are without effect (Witkovsky, 1967; Naka and Rushton, 1968). Ganglion cells, on the other hand, are responsive to incremental flashes at adapting levels well above those which produce saturation of the S-potential (Witkovsky, 1967).

It is important to note, however, that studies on the S-potential have been performed almost exclusively on animals having more than one receptor mechanism, where the relative contribution of each mechanism to the recorded response may vary significantly from one neuronal layer to the next (Witkovsky, 1967). In these circumstances, it is difficult to isolate the response properties of a given mechanism at different levels of observation (cf. Ripps and Weale, 1970). Furthermore, visual adaptation to both light and dark can be surprisingly slow (Dowling and Ripps, 1970), and it is no simple matter to hold a pipette intracellularly for the times required to establish thresholds.

The skate retina offers several advantages over other species in regard to many of the difficulties cited above. The retina is easily maintained in the eyecup for several hours, it appears to contain only rods, and it has a relatively uniform layer of extraordinarily large horizontal cells situated just below the receptor terminals (cf. Fig. 3 of Dowling and Ripps, 1970). We have previously reported the changes in sensitivity that occur in the electroretinographic b-wave and ganglion cell discharge during light and dark adaptation of the skate retina and correlated these changes with measurements of rhodopsin kinetics (Dowling and Ripps, 1970). The present paper describes the recording of S-potentials in this species, and compares their adaptation properties with those observed in more proximal responses.

MATERIALS AND METHODS

Small pieces of eyecup ($\sim$1 cm square), excised under dim red light from the tapetal region of a dark-adapted skate (either *Raja erinacea* or *R. oscellata*), were prepared and maintained as described previously (Dowling and Ripps, 1970). The tissue was placed on a Ringer-soaked cotton pad in contact with a chlorided silver plate that served as

the reference electrode. Glass micropipettes containing several glass fibers were drawn on a modified Livingston puller (Werblin and Dowling, 1969) and filled by injection with 2 M KCl (Tasaki et al., 1968); best results were obtained with pipettes having resistances of 35–75 megohms. The micropipettes were mounted on a hydraulic microdrive (David Kopf Instruments, Tujunga, Calif.) featuring a remote, pulsed stepping motor which enabled depth adjustment of the electrode in steps of 1 μ. Signals were led through the salt bridge to a Ag/AgCl electrode connected to the input stage of a Bak ELSA-4 negative-capacitance amplifier (Electronics for Life Sciences, Rockville, Md.). The responses were displayed on a Tektronix Model 502 oscilloscope (Tektronix Inc., Beaverton, Oreg.) and recorded on a Honeywell Model 1508A Visicorder (Honeywell Inc., Denver, Col.); the latter employs a fluid-damped mirror galvanometer with a flat frequency response ($\pm 5\%$) from DC-2000 Hz.

The Optical System   Light was projected onto the preparation from a photostimulator (Fig. 1) consisting of two optically equivalent pathways that provided independent control of "test" and "adapting" fields. The light sources S, were 12 v, 100 w concentrated filament, tungsten-halogen lamps (Philips 7023) connected in series to a current-stabilized DC supply and operated slightly below rated amperage to prolong lamp life. The apparent blackbody temperature of the lamps was measured with an optical pyrometer, and the value corrected for tungsten emission (Harnwell and Livingood, 1933); this gave a true temperature of 2750°K. Both sources were filtered through BG 38 and KG 1 heat filters H to eliminate completely ($D > 10^5$) infrared radiation.

Condensing lenses, C, formed reduced images of the filaments in the plane of electromagnetic shutters T. The beams were then collimated by lenses, L_1, and refocused by lenses, L_2, at a pellicle beam splitter, P, which made the two optical paths collinear. Lens, L_3, formed superimposed images of the filaments at the surface of a
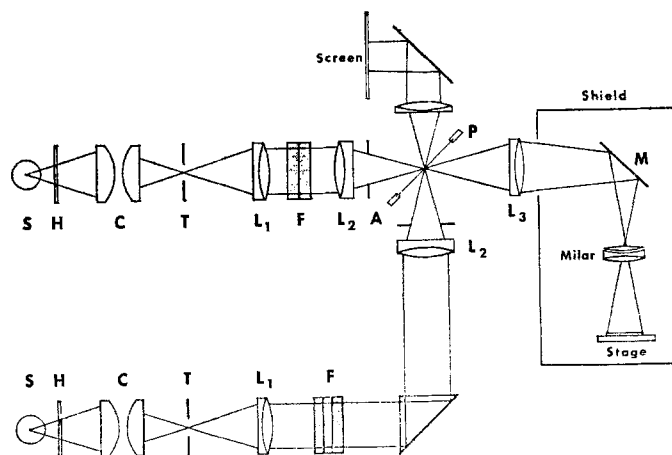

FIGURE 1.   Schematic view of the dual-beam photostimulator. Test and adapting fields are defined by apertures at A, and focused on the preparation which is mounted on the microscope stage. See text for details.

65 mm Milar objective (E. Leitz, Rockleigh, N. J.) after reflection from mirror M. The stimulus fields could be varied in size by apertures inserted at A, the plane conjugate with the retina, and positioned with respect to the recording electrode by means of X-Y micrometer drives. Neutral density and interference filters at F controlled the intensity and spectral composition of the stimuli. In order to exclude ambient illumination and reduce electrical interference, the final elements of the optical system, together with the preparation, viewing microscope, micromanipulator, and amplifier probe were housed in a shielded enclosure. However, the two beams could be monitored externally on a calibrated screen which received enlarged images of the stimulus fields.

Calibration   The irradiance in the plane of the retina was measured with a thermopile and microammeter. Unattenuated, the energy flux delivered by the test and adapting field was 1.22 mw/cm^2 and 1.71 mw/cm^2, respectively. Spectral sensitivity data were obtained by determining thresholds for 10 spectral regions isolated by narrow-band interference filters (half-band width = ± 4 nm). The relative energy E_λ transmitted by the filters was measured with a calibrated photomultiplier, and the reciprocal of log E_λ that produced a criterion S-potential was used in the determinations of quantum sensitivity plotted in Fig. 10. Flash irradiation preceded some experiments on the course of dark adaptation. For these experiments, a heat-filtered xenon discharge lamp (Strobonar 65D, Honeywell, Inc., Denver, Col.) was used to light adapt the retina; a single flash ($t \approx 0.8$ msec) bleached 75–82% of the available rhodopsin in the test region (Dowling and Ripps, 1970).

Recording the S-Potential   In the skate eyecup, depth measurements did not provide a wholly satisfactory method for locating the source of S-potentials, since micrometer readings of the distance between the edge of the retina and entry into S-units varied from 75–105 μ. Several factors probably contributed to this variability; e.g., deformation of the retina by the electrode, the angle of the pipette with respect to the retina, settling of the preparation into the moist cotton on which it rested, and changes in retinal thickness due to dehydration. However, advancing a micropipette through the dark-adapted retina from the vitreal side produced a fairly consistent sequence of events that aided intracellular placement of the electrode in S-units. At the surface of the retina, a small (1–2 mv) positive b-wave was invariably observed in response to a moderately dim light flash that illuminated most of the retina. After penetrating the internal limiting membrane, the pipette occasionally entered ganglion cells, most of which discharged spikes spontaneously. Beyond the ganglion cell layer, the electrode passed through a region (presumably the inner plexiform layer) in which only the b-wave was detectable, and then to a deeper (inner nuclear ?) layer where shifts in DC potential indicated the penetration of cell membranes. Many of these cells gave no light response; others responded with slow potentials, no larger than 5–10 mv, of either polarity. S-units were usually encountered by advancing the pipette an additional 10–15 μ.

Resting potentials of S-units in the skate were typically 20–35 mv, inside negative. In response to light, there was a further negative shift in transmembrane potential, bright stimuli eliciting S-potentials with maximal amplitudes of 25–55 mv. The responses were of the L-type only, i.e. hyperpolarizing regardless of stimulus wave-

length, and were extremely stable for intracellular recordings in the vertebrate retina. We could often record from a cell for 40–50 min, and on occasion have held a single unit for over 90 min.

RESULTS

Responses in the Dark-Adapted Retina

THE EFFECT OF INTENSITY ON RESPONSE AMPLITUDE   Fig. 2 shows a series of S-potentials elicited by 0.2 sec light flashes of increasing intensity. In this and most other preparations, a threshold response (1 mv) to a 4 mm test spot centered on the recording electrode was obtained with a retinal illumin-ance of about 3.86×10^{-9} mw/cm^2; i.e., 8.5 log units below full intensity of the stimulus field. As the stimulus intensity is raised, the response latency
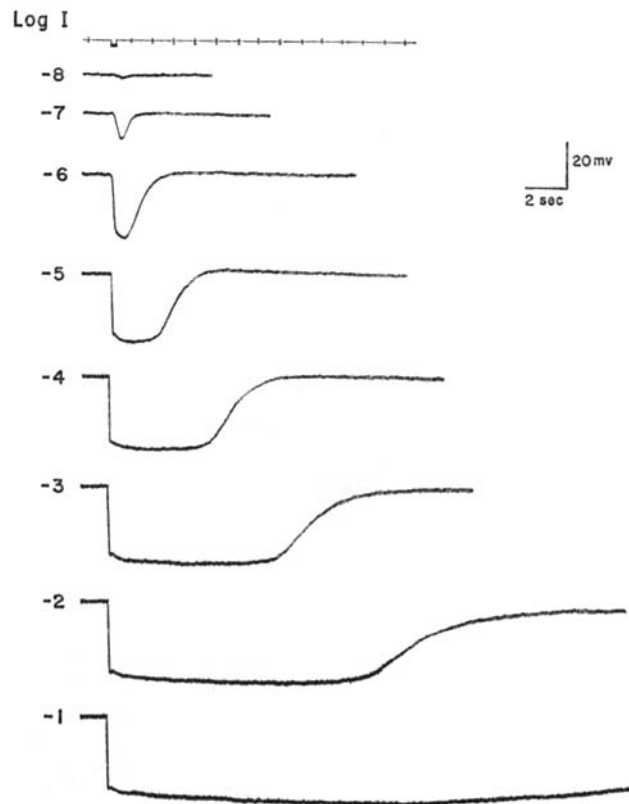

FIGURE 2.   S-potentials recorded from the dark-adapted skate retina in response to 0.2 sec test flashes. With increasing stimulus intensity the responses grow in amplitude, and their duration increases. In this and subsequent figures a downward deflection indicates negativity of the active electrode, and the log I values refer to the densities of neutral filter interposed in the test beam; unattenuated, the latter produced a retinal irradiance of 1.22 mw/cm^2.

decreases, while its duration and amplitude increase. The growth in amplitude is rapid at first, and then approaches asymptotically a maximal value ("saturation") with intensities about 4.0 log units above threshold (cf. Fig. 3). Except for very dim flashes (−8, −7), the potentials are clearly asymmetrical, exhibiting a fast onset and a much slower decay phase. Of particular interest are the prolonged period of hyperpolarization and extension of the decay phase when the stimulus intensity exceeds the saturation level.

A number of authors (cf. Naka and Rushton, 1966; Glantz, 1968; Naka, 1969 a; Steinberg, 1969; Baylor and Fuortes, 1970) have reported that the voltage vs. intensity (V–log I) functions for the graded responses of S-units and receptors can be described by a nonlinear equation of the form:

$$V/V_{max} = I^a/(I^a + K) \tag{1}$$

where V is the response amplitude evoked by light of intensity I; V_{max} is the saturation potential; and the constants a and K determine, respectively, the slope of the function and its position on the abscissa.

Although voltage functions were not systematically examined in the present study, V–log I data were culled from eight experiments in which recordings were obtained over a range of intensities sufficient to elicit the full gamut of responses, from threshold to saturation. The averaged results of these experiments are plotted as open circles in Fig. 3 where the continuous curve represents equation (1) for $a = 0.7$, $K = 1.2 \times 10^{-5}$ millijoules/cm². The dashed-line curve is a graph of equation (1) when $a = 1.0$, the value obtained by Naka and Rushton (1966) for cone-driven L-units in the tench retina, and by Baylor and Fuortes (1970) for intracellular responses of turtle cones. It would appear that the scotopic S-units of skate respond to a broader
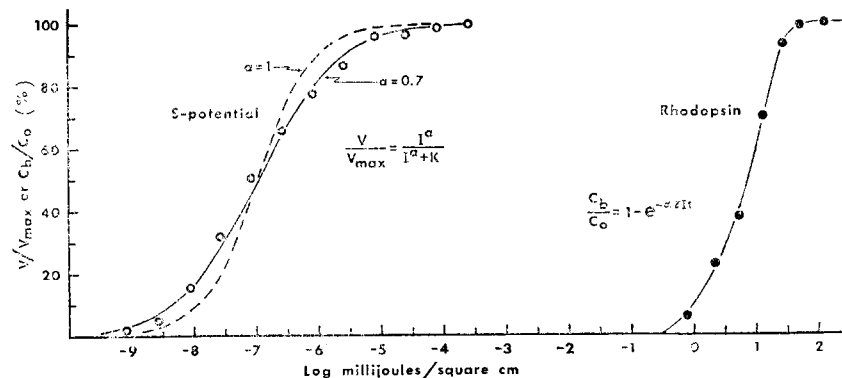

FIGURE 3. The effect of light quantity on S-potential amplitudes and the bleaching of rhodopsin in the skate retina. Changes in voltage and concentration are expressed as percentages of their respective maxima. S-potential results were obtained with a 4 mm test field, 0.2 sec flash duration, and represent the average of eight experimental runs; values of a for the individual V–log I curves ranged from 0.6 to 0.85.

range of intensities than is true for the cone system in other vertebrates. However, it should be noted that Boynton and Whitten (1970) reported recently that a assumed values of 0.7–0.73 for V–log I curves derived from late receptor potentials in the monkey fovea.

A simple computation shows that the amount of light required to saturate the S-unit is *far* below that which effects a significant reduction in photopigment concentration. However, the relative quantities involved can be obtained directly by comparing the V–log I curve with the function describing the bleaching of rhodopsin in the skate retina derived from fundus reflection measurements (Dowling and Ripps, 1970). In order to facilitate the comparison between electrical responses to brief flashes 0.2 sec in duration, and density changes produced by 30 sec exposures, the retinal irradiances (mw/cm²) of the separate studies have been time integrated to give equivalent units (millijoules/cm²) for the scale of the abscissae. When plotted (Fig. 3) as the fraction of pigment bleached C_b/C_o, the photochemical results (solid circles) follow closely the exponential relation $C_b/C_o = 1$-exp $(-\alpha\gamma It)$. Since 1 millijoule/cm² $\approx 1.6 \times 10^6$ scotopic troland-sec, the photosensitivity $\alpha\gamma$ of skate rhodopsin is found to be $10^{-7.2}$ scot td sec^{-1}, very nearly the same as that obtained from in vivo measurements on human rhodopsin (Ripps and Weale, 1969). Moreover, it is immediately apparent that the whole of the S-potential function is generated by radiant exposures that bleach trivial fractions ($<0.1\%$) of the visual pigment in the test area. From measurements on the influence of stimulus area (cf. Fig. 4), it is clear that reducing the size of the test field will cause the V–log I curve to be shifted to the right on the abscissa. But even with a test spot 0.25 mm in diameter, the displacement would amount to less than 3 log units; i.e., the voltage function does not encroach upon the bleaching curve. However, stray light effects (see below) precluded accurate determinations of the minimum stimulus diameter capable of generating the full range of S-potentials.

RECEPTIVE FIELDS The marked dependency of response amplitude on the area of stimulation is considered generally to be characteristic of S-potentials (Tomita et al., 1958; Gouras, 1960; Norton et al., 1968). Indeed, this feature is quite useful in distinguishing S-potentials from the graded, hyperpolarizing responses of vertebrate photoreceptors which do not exhibit a substantial area effect (Tomita, 1965; Werblin and Dowling, 1969; Kaneko, 1971; but compare Baylor et al., 1971). The large extent of the receptive fields for S-units in the skate retina was demonstrated in two ways. In the first (Fig. 4, left), stimulus intensity was held constant, and the area of the stimulus field was increased symmetrically about the recording electrode. Using bright test flashes (log $I = -4$), small increments in field size produced large increases in amplitude, so that the potential rapidly approached its saturation level (-35 mv). In these circumstances, the field size above which
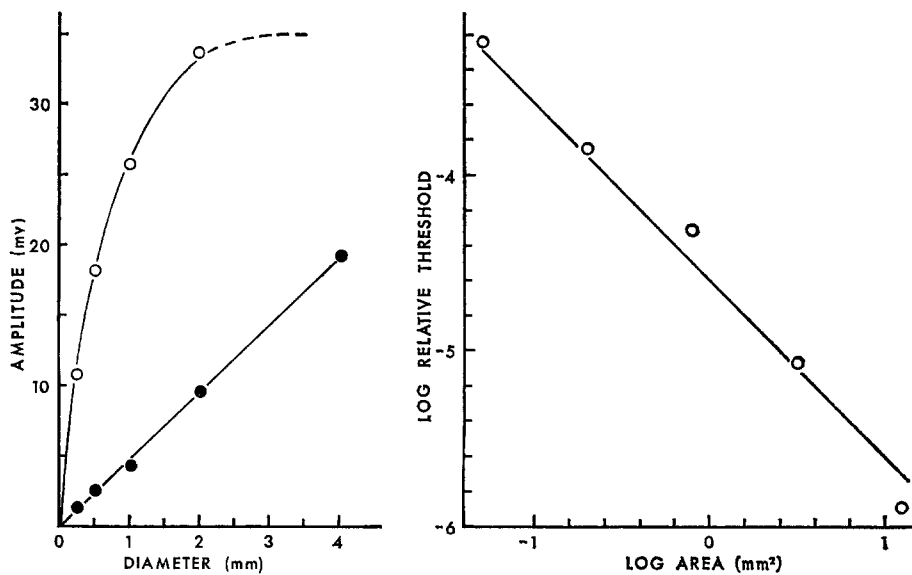
FIGURE 4. The effect of stimulus size on the S-potential. On the left, response amplitude is shown as a function of stimulus diameter with constant-intensity stimuli: open symbols, $\log I = -4$; solid symbols, $\log I = -6$. On the right is the area-intensity function derived for a response criterion of -20 mv.
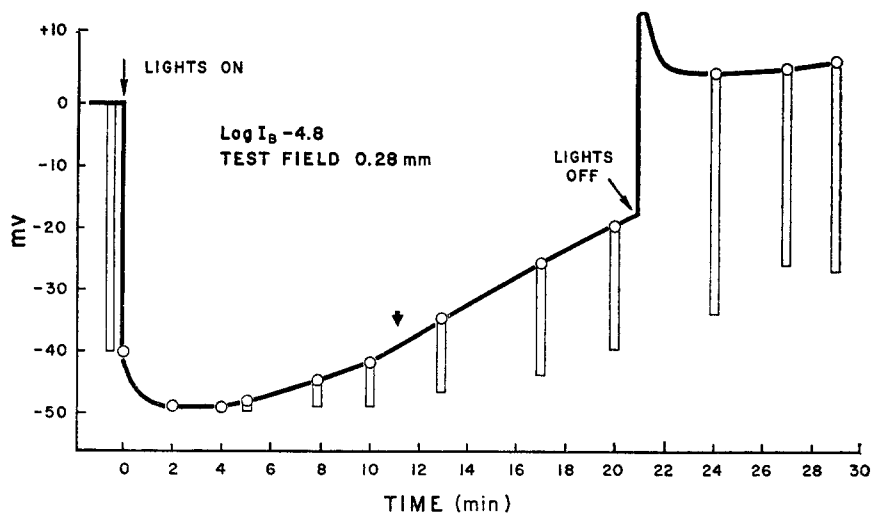

FIGURE 5. The effects of light and dark adaptation on an S-unit. The retina was exposed to a background light of saturating intensity at time zero, and the adapting field was extinguished after 21 min of light adaptation. The heavy line indicates the membrane potential during the course of the experiment; dark-adapted (resting) potential is plotted as zero on the scale of ordinates. The open bars represent the maximum S-potential amplitudes that could be elicited at various times, beginning with the maximum response of the dark-adapted unit. Note that during the first 4 min of light adaptation, the S-potential was saturated, and our brightest test flashes were without effect. Subsequently, the membrane potential moved towards the resting level, and superimposed flashes elicited incremental responses of increasing amplitude. The heavy arrow at 11 min of light adaptation shows the time at which a stable increment threshold was attained (see Fig. 6). Further details in text.

there was no further increase in potential appeared to be about 3 mm in diameter. But as Gouras (1960) has convincingly argued, the value obtained with bright test flashes must represent a lower limit to the size of the receptive field, since much of the response is probably due to stray light falling outside the borders of the test field; elimination of nonfocal stray light can only increase this value. Consequently, when the procedure was repeated with the stimulus intensity reduced 100-fold (log I = −6), there was a gradual increase in potential over the entire 4 mm area tested, and there was no indication that this represents the full extent of the receptive field.

The results of a second experiment, in which the organization of the receptive field was examined, also showed that an extremely large retinal area contributes to the S-potential in skate. A series of V–log I functions were recorded at each of several test field diameters, and the intensities required to evoke a constant-amplitude response read from the curves. The results, plotted in Fig. 4, right, show that Ricco's law (area × intensity = C) holds for stimulus diameters from 0.25 to 4.0 mm. Furthermore, as there is no sign of a departure from the reciprocal relation between stimulus area and intensity, it is likely that the receptive field over which complete summation occurs is considerably larger than 4.0 mm.

Light Adaptation

THE EXPERIMENTAL PROTOCOL Our findings on the adaptation properties of S-units in the skate retina are most conveniently presented by first describing the kinds of information obtained in a typical experimental run. Results, such as those illustrated in Fig. 5, provide data on: (a) the behavior of the membrane potential (solid line) during exposure to a steady background; (b) maximum S-potential amplitudes (rectangular bars) in response to a bright test flash delivered before, and at the times indicated after, onset of the adapting field; (c) the membrane potential and response amplitude after extinguishing the background; and (d) threshold changes during the course of light and dark adaptation (Fig. 6).

The adapting field in this experiment illuminated the entire retina, and its intensity, log I_B = − 4.8, was at the saturation level of this S-unit (cf. Fig. 14); the test field was 0.28 mm in diameter and centered on the recording electrode. The dark-adapted resting potential of the unit (−25 mv) is indicated as zero on the millivolt scale, and the maximum S-potential amplitude obtained in the dark-adapted state (−40 mv) is shown by the initial bar in Fig. 5. When the adapting light was turned on (time zero), the transmembrane potential immediately went 40 mv negative, and drifted further downward over the next minute and a half to −48 mv where it remained for the next 4 min. During this time the S-unit was "saturated," in the sense that superimposed flashes of maximal intensity (i.e., 10^8 above dark-adapted threshold) were totally ineffective in eliciting a response.

After 5 min of light adaptation, however, the membrane potential began to return slowly toward the dark level, and light-evoked responses of low amplitude (1-2 mv) could be recorded. The membrane potential continued to rise for the duration of the light-adapting period (21 min), during which time the maximum light-evoked potentials grew appreciably larger (up to — 20 mv). When the background light was extinguished, the membrane potential jumped up instantaneously and overshot the dark-adapted potential, but returned close to the resting level within 2 min of dark adaptation. Subsequent recordings of the maximum amplitude responses were somewhat variable, but they were nearly as large as those obtained in the dark-adapted retina.

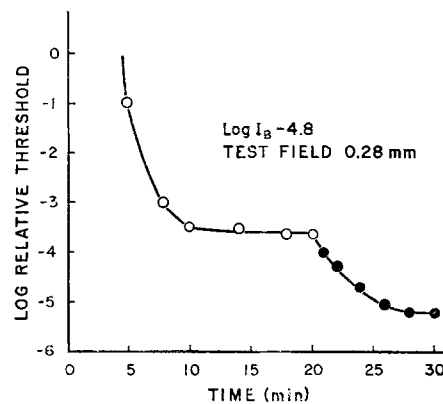Threshold measurements made during the course of this experiment are

FIGURE 6.   Thresholds of the S-potential measured during the experiment illustrated in Fig. 5. After the saturation period, thresholds in the light (open circles) fell rapidly to a stable level (the final increment threshold) about 1.5 log units above the dark-adapted value. Recovery of sensitivity during dark adaptation (solid circles) required 8-10 min.

shown in Fig. 6; a response of 1 mv was easily detectable and therefore used as the criterion for threshold. As noted previously, a response from an S-unit could not be elicited during the initial stages of the light adaptation period corresponding to the period of S-unit saturation. After 5 min of light adaptation, when the unit again became responsive, the threshold was elevated about 5 log units above its dark-adapted threshold. However, within the next 5 min, the increment threshold fell rapidly over nearly 3 log units to a level that was maintained until the light was extinguished. In darkness, the S-unit regained dark-adapted sensitivity in 8-10 min.

Another example of the effects of light adaptation on an S-unit is shown in the recordings of Fig. 7. Although the stimulus parameters differ from those of the previous experiment (see figure legend for details), some of the significant features described above are well illustrated. For example, the large amplitude responses recorded in the dark-adapted preparation were com-
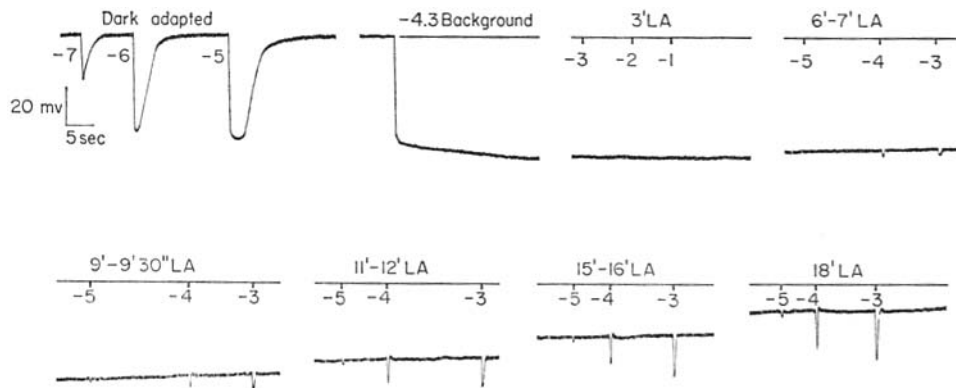
FIGURE 7.   S-potentials elicited from the dark-adapted preparation, and at various times during the course of light adaptation (log I_B = -4.3). The intensities of the test flashes (0.2 sec in duration, 4.0 mm in diameter) are indicated along a line representing the resting potential of the dark-adapted unit. Onset of the background saturates the S-unit to a voltage level that is maintained in the record taken after 3 min of light adaptation. Each succeeding time segment shows the recovery of the membrane potential and the increase in response amplitudes to a series of test flashes. Note that the responses to a bright flash (log I = -3) increase in light adaptation more rapidly than do the responses to a dim flash (log I = -5).

pletely suppressed by the onset of an adapting field that saturates the S-potential, and the unit remained unresponsive for more than 5 min. But in each succeeding time segment, the membrane potential was observed closer to the resting level, and response amplitudes grew larger. It is important to note, however, that the responses to the weaker stimulus (log I = -5) did not increase in the same proportion as those evoked by brighter flashes; hence, threshold remained relatively unchanged after approximately 15 min of light adaptation in spite of a continued rise of membrane potential toward the dark-adapted level.

THE WAVE FORM OF THE S-POTENTIAL AND SPECTRAL SENSITIVITY   Another interesting feature of the recordings in Fig. 7 is the change in wave form which the S-potential undergoes as a result of light adaptation. This phenomenon is shown in greater detail in Fig. 8, where S-potentials recorded after 45 min of light adaptation to a moderately bright background (log I_B = -3.8) are compared with responses obtained from the dark-adapted preparation. Each series begins near threshold intensity, which is about 3 log units higher after this degree of light adaptation. But even in its threshold activity, the light-adapted response exhibits faster "on" and "off" components. As flash intensity is increased, the differences in wave form become more obvious; brighter stimuli (-4, -3) bring about an inflection in the decay phase of the response, and cause the sharp "off" component to over-

shoot the unit's light-adapted resting potential. Once the light-adapted response has reached its saturation level, further increases in intensity (-2, -1) act, as on the dark-adapted unit, to extend the duration of the response and the time required to recover to the resting level.

It has been suggested that the change in response wave form induced by light adaptation reflects a change in input to the S-unit, i.e. a switch from rod dominance in the dark-adapted state, to a predominantly cone input in
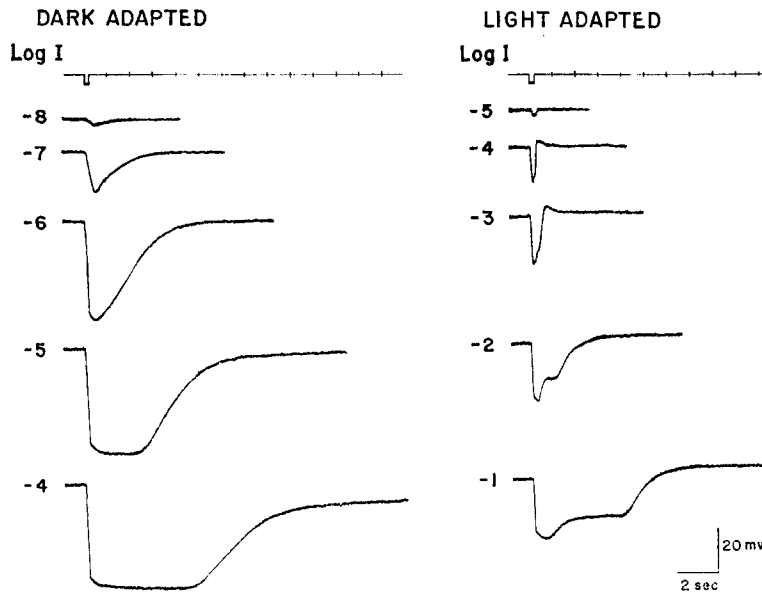

FIGURE 8.   S-potentials recorded from the dark- and light-adapted retina; flash duration was 0.2 sec. Recordings in the light-adapted state were taken after about 40 min exposure to log I_B = -3.8. The wave forms of the light-adapted responses are generally more complex; they exhibit an early transient component, decay more rapidly, and the "off" phase often overshoots the base line.

the light-adapted retina (Brown and Murakami, 1968). This interpretation is not without merit; S-units are almost certainly driven by receptors, and the late receptor potential of the cone system (e.g. in cat and monkey) decays rapidly, while that of the rods follows a slow time course (cf. Brown et al., 1965). But the situation cannot be quite so simple, because the tench L-potentials, which also change form in light adaptation (Naka, 1969 b), seem to receive information only from cones (Naka and Rushton, 1966), and our histological observations make it appear unlikely that the skate retina has any cones at all (Dowling and Ripps, 1970). Nevertheless, we have tried to determine whether one or more types of receptor are involved in the adaptation process by comparing spectral sensitivity functions of the dark- and

light-adapted retina. A moderately bright background (log I_B = −3.8) was employed for light adaptation, and 20 min of continuous exposure preceded the measurements.

For each of 11 test wavelengths (from 420 to 620 nm), S-potentials were elicited over a range of intensities sufficient to generate the low end of the
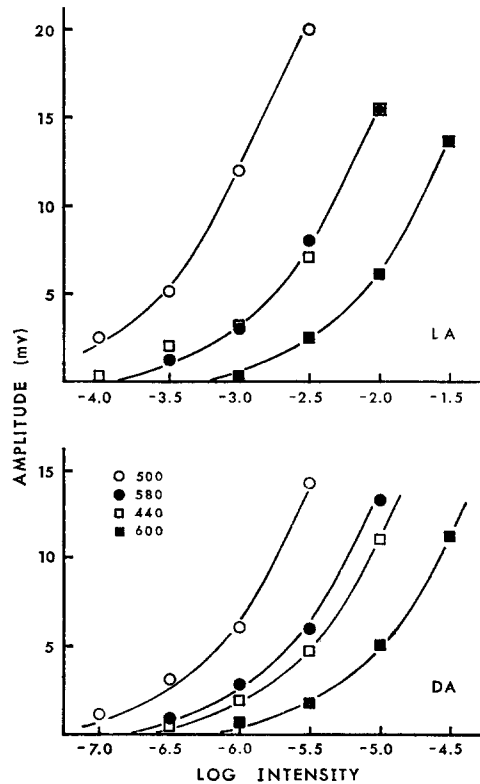

FIGURE 9. Voltage-intensity data at several test wavelengths for S-potentials recorded in the dark- and light-adapted (log I_B = −3.8) retina. Note that light adaptation shifts the V–log I functions along the log I scale (compare LA with DA for a given wavelength), but does not alter their shapes; i.e., all curves were constructed from a single template. These curves are the raw data, uncorrected for filter transmissivity or emission characteristics of the source.

V–log I function. Responses to a "standard" stimulus flash were recorded before and after each spectral series to ensure stability of the cell's response properties throughout the experimental run. Peak amplitudes were plotted as a function of log intensity for each wavelength (Fig. 9), and the relative energy to attain a criterion response of 5 mv was determined from the curves (after correcting for the spectral transmissivity of the filters and emission characteristics of the lamp). The results, converted to relative quantum flux,

and expressed as its reciprocal, i.e. log relative quantum sensitivity, are shown in Fig. 10. There is clearly no change in spectral sensitivity as a consequence of light adaptation; both sets of data match very well a rhodopsin nomogram curve with λ_{max} = 500 nm (Dartnall, 1953). That this pigment is, in fact, the light-sensitive substance in the skate retina has been demonstrated previously (Dowling and Ripps, 1970).

We have collected spectral sensitivity data on nine preparations, only two of which were run at all wavelengths. However, the results obtained were in every instance in good agreement with those shown in Figs. 9 and 10. Thus,
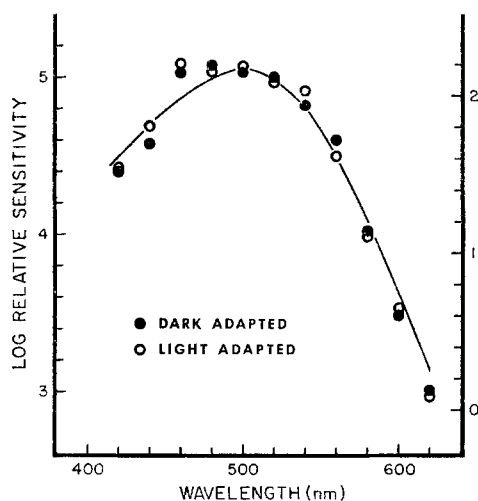

FIGURE 10. Spectral sensitivity of the S-potential in the dark-adapted retina (solid symbols and ordinates on the left) and after light adaptation to log I_B = −3.8 (open symbols and ordinate scale on the right). The continuous curve was constructed from the Dartnall nomogram for a rhodopsin pigment with λ_{max} = 500 nm. Light adaptation decreased sensitivity by about 3 log units, but did not induce a Purkinje shift.

light adaptation in skate affects the lateral position of the V–log I curve (cf. Naka, 1969 a; Boynton and Whitten, 1970; and Fig. 9), but not its shape or the spectral location of maximum sensitivity (Purkinje effect). It appears, therefore, that S-units in the skate receive signals from receptors having but one spectrally distinct photopigment.

SATURATION OF THE S-POTENTIAL   It is generally thought that the S-potential is a *sustained* response to illumination (Svaetichin, 1953; Witkovsky, 1967; Naka and Rushton, 1968). In the cyprinid retina, for example, Naka and Rushton (1968) found that a bright adapting light *clamped* the S-potential to a fixed level of hyperpolarization (saturation). But we have already seen that this observation does not adequately describe the dynamic properties of S-units in the skate. Saturation of the S-potential is clearly a temporary con-

dition, even when the illumination producing saturation is maintained (Figs. 5 and 7). It is true, of course, that when the S-unit has reached the limit of its capacity to hyperpolarize, it becomes completely incapable of responding to the arrival of additional signals (i.e., threshold $\rightarrow$ ∞), and this situation may hold for many minutes (the "silent" period). But at some point in the course of light adaptation, the membrane potential begins to march gradually back towards the resting level, and the S-unit becomes responsive to stimulus flashes superimposed on the background field.

Since we will have occasion to refer to this recovery phenomenon again, it is important to establish that the factors producing it are not simply mechanical in nature, e.g., withdrawal of the pipette from the S-unit, or disruption of the cell membrane. The following points argue against a mechanical origin. Whenever a microelectrode became dislodged from an S-unit or was intentionally withdrawn, the polarization level moved invariably in the positive direction, but light-evoked responses always *decreased* in amplitude; the less negative the membrane potential, the smaller were the responses. This is precisely the reverse of what is observed in the course of light adaptation. Furthermore, when a unit was lost, for whatever reason, extinguishing the adapting field had no effect on the prevailing potential, not did stimulus flashes delivered in dark adaptation. On the other hand, with a viable unit in which the electrode maintained its position, the membrane potential responded quickly to the offset of the light, and responses recorded subsequently in the dark were similar in amplitude to those recorded before the start of light adaptation (Fig. 5).

THE TEMPORAL COURSE OF LIGHT ADAPTATION   Although Figs. 5 through 7 present a fairly descriptive picture of the light adaptation process, the experiments of Fig. 11 serve to illustrate the effects of increasing background intensity on the various response parameters. Thus, with the dimmer adapting field (log I_B = $-$ 4.8), the S-unit remained saturated (and unresponsive) for only about 2 min, whereas a 10-fold increase in background intensity (log I_B = -3.8) saturated the potential for more than 10 min. Although clearly related to I_B, the duration of the silent period induced by a given background intensity was somewhat variable between experiments (compare Figs. 5 and 11). Another puzzling finding concerns the saturation level of the S-unit. The vertical bars to the left of the curves in Figs. 5 and 11 represent the maximum light-evoked response of the dark-adapted unit to a 0.2 sec flash. But in every instance, we have found that during exposure to a sustained adapting field, the floor of hyperpolarization drifts further down for as long as 2 min after the onset of the background. Thus, a brief flash, no matter how bright, could not hyperpolarize the S-unit to the depths reached during prolonged exposures.

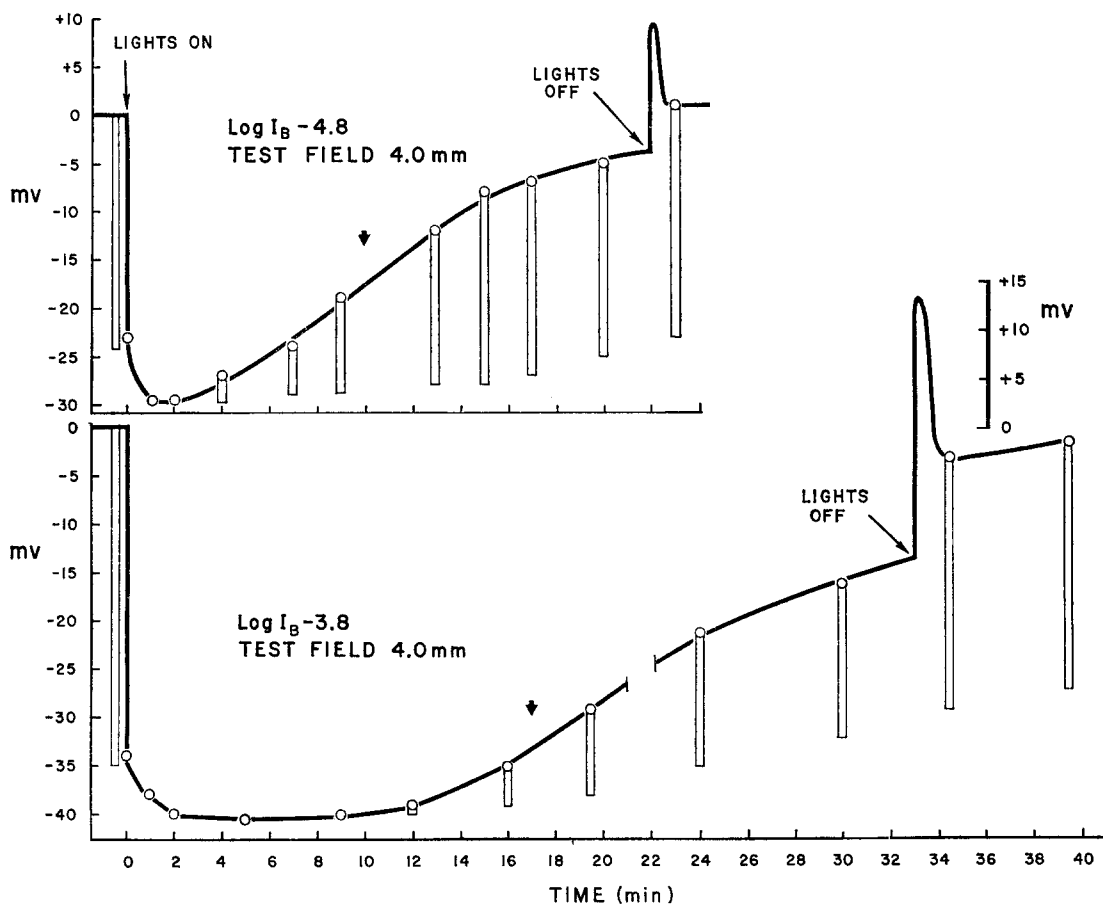After the period of S-potential saturation, there was the gradual membrane

FIGURE 11.   S-potentials and transmembrane voltage levels in the course of light and dark adaptation. The data were obtained at two background intensities (log I_B = −4.8 and −3.8), both of which produced an initial saturation of the S-unit. Note that with the brighter adapting field the saturation period is longer, the recovery of membrane potential is slower, and a longer time is required to attain a stable increment threshold. The unit was lost momentarily between 21 and 22 min of light adaptation (indicated by a break in the record). See caption to Fig. 5 for key to symbols.

recovery which, given sufficient time, brought the voltage level to within a few (3–5) millivolts of the resting value. Although this process appeared to continue for as long as the background light shone on the preparation, the recovery slowed with time and we cannot say with certainty whether a light-adapted unit is capable of recovering *completely* the membrane potential of its dark-adapted state.

Threshold measurements taken in the course of light adaptation are shown in Fig. 12 (open circles). The resultant curves, like that of Fig. 6, are in remarkably good agreement with those derived from threshold data on the
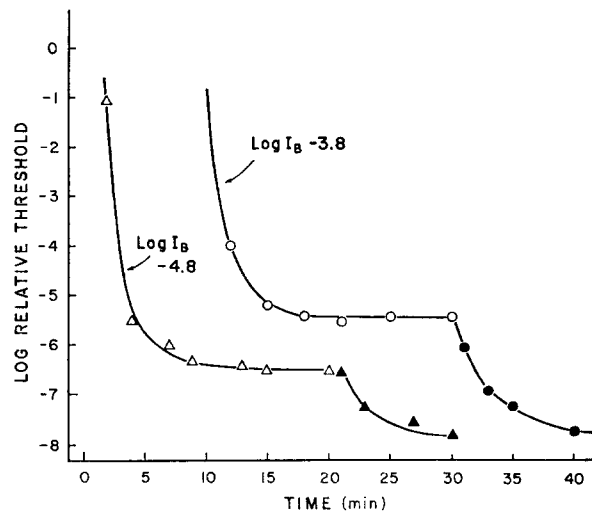
FIGURE 12. Threshold measurements on the S-potential in the course of the experiments illustrated in Fig. 11. The extended saturation period induced by the brighter background (log I_B = -3.8) is reflected in the long time interval during which thresholds were indeterminate. Note also that the final increment threshold level (plateau) was reached sooner, and the threshold was lower with the weaker adapting field. Since the backgrounds bleached insignificant fractions of the available rhodopsin, dark adaptation (solid symbols) progressed rapidly and was completed in 8–10 min.
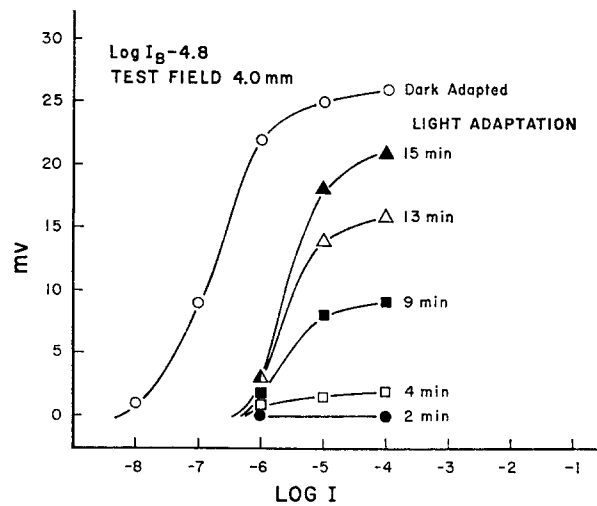

FIGURE 13. V–log I data obtained from a dark-adapted retina, and in the course of light adaptation (log I_B = -4.8). 2 min after the onset of the adapting field the S-unit was still saturated, and superimposed flashes did not evoke a response (solid circles). After 4 min of light adaptation, small responses were elicited, but only with test flashes 2 log units above dark-adapted threshold. As light adaptation proceeded, the V–log I curve became steeper, but threshold was relatively unchanged and the curves shifted only slightly along the abscissa.

b-wave and ganglion cell discharge during light adaptation to comparable luminance levels (Figs. 13 and 15 of Dowling and Ripps, 1970). In every case there is a silent period at first, corresponding to saturation of the S-unit, and during which time thresholds cannot be determined. When the unit is again responsive, thresholds fall rapidly to a plateau that is maintained for the duration of light adaptation. In general, the brighter the adapting field, the longer the period of insensitivity and the higher the incremental threshold plateau. Furthermore, comparison of Figs. 11 and 12 reveals that the plateau is reached for both units long before the recovery of membrane potential is complete; i.e., over most of its course, changes in sensitivity of the light-adapted S-unit appear to be entirely unrelated to the changes in its membrane potential.

We alluded earlier to the fact that whereas suprathreshold responses grow more or less continually as membrane negativity decreases, the incremental threshold plateau is established early in the course of light adaptation (Fig. 7). This is evident also in Fig. 11, where the arrows indicate the times at which the increment thresholds stabilized as compared with membrane levels and response amplitudes. Thr manner in which this surprising result comes about is illustrated in Fig. 13, where V–log I data were obtained in the course of light adaptation. Since the adapting field saturated the S-unit for about 3 min, responses could not be recorded earlier. At 4 min, however, small amplitude S-potentials were elicited; but the change in amplitude as a function of intensity was small (cf. Fig. 7), and the threshold had already dropped to within 2.5 log units of the dark-adapted value. The slope of the V–log I function increased sharply after 9 min of light adaptation, but the curve was shifted only a little to the left along the log I scale, reflecting the small drop in threshold that occurred between 4 and 9 min of light adaptation. Although the V–log I curve became steeper with further light adaptation, thresholds reduced only slightly before stabilizing at about 1.7 log units above the dark-adapted value.

THE INCREMENT THRESHOLD   It is apparent from the preceding sections that the unique capacity of the S-unit to "recover" from its saturation level enables one to determine increment thresholds in the presence of adapting fields that initially saturate the S-potential. In fact, increment thresholds can be measured upon backgrounds that bleach more than 95% of the available rhodopsin; e.g., when a background illuminance $I_B = -1.8$ is maintained for more than 20 min.

We had found previously, in recording light-adapted b-wave and ganglion cell responses of the skate (Dowling and Ripps, 1970), that if the background field bleached significant amounts of visual pigment it was necessary to wait

as much as 40 min for the increment threshold to stabilize. This was our experience with the skate S-potentials. Accordingly, at every background level tested, sufficient time was given to achieve a stable increment threshold (plateau); these are the Δi values plotted in the right half of Fig. 14. The left side of the figure presents the V-log I data when the background, acting as the test field, was flashed on. Clearly, saturation of the S-potential amplitude occurred when log $I_B \geqslant -4.8$; but increment thresholds could be measured on background fields *10,000 times more intense.*
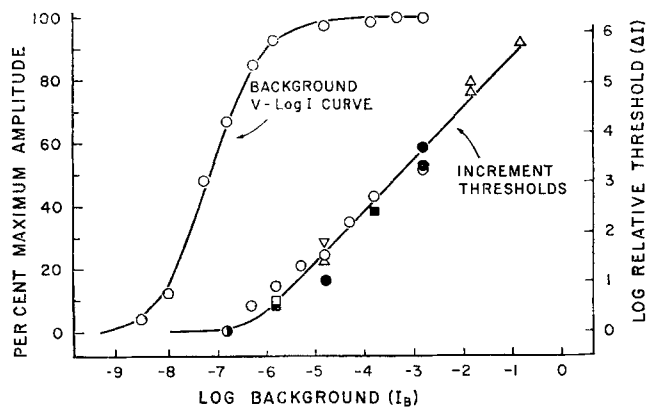

Figure 14.   Increment thresholds (ΔI) as a function of background illuminance (I_B) Also shown (to the left) are the response amplitudes elicited by the onset of the background fields. The increment threshold was hardly at all affected by adapting fields that produced large responses (up to 80% of maximum) in the S-unit. With brighter backgrounds, the increment threshold increased linearly as a function of I_B. See text for details.

Note that within the range of backgrounds which hyperpolarize the S-unit to approximately 80% of its maximum (i.e., log I_B from -9.0 to -6.5), the increment threshold is not at all elevated above the dark-adapted level (arbitrarily set at log ΔI = 0). With higher background luminances, increment thresholds rise as a linear function of intensity over the additional 5.7 log units tested. The slope of this function is unity, and is therefore virtually identical with the increment threshold relation obtained for both the b-wave and ganglion cell responses in the skate retina (Dowling and Ripps, 1970).

DARK ADAPTATION   Some aspects of S-unit activity associated with dark adaptation were described with reference to Figs. 5 and 6, and there are obviously comparable features in the data of Figs. 11 and 12. After the retina had been light adapted to a moderately strong background field, extinguish-

ing the background elicited a characteristic off-response: the S-potential rebounded immediately to its resting level, overshot it, and then returned within a minute or so to within a few millivolts of the dark level, where it remained (Fig. 11). On the other hand, dark adaptation (measured by the stimulus intensity required to elicit a constant response) followed a completely independent course (Fig. 12). In darkness, the recovery of sensitivity required at least 8–10 min for completion. But for most of this time, as thresholds fell 2–3 log units, the membrane potential held at a constant level. Thus, during dark adaptation as well as during light adaptation, there seems to be no correlation between sensitivity and the membrane potential of the S-unit.

The relatively rapid rate of dark adaptation illustrated in Figs. 6 and 12 is to be expected when the adapting light bleaches a small fraction of the visual pigment (Naka and Rushton, 1968; Dowling and Ripps, 1970). In order to bleach away quickly large amounts of pigment, we adopted the procedure described previously (Dowling and Ripps, 1970) whereby flash photolysis is employed to bleach about 80% of the available rhodospin. Thresholds, membrane levels, and maximum amplitude responses were again monitored after the exposure. As shown in Fig. 15, the flash drove the membrane potential of the S-unit (heavy line) to its saturation level of −30 mv, where it stayed for almost 10 min; during this time, our most intense test flashes were without effect and thresholds were indeterminate. However, over the next 15 min, the membrane potential rose towards its resting level along a steep, nearly exponential curve.

But it is again apparent that there are marked discrepancies between membrane levels and thresholds (squares). For an abrupt drop in threshold occurred during a time when the membrane potential was only starting to move from its saturation voltage. And at about the 15 min mark, when the membrane level was changing most rapidly, thresholds showed signs of embarking on the slow (photochemical) phase of dark adaptation (Dowling, 1963). Furthermore, thresholds were still more than 2 log units above the dark-adapted value after the S-unit had been restored completely to its original resting level. Similar results were obtained in four such experimental runs; in each, the membrane potential recovered to ±5 mv of its resting level 20–30 min after the flash, whereas the thresholds at that time were still elevated by more than 2 log units. We observed also that the maximum response that could be elicited (rectangular bars of Fig. 15) increased as the membrane voltage decreased, but the response amplitude was not nearly as great as might be predicted from the membrane level. For example, with the S-unit at or near its resting level, a test flash 10,000 times brighter than that required to saturate the dark-adapted unit, evoked a response only half as large. The maximum potential increased slowly with further dark adaptation while the membrane level remained relatively unchanged.
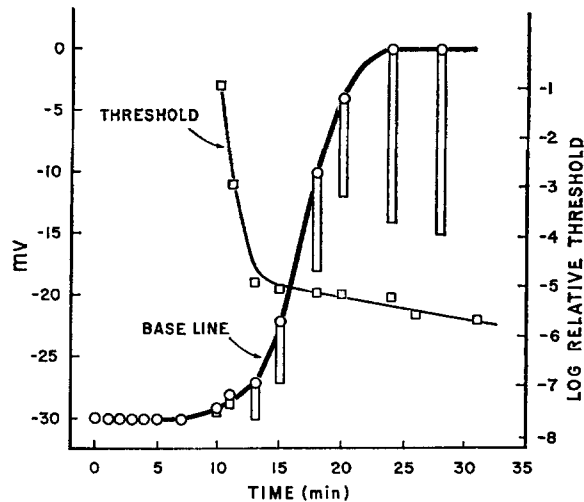
FIGURE 15. The dark adaptation of an S-unit after flash bleaching. For the first 9 min after the flash exposure, the membrane potential (circles and heavy line) remained at its saturation level, and thresholds (squares and thin line) were indeterminate. Over the next 10–12 min, the membrane potential rose rapidly to the level of the dark-adapted unit where it stabilized. Maximum S-potential amplitudes (bars) increased with time in the dark, but neither amplitudes nor thresholds could be correlated with the level of the membrane potential. Note that although the membrane potential was close to the dark-adapted level after 30 min in darkness, the threshold of the S-unit was still elevated by about 2 log units.

Experiments like the one shown in Fig. 15 usually terminated after 30–50 min, for we could only rarely obtain reliable recordings from skate S-units for longer periods of time. However, the skate retina requires almost 2 hr to dark-adapt completely after flash bleaching (Dowling and Ripps, 1970). Consequently, in an attempt to track dark adaptation over most of its course, we arrived at the compromise solution depicted in Fig. 16. An S-unit was penetrated in the dark-adapted retina and tested for the first 40–50 min after flash bleaching (open symbols). When the cell was lost or the responses deteriorated, the electrode was moved slightly to one side and inserted into another S-unit; the latter produced the results plotted as solid symbols in Fig. 16. Some justification for this unconventional maneuver stems from the fact that in the more than 80 S-units tested in the course of this study, the absolute thresholds never varied by more than 0.7 log unit, and were more often less than 0.3 log unit of the mean.

The results illustrated in Fig. 16 are similar to the threshold data of Fig. 15, but include also measurements on the course of dark adaptation for about 90 min after the flash. We reported previously that during the slow phase of the dark adaptation process (i.e., between 20 and 100 min), the changes in b-wave and ganglion cell sensitivities correlated closely with the regeneration
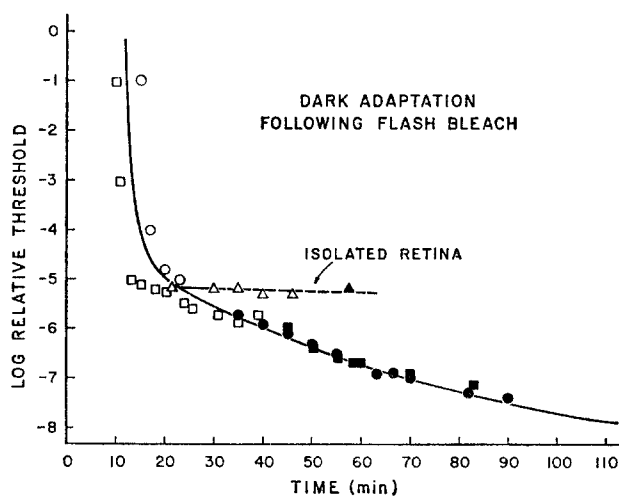
FIGURE 16. The temporal course of dark adaptation after flash bleaching. The S-unit was saturated for the first 10 min after the exposure; but immediately thereafter, thresholds fell rapidly to within 3 log units of absolute sensitivity. With further time in darkness, the recovery of sensitivity was slow in the eyecup preparation (circles and squares), but absent in the isolated retina (triangles). The solid line drawn through the data represents the dark adaptation function measured on the ERG b-wave and ganglion cell response of skate after a similar bleaching exposure (Dowling and Ripps, 1970). The various symbols indicate different experimental sessions, but in each session the results were derived from two separate S-units (open and solid symbols).

of rhodopsin in the skate retina; specifically, log threshold was inversely proportional to the rhodopsin concentration throughout the time course of this phase of dark adaptation (Dowling and Ripps, 1970). Now if we take the curve describing the changes in b-wave and ganglion cell thresholds after flash bleaching (cf. Fig. 11 of Dowling and Ripps, 1970), and superimpose it (continuous line) on the S-potential measurements (symbols), we obtain an excellent fit to present data. This suggests that a linear relation between log threshold and rhodopsin concentration during the slow phase of dark adaptation applies also at the level of the S-unit.

Further evidence that the slow recovery of S-potential threshold depends upon the regeneration of rhodopsin was obtained in an experiment on the skate retina isolated from the eyecup (triangles of Fig. 16). It is well known that when separated from its pigment epithelium, the vertebrate retina does not ordinarily regenerate a significant fraction of its rhodopsin after bleaching (Weinstein, Hobson, and Dowling, 1967; Cone and Brown, 1969). In these circumstances, the slow component of the dark adaptation curve should be eliminated, and final thresholds determined solely by the amount of pigment bleached during the flash (Weinstein et al., 1967). Our results confirmed this expectation. The threshold measured 20 min after flash bleaching had already

fallen to about 3 log units of the absolute threshold, but it remained at this level over the next 25 min (open triangles). After 60 min in darkness, a second S-unit was impaled; but its threshold (solid triangle) was elevated to the same extent.

DISCUSSION

In this study we have examined the adaptation properties of skate S-units and found them to be strikingly similar to those obtained from measurements on the ERG b-wave and ganglion cell discharge. A relation between the S-potential, the b-wave, and the ganglion cell response is perhaps to be expected in view of recent results showing that current injected into the horizonal cells can alter the amplitude of the ERG and evoke a spike discharge of the ganglion cells (Byzov, 1967: Maksimova, 1969; Naka, 1971). But far from acting as a regulatory center for controlling the sensitivity of the more proximal responses, S-unit activity appears to be governed by adaptive processes that have occurred *before* the S-unit is reached. We have not positively identified the cells from which the present recordings were obtained, but there is good reason to believe that the S-potentials arise from the horizontal cells in the skate retina. If this assumption is valid, we are led to the surprising conclusion that the principal adaptive machinery in the skate retina probably resides within the receptors themselves—for these are the only elements situated distal to the horizontal cells.

Evidence for the view that adaptation occurs distal to the origins of the S-potential stems from the fact that, during both light and dark adaptation, there is no apparent relation between the activity level (membrane potential) of the S-unit and its sensitivity to light. We have shown (in confirmation of Naka and Rushton, 1968) that in dark adaptation gross changes in sensitivity can occur when the S-potential is at or near its resting level. Conversely, the increment threshold of the light-adapted S-unit can have already stabilized at a constant value, while its membrane level is changing significantly (Fig. 11). Indeed, even during the silent period when there is by definition a correlation between the saturation time and the time during which threshold is indeterminate, there is evidence that the suppression of retinal excitability is expressed at the receptor level. For we reported previously (Dowling and Ripps, 1970) that during the silent period we could not, with our brightest stimuli, elicit the a-wave of the electroretinogram, a component which is derived from rod activity (Penn and Hagins, 1969).

The careful measurements made earlier by Naka and Rushton (1968) on the tench retina made it seem unlikely that the S-potential was responsible for mediating the changes in visual sensitivity that accompany the dark adaptation process. For they too found that, after a bleaching exposure, the membrane potential of the S-unit returned rapidly to its resting level, whereas

the amplitude and sensitivity of the S-potential underwent large and progressive changes for some time thereafter. They interpreted this important finding to mean that bleached photopigment somehow attenuates the receptor signals *before* they impinge upon the S-units. With these observations our findings are in complete accord.

But our results on the effect of "real" light (i.e., background illumination) on the skate retina are clearly at odds with some of the widely held views concerning the nature of the S-potential and the light adaptation process of the rod mechanism. Svaetichin (1953), for example, first reported that under illumination the S-potential showed no significant adaptation, and Naka and Rushton (1968) contend that a background field produces essentially a maintained change of S-potential that does not alter with time. Using strong background fields, Witkovsky (1967) reported that S-potentials saturate and that superimposed flashes are without effect. While these observations accurately describe the initial effects of an adapting field, we have shown unequivocally that the saturation potential is maintained only for a limited period of time. We have dubbed this the silent period, since the S-unit is completely unresponsive to incremental flashes for as long as it remains saturated. Although the silent period may last for as long as 20 min in the presence of intense illumination, it is followed invariably by a recovery cycle during which the membrane potential recedes toward its resting level, and the S-unit becomes increasingly more sensitive to photic stimuli.

A similar phenomenon has been observed recently in recordings from the horizontal cells of the mud puppy retina (Werblin, 1971), but the time course of light adaptation is appreciably faster in mud puppy than in skate. Since it is characteristic of some retinas to light-adapt slowly, as in the skate and the goldfish (Dowling and Ripps, 1970; Reynaud, 1969), it becomes necessary to allow sufficient time for the S-unit to recover from the initial effects of an adapting field before a stable increment threshold can be established (Figs. 6 and 12). Under these conditions, measurements can be made in the skate retina upon backgrounds that bleach more than 95% of the visual pigment, and the increment threshold data describe a linear function of unit slope (Fig. 14).

Rod Sensitivity and the Adaptation Pool   Our results on the behavior of S-units in the skate during both light and dark adaptation strongly suggest that the principal changes in sensitivity are occurring in the rods themselves and not elsewhere in the retina as several recent theories have postulated (Rushton, 1963; Dowling, 1967). The present findings would also seem to exclude a regulation of receptor sensitivity by horizontal cells feeding back onto receptors (Byzov, 1969; Svaetichin et al., 1971).

Dark-adapted rods have attained the ultimate in sensitivity, for they can respond to the absorption of a single quantum of light, and a half dozen or

so such events occurring nearly simultaneously in a cluster of elements leads to a visual sensation (Hecht et al., 1942). It has been suggested, however, that the rod maintains this exquisite sensitivity irrespective of its previous light history or that of its neighbors, i.e., the whole of visual adaptation occurs at an adaptation pool to which the cluster of rods deliver their signals, and in which the level of visual sensitivity is established (Rushton, 1965 *a*). On this view, a rod sends a message to the pool for every quantum caught; hence, "adaptation of the *rods* is irrelevant, the adaptation of the *pool* everything" (Rushton, 1965 *b*).

Although the adaptation properties of vertebrate photoreceptors have not been studied extensively (but compare Werblin, 1971), Boynton and Whitten (1970) have shown adaptation of the late receptor potential in the monkey fovea, and their experiments appear to provide direct evidence that vertebrate cone receptors can display considerable adaptation. The present experiments indicate that the sensitivity of rods also can be reduced independently of any significant change in their light-absorbing characteristics (e.g., lowering the concentration of photopigment). Of particular importance in this regard is the observation that in light adaptation the V–log I curve of the S-potential shifts laterally along the log I scale (Fig. 9), and this shift is *not* due to a change in the resting potential of the S-unit.

Interestingly, intracellular measurements on some invertebrate photo-receptors reveal analogous shifts in the position of the V–log I curve during both light and dark adaptation (Naka and Kishida, 1966; Glantz, 1968). With background intensity as a parameter, raising the level of I_B shifted the curves further to the right on the log I scale; in dark adaptation, they moved to the left with increasing time in darkness (Glantz, 1968). In these circumstances, however, the changes in sensitivity were roughly proportional to a displacement of the cell's resting potential. Invertebrate receptors depolarize and vertebrate receptors hyperpolarize to light, but in both, the V–log I curve is of the form described by equation 1. It is possible, therefore, that a maintained hyperpolarization (resulting in a response compression) will be shown to accompany sensitivity changes of vertebrate receptors, and such a result is suggested by the experiments of Boynton and Whitten (1970) and Werblin (1971). The techniques are now available to study adaptation both intra- and extracellularly at the photoreceptor level in vertebrates; further experiments should provide insight into the mechanisms of receptor adaptation.

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