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THE ROD AFTER-EFFECT IN S-POTENTIALS FROM CAT RETINA

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U. S. ARMY AEROMEDICAL RESEARCH LABORATORY

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## SUMMARY PAGE\*

### THE PROBLEM

S-potentials in response to intense flashes of light slowly recover their resting levels. In cat, this after-effect originates mainly in the rods. How does it relate to percentage rhodopsin bleached?

### FINDINGS

At threshold, flashes which produced the rod after-effect bleached only very small quantities of rhodopsin; and at a fixed flash duration, the duration of the after-effect increased as a function of log intensity. The after-effect's threshold occurred at about the intensity which saturated the maintained voltage.

The principal results were obtained from 29 bleaches in the same number of S-potentials. The duration of the after-effect, measured as time to recover one-half voltage, was a linear function of exposure time (0.5 to 64.0 sec) to a strong light (6.5 log td. scotopic). The duration continued to increase after an exposure of 16 sec, even though at least 99 per cent of the rhodopsin had been bleached.

After strong bleaches the S-potential returned to the baseline well before the recovery of either cone or rod excitability, as evidenced by V-Log I curves.

It is concluded that the after-effect originates from something which accumulates after the maintained voltage in rod pathways reaches a ceiling. The accumulation can continue at a fixed rate irrespective of the bleaching rate.

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\*The findings in this report are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

The animals used in this study were handled in accordance with the "Principles of Laboratory Animal Care" established by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

## INTRODUCTION

When flashes of light are sufficiently intense, an S-unit does not immediately return to its resting potential. Both in fish (4, 5) and mammals (13) a negative potential remains after the flash, and it may take minutes for it to return to the baseline. Recently, Naka and Rushton (5) investigated whether this persistent elevation of potential represents the signal which lowers the excitability of responses after a bleach. They showed, however, that the potential returns to the resting level much before test responses reestablish their prebleached amplitudes. Thus, the level of potential following a bleach does not carry information about the level of excitability. These two after-effects, level of excitability and the persistent negative potential, appear to be unrelated following bleaching; and the former, when measured as log threshold, is proportional to the quantity of bleached pigment remaining after strong bleaches (3, 7). Does this mean that the negative after-effect is unrelated to quantity of pigment bleached? Fortunately, in the cat, the rods were identified as the source of this effect (12), and its relation to bleaching could be studied; although this was the purpose of the present investigation, the relation of the negative after-effect to the level of rod and cone excitability also was investigated.

The preparation and maintenance of the cat, recording technique from the intact eye, conditions of stimulation, and techniques relating to S-potentials already have been described (10, 12, 14). The percentage rhodopsin bleached was not directly measured but it was estimated from the values obtained by Rushton (6) in the human eye. It was assumed that a retinal illumination of about 6.8 log td. sec (scotopic) bleached 50 per cent of the rhodopsin (6, 7).

## RESULTS

Figure 1 shows the duration of the rod after-effect as a function of log intensity. The superposed responses illustrate that at first, although duration progressively increased, the rate of recovery, measured as the swing toward the baseline, did not change (responses 1 to 3). At higher intensities, however, rate of recovery decreased and the break between the maintained negative potential and the point of recovery disappeared (response 5). The threshold for these effects was about 3.0 log above the intensity at which dark-adapted responses could be first distinguished (12); but these flashes only bleached very small quantities of rhodopsin. For example, in Figure 1, the threshold after-effect was produced by a flash having 1/1,000 th the bleaching intensity of one which bleached 0.1 per cent of the rhodopsin.

To investigate the relation to pigment bleached, I used the following procedure. After obtaining an S-unit in the dark-adapted retina a brief V-Log I series was performed in order to establish response reliability. The same retinal area (2.00 diam) was then exposed to a strong light of 6.5 log td. scotopic, varying in duration from 0.5 to 64.0 sec. The potential was recorded continuously before, during, and after

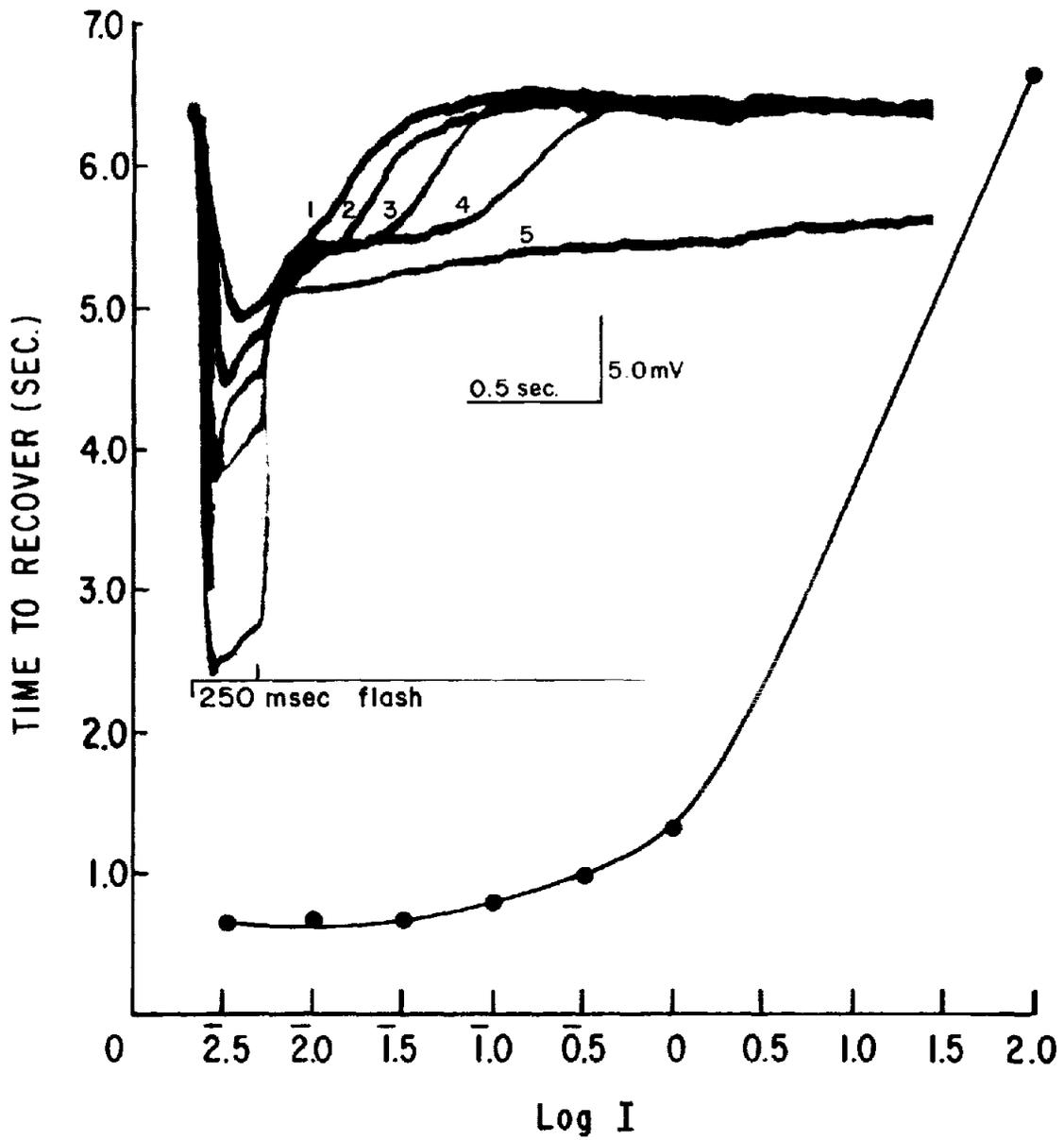


Figure 1

The rod after-effect as a function of log intensity. Five responses of an S-unit to 245-msec flashes of white light, below and above the threshold for the after-effect, are superposed. Response 2 is at threshold for the after-effect and  $\sim 3.0$  log above the response threshold while 1, 3, 4, and 5 are, respectively, 2.5, 3.5, 4.0, and 6.5 log above response threshold. Graph: This curve was plotted from the responses partially presented in the inset. Time to recover was measured as the time taken to reach the baseline after the initial, abrupt recovery.

the bleach, and where possible, V-Log I sequences were repeated at intervals following the bleach.

Only one bleach could be performed for each S-unit and more than one half of the bleaches were unacceptable. The principal difficulties were loss of the S-unit following the bleach and failure to sustain the response while the flash was on. An acceptable result was obtained in 29 bleaches which produced an average hyperpolarization of 28.0 mV (15.0 to 41.5) while the voltage during the flash dropped on average of only 15 per cent from the peak.

The total time taken to return to the baseline following the bleach could not be reliably measured. A shorter time measurement was chosen, therefore, which was much less affected by drift and other sources of variability. Figure 2 illustrates the method of measurement for a 16.3-sec bleach. The bracket following the flash indicates one-half voltage between the end of rapid recovery and the baseline. I measured the time taken to recover this voltage, in this case, 15.0 mV in 19.2 sec.

Figure 3 presents the results of these experiments. Each point indicates a single bleach. The scatter, increasing with duration of exposure was not unexpected, for the data were derived from 29 S-units in 10 eyes. The open circles show the means for six durations of the flash: 0.5, 4, 8, 16, 32, and 64 sec. Although percentage pigment bleached increases with duration of exposure, 99 per cent of the pigment already had been bleached by the 16-sec exposure. The 32- and 64- sec exposures could not have appreciably increased the percentage bleached, but they did increase the duration of the after-effect. Observe, therefore, that the time to recover one-half voltage increased linearly with duration of exposure and not with percentage pigment bleached.

Figure 4 displays a representative series of V-Log I curves, in this case, from the bleach already illustrated in Figure 2. Six minutes following the "off" of the bleaching light the entire V-Log I curve (o) was still shifted more than 2.0 log along the horizontal axis, although the potential had returned to the baseline at the end of 2 min. The responses at 6 min exhibited the cone form (short "on" and "off" latencies, even at threshold) and threshold was at a photopic level. Some additional recovery of cone excitability occurred at 8 min (◊) but little further recovery at 12 (Δ) and 16 (□) min. The stationary position of the V-Log I curves corresponded to the plateau between rod and cone recovery in dark-adaptation curves. After strong bleaches, evidence for rod recovery (increase in latency of the threshold responses, lowering of threshold to scotopic levels) usually was first observed at 15 to 20 min. Thus, in confirmation of Naka and Rushton (5) the S-potential returned to the baseline long before excitability recovered. In addition, the rod after-effect appeared to be unrelated to the recovery of either cone or rod excitability.

Recovery of rod threshold was very difficult to follow by this technique because of the difficulty in holding the recording for 20 min or longer following strong bleaches.



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Figure 2

Response of an S-unit to a 16.3-sec flash of white light which bleached 16 per cent of the rhodopsin. Pen-writer record, dc amplification. Negative responses deflected the pen downward. The bracket after the flash indicates one-half voltage between the beginning of slow recovery and the baseline. The brief positive displacement at 13.2 sec is an artifact.

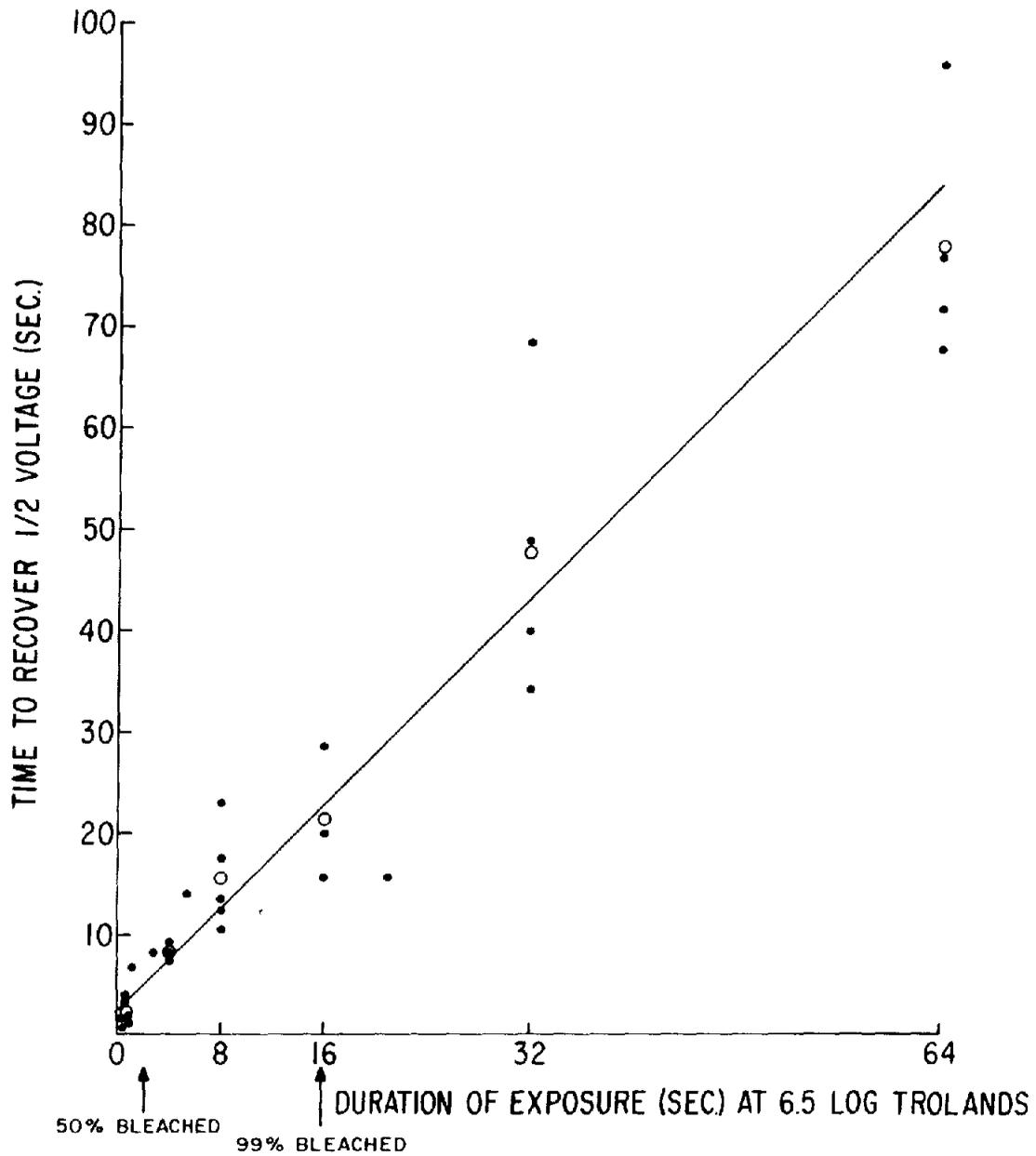


Figure 3

The linear relation between the rod after-effect and duration of exposure at 6.5 log td. The after-effect was measured as the time to recover one-half the voltage between the end of abrupt recovery and the baseline. Each measurement (●) was obtained from a separate bleach in 29 different S-units from 10 eyes. (○),  $\bar{X}$  for 0.5, 4.0, 8.0, 16.0, 32.0, and 64 sec bleaches.

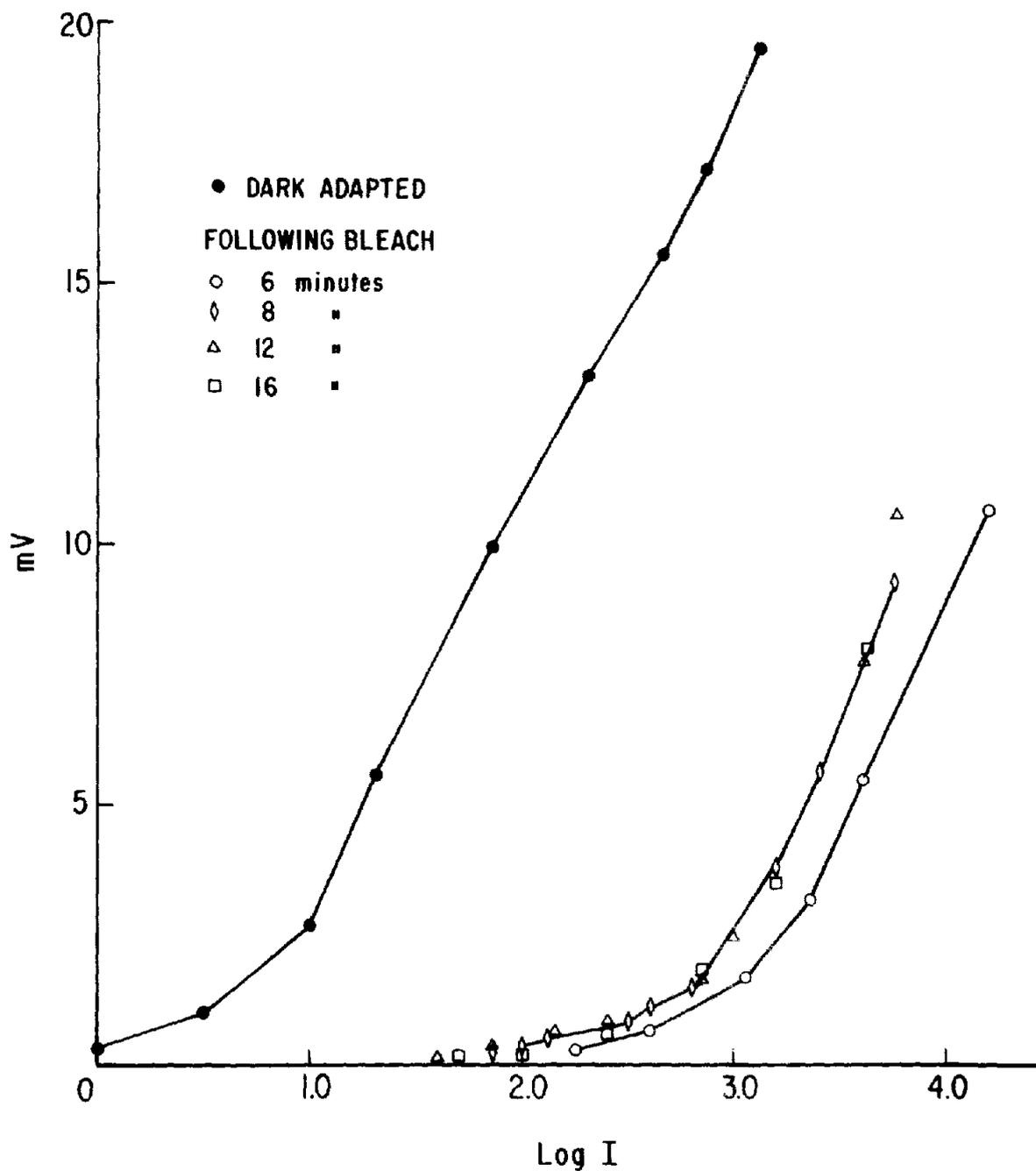


Figure 4

V-Log I curves before and after bleaching, from the 16.3-sec bleach illustrated in Figure 2. The flashes were 465 msec in duration, 2.00 mm diam; and the amplitude (mV) of the initial peak voltage was measured.

Within this period of time, however, striking changes occurred in the after-effect. In the experiment illustrated in Figure 5, intense 465-msec flashes were administered at 1- to 3- min intervals following a flash which bleached 99 per cent of the rhodopsin. The sample of responses was selected to show the return of the after-effect 1 to 22 min following the bleach. In a fully dark-adapted retina the potential would have taken 25 to 50 sec to return to the baseline following the 465-msec test flash. Immediately following the bleach, however, it returned to the baseline in about 2.5 sec (Figure 5, 1 min). This time progressively increased in the next 21 min as indicated by the progressive increase in the elevation of the potential above the baseline in the 4-sec period following each flash.

## DISCUSSION

### ORIGIN OF THE ROD AFTER-EFFECT

After a flash is turned off, the S-unit continues responding to a signal that is somehow related to the previous excitation of rods. The after-effect's threshold is only about 3.0 log above the threshold of the dark-adapted S-potential, and these flashes bleach very small amounts of rhodopsin. In terms of bleaching, then, it is not a "high-intensity" effect (11, 13); but it does occur at about the same intensity at which the maintained voltage reaches a ceiling (13).

It appears, therefore, that when voltage (maintained) can no longer increase, the duration of the response can. As intensity is increased further, with flash duration fixed, response duration increases, suggesting that "something" continues to be produced even though it does not result in a voltage change. It may be the accumulation of this "something" which prolongs the response.

With flash intensity fixed at a high level (6.5 log td. scotopic) and flash duration progressively increased, the duration of the after-effect increased linearly irrespective of whether the bleaching rate (quantum catch rate) was great, as at first, or small (after 16 sec). Whatever accumulates, therefore, must be produced at a fixed rate when the light is on regardless of the quantum catch. But a process of fixed size irrespective of light strength is a saturated one. We must postulate then that the process leading to the after-effect was saturated at every stage of bleaching, even during 0.8 min after 99 per cent bleaching. During this time the rate of regeneration of cat's rhodopsin must have been sufficient to maintain the accumulation at a fixed rate.

What is it that builds up and is completely integrated over time, resulting in a recovery time proportional to this integral? Accumulation of a photoproduct is unlikely because almost all of the photoproduct is produced in the initial 16 sec and little thereafter. The build-up is closely related to the production of a maximum signal in rod pathways since the after-effect's threshold is at rod saturation (maintained voltage). Elsewhere, I suggested that a membrane recovery process might be overloaded (13); similarly, overloading of transmitter inactivation might lead to a prolonged response.

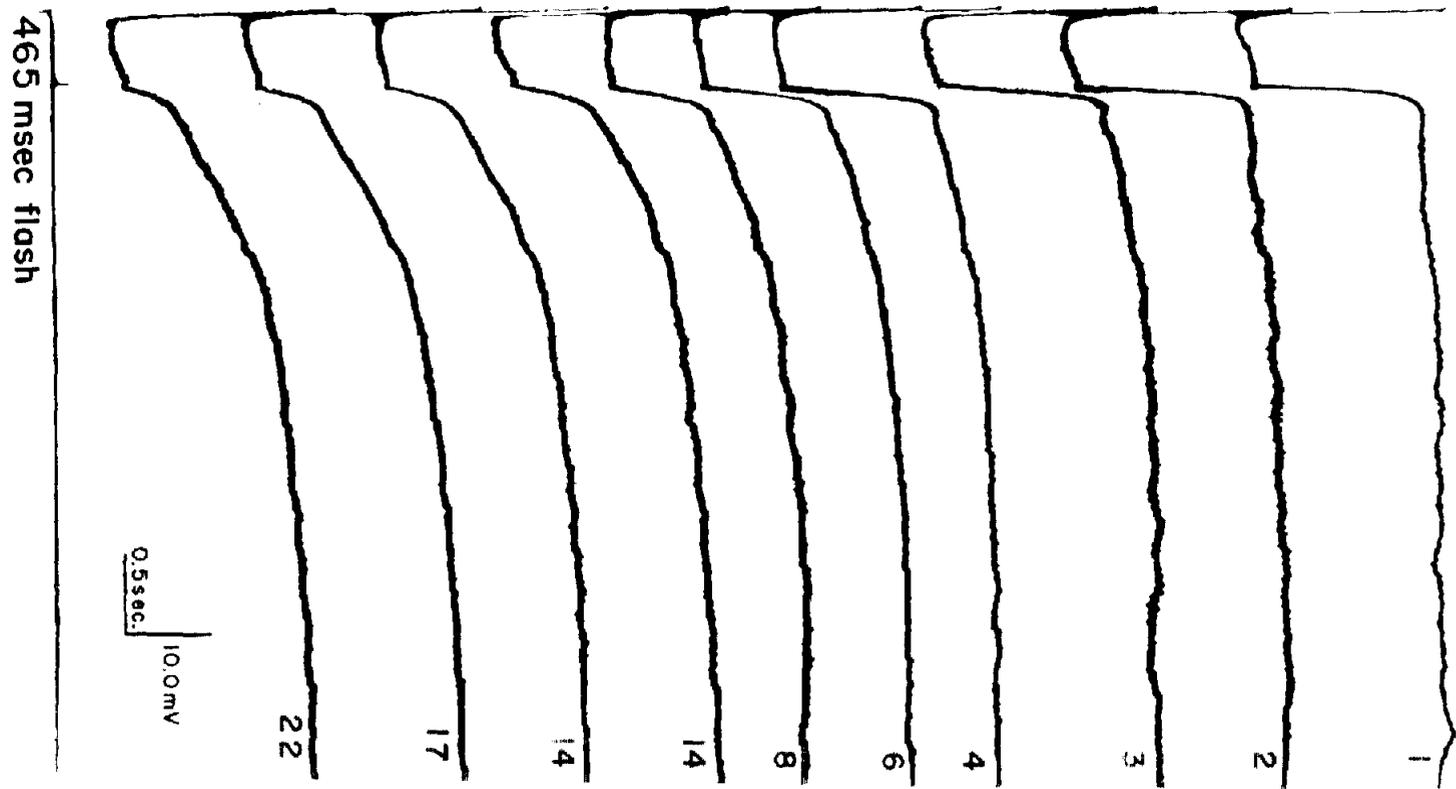


Figure 5

Recovery of rod after-effect following a flash which bleached at least 99 per cent of the rhodopsin. A test flash was administered at 1-min intervals following the bleaching flash. Each test flash would have bleached 16 per cent of the rhodopsin in a dark-adapted retina and produced a response which would have taken 25 to 50 sec to return to the baseline. Selected responses to the test flash are presented from 1 to 22 min following the bleach. Time after bleach, in minutes, is indicated in the right margin.

## Relation To Other Neuronal Events

In other recent work from this laboratory the dc component of the local electroretinogram (LERG) and the on-response of ganglion cells were both shown to persist into the off-period following sufficiently intense flashes (9-11). Since the same effect was observed in the late receptor potential (late RP) (1, 11), it was assumed to have originated in the receptors and to have been transmitted to ganglion cells via bipolar cells. Although the spectral sensitivity of the after-effect in the late RP, LERG, and ganglion cells was not determined, it had essentially the same characteristics with regard to intensity, duration, and light adaptation as the S-potential's after-effect. It seems therefore that the after-effect originating in the rods is transmitted to bipolar cells as well as horizontal cells (L-type S-units).

The short-latency off-responses which appeared in the dc component (11) at high intensities can now be assigned to the cones because of the photopic sensitivity of S-potentials. The splitting of ganglion cell off-responses at high intensities also can now be understood (11). A short-latency remnant of the off-response stayed intact while the main body of the off-response increased in latency, weakened, and then disappeared as flash-intensity increased. Intracellular records from ganglion cells showed that at relatively high intensities (light-adapted retinae), the postsynaptic potential (PSP) present during the on-period tended to persist further and further into the off-period. A short-latency repolarization remained, however, at the latency of the short-latency off-response. These data now suggest that in light-adapted retinae, the on-response consists of both rod and cone components. When the rod component increases in duration its decay, which is directly associated with the off-response, increases in latency and the off-response is gradually lost as the rate of decay becomes slower. But cone on-responses still tend to decay abruptly at these intensities, and the associated cone off-response remains. At the highest intensities, however, the cone off-response of ganglion cells also was lost which is reminiscent of the effect on S-potential cone-responses (11).

None of the previously reported ganglion cell recordings were obtained from dark-adapted retinae, and if the proposed explanation is correct, we should be able to make some predictions for the dark-adapted responses. Depending on the degree to which a particular light stimulates cones, the cone off-response should have a distinct threshold as intensity is increased. Depending on the degree to which rods are stimulated, the rod off-response, beginning at some intensity, should progressively increase in latency and weaken. This is exactly what happens as illustrated in Figure 6. A small spot (diam 0.5 mm) of orange light (615 nm) was flashed (465 msec) at the most sensitive location in a receptive field. The cell responded with inhibition during the on, excitation following the off, and the long latency of both responses ( $\sim 100$  msec) implicated the rods. The off-latency could be readily measured and it changed very little over a 4.0-log range above threshold (0.2 to 4.0). As predicted, the off-response weakened, here at 3.4 log above threshold, increased in latency (4.4) and disappeared (4.8) at higher intensities. Finally at  $> 5$  log above the threshold of the rod response a short-latency brief off-response appeared (6.0) which must have been derived from the cones.

615 nm

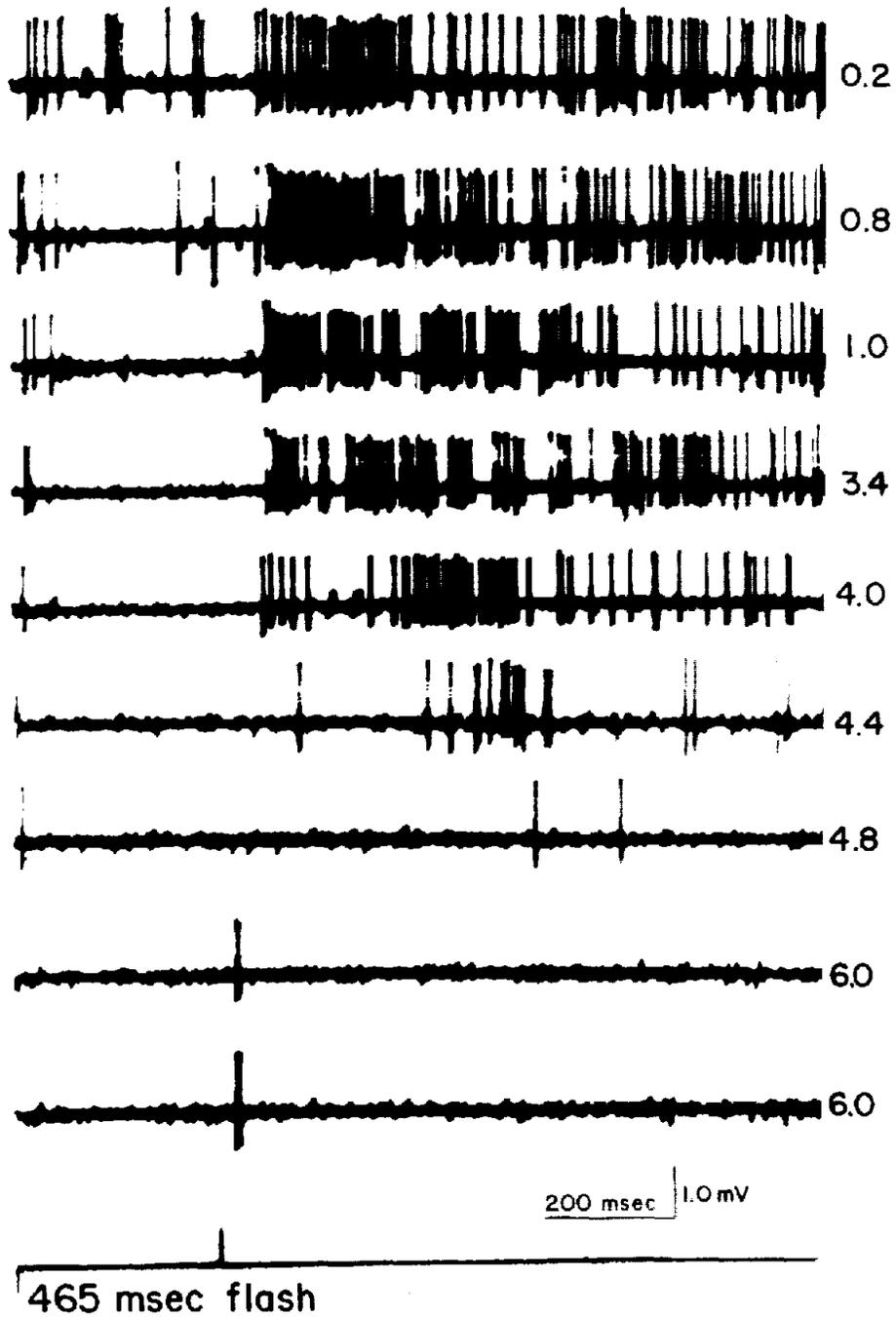


Figure 6

Intensity sequence in response to orange light (615 nm) from a ganglion cell in a dark-adapted retina. A 0.5-mm diam spot was centered at the point of lowest threshold. The intensity of a 465-msec flash was increased in log steps above threshold as indicated in the right margin. Photographed on a storage oscilloscope. Negative responses are displayed upward.

Although dark-adapted ganglion cells were not extensively surveyed, their responses differed strikingly from S-units. We know from S-unit results that 615-nm flashes in dark-adapted retinæ excite cones at about 0.5 to 1.0 log above the rod threshold (13). In ganglion cells, however, cone responses were not observed until the intensity was raised several log units above this point. There would seem to be two principal explanations, either our sample of ganglion cells receive a very weak cone component or the cone input is blocked in dark-adapted retinæ. In addition, the rod after-effect at the ganglion cell might also contribute to the elevated cone threshold. By continuing into the off-period, the PSP of the on-response could prevent the cone PSP from repolarizing enough to trigger an off-response. In any case, this finding tends to support the conclusion of Witkovsky (15) that S-units and ganglion cells exhibit strikingly different response properties.

Recently, Crescitelli and Sickel (2) described an intensity-dependent delay of an off-effect in the transretinally recorded slow wave of the isolated frog retina. This phenomenon seems identical to the one presently described because the former also exhibited rod spectral sensitivity and a raised threshold after bleaching. Furthermore, a similar effect also had been observed in ganglion cell responses from frog retina (3). They considered, however, that the delay was evidence for inhibition, cone-rod in the photopic state and rod-rod under scotopic conditions (2). Basic to this explanation is the assumption that an off-effect is a circumscribed response which when suppressed can be delayed.

In the present work it has been possible to observe on-responses in continuity with their decays (or recoveries). In slow potentials, an off-response seems to be identical with the decay of the on-response to the baseline, possibly plus an overshoot; and increases in the latency of off-responses always originate from increases in the duration of on-responses. Is it possible that rod on-responses are prevented from decaying because of rod-rod or cone-rod inhibition? I have not obtained any data to support this hypothesis. In dark-adapted S-units to blue flashes (433 nm) rod responses increased in duration at intensities that seemed to be below the cone threshold (13). At other wavelengths the increase in latency always seemed to be a function of the amount of stimulation received by the rods (13). Furthermore, rod-cone interaction in S-units seems to be additive and not subtractive (13). Finally, in ganglion cell recordings delay and loss of the off-response did not result from inhibition of the off-response but from its weakening when the PSP of the on-response increased in latency and decayed slowly (11). Certainly, cone-rod inhibition may be a prominent retinal mechanism but it is probably not the origin of the rod after-effect in S-units, ganglion cells, and the LERG.

## REFERENCES

1. Brown, K. T., and Murakami, M., Delayed decay of the late receptor potential of monkey cones as a function of stimulus intensity. Vision Res., 7:179-189, 1967.
2. Crescitelli, F., and Sickel, E., Delayed off-responses recorded from the isolated frog retina. Vision Res., 8:801-816, 1968.
3. Dowling, J. E., Chemistry of visual adaptation in the rat. Nature, Lond., 188:114-118, 1960.
4. Gouras, P., Graded potentials of bream retina. J. Physiol., 152:487-505, 1960.
5. Naka, K. I., and Rushton, W. A. H., S-potentials and dark adaptation in fish. J. Physiol., 194:259-269, 1968.
6. Rushton, W. A. H., The difference spectrum and the photosensitivity of rhodopsin in the living human eye. J. Physiol., 134:11-29, 1956.
7. Rushton, W. A. H., Rhodopsin measurement and dark-adaptation in a subject deficient in cone vision. J. Physiol., 156:193-205, 1961.
8. Sickel, W., and Crescitelli, F., Delayed electrical responses from the isolated frog retina. Pflüg. Arch. ges. Physiol., 297:266-269, 1967.
9. Steinberg, R. H., Relation between ganglion cell activity and the local electroretinogram of cat retina. Nature, Lond., 216:1008-1010, 1967.
10. Steinberg, R. H., Comparison of the intraretinal b-wave and d.c. component in the area centralis of cat retina. Vision Res., 9:317-331, 1969.
11. Steinberg, R. H., High-intensity effects on slow potentials and ganglion cell activity in the area centralis of cat retina. Vision Res., 9:333-350, 1969.
12. Steinberg, R. H., Rod and cone contributions to S-potentials from cat retina. NAMI-1071. Pensacola, Fla.:Naval Aerospace Medical Institute and U. S. Army Aeromedical Research Laboratory, 1969.
13. Steinberg, R. H., Rod-cone interaction in S-potentials from cat retina. NAMI-1072. Pensacola, Fla.:Naval Aerospace Medical Institute and U. S. Army Aeromedical Research Laboratory, 1969.

14. Steinberg, R. H., Walker, M. L., and Johnson, W. M., A new microelectrode positioner for intraretinal recording from the intact mammalian eye. Vision Res., 8:1521-1523, 1968.
15. Witkovsky, P., A comparison of ganglion cell and S-potential responses in carp retina. J. Neurophysiol., 30:546-561, 1967.