Neural and Photochemical Mechanisms of Visual Adaptation in the Rat

JOHN E. DOWLING

From the Biological Laboratories, Harvard University, Cambridge, Massachusetts

ABSTRACT The effects of light adaptation on the increment threshold, rhodopsin content, and dark adaptation have been studied in the rat eye over a wide range of intensities. The electroretinogram threshold was used as a measure of eye sensitivity. With adapting intensities greater than 1.5 log units above the absolute ERG threshold, the increment threshold rises linearly with increasing adapting intensity. With 5 minutes of light adaptation, the rhodopsin content of the eye is not measurably reduced until the adapting intensity is greater than 5 log units above the ERG threshold. Dark adaptation is rapid (*i.e.*, completed in 5 to 10 minutes) until the eye is adapted to lights strong enough to bleach a measurable fraction of the rhodopsin. After brighter light adaptations, dark adaptation consists of two parts, an initial rapid phase followed by a slow component. The extent of slow adaptation depends on the fraction of rhodopsin bleached. If all the rhodopsin in the eye is bleached, the slow fall of threshold extends over 5 log units and takes 2 to 3 hours to complete. The fall of ERG threshold during the slow phase of adaptation occurs in parallel with the regeneration of rhodopsin. The slow component of dark adaptation is related to the bleaching and resynthesis of rhodopsin; the fast component of adaptation is considered to be neural adaptation.

INTRODUCTION

It is a matter of common experience that the eye loses sensitivity in the light and regains sensitivity in the dark. Hecht was the first to emphasize the idea that recovery in dark adaptation was related to the resynthesis of the visual pigments. Hecht, however, could never convincingly formulate what the relation of sensitivity to pigment concentration might be because he had little data with which to work. This was primarily because visual thresholds during dark adaptation were usually measured on human subjects in which pigment concentrations could not be measured. In animals, on the other hand, visual pigment levels can be accurately measured, but visual thresholds are difficult to determine. In the few attempts made to correlate the regenerating pigment with the electrical activity of the eye, there seemed to be

no simple relation between pigment concentration and size of b-wave potential of the ERG in the dark-adapting eye (Granit *et al.*, 1939). In 1942, Hecht summed up the position as follows:—

"In general, human visual dark adaptation runs roughly parallel with the accumulation of visual purple in the dark-adapting animal retina. Efforts to study this parallelism have not been successful. . . ."

With the achievement of the synthesis of visual pigments in solution, it has since been possible to compare the regeneration of visual pigments in solution with dark adaptation. Wald, Brown, and Smith (1955) in a study of regeneration of chicken photopigments showed that the cone pigment, iodopsin, regenerates very much faster than the rod pigment, rhodopsin, and they pointed out the striking qualitative similarity between the test tube regeneration of these rod and cone pigments and the course of human dark adaptation. They further pointed out that this relationship is valid only provided that one compares the concentration of pigment with the *logarithm* of the visual threshold. They suggested that this was the relation between pigment concentration and threshold in the living eye.

Recently, it has been possible to get direct evidence on this important relation. While studying the effects of vitamin A deficiency on the retina of the rat, we found that the logarithm of the ERG threshold rose in parallel with the fall of visual pigment concentration in the eye (Dowling and Wald, 1958). In human vitamin A deficiency there is a similar rise of log visual threshold (Wald *et al.*, 1938; Hecht and Mandelbaum, 1940), and it appeared that the ERG threshold in the rat is a good index of visual cell function and sensitivity. Using the ERG threshold as a criterion of sensitivity, we subsequently compared the regeneration of visual pigment during dark adaptation with the fall of ERG threshold and found that in dark adaptation also, the logarithm of the ERG threshold is linearly related to the concentration of visual pigment in the eye (Dowling, 1960).

Rushton (1961) has now unequivocally demonstrated this relation in the living human eye by comparing the psychophysical threshold with visual pigment concentration determined by retinal densitometry. A linear relation between log threshold and visual pigment concentration is found to hold for both the rods and cones, although the range of adaptation is very different in the two instances. With an increase from 60 to 100 per cent rhodopsin during dark adaptation, Rushton showed that the threshold of his rod monochromat subject fell 6.5 log units, indicating that the entire rod adaptation range is about 18 log units. In the cones, on the other hand, the adaptation range is about 3.5 log units (Rushton, 1962). In the rat rods the range of adaptation due to visual pigment bleaching and resynthesis is about 5 log units.

It appears clear, therefore, that after bleaching away the visual pigments

in the eye, the subsequent recovery of the eye awaits the visual pigment resynthesis, and that it is the logarithm of the sensitivity that varies linearly with the pigment concentration. What is not nearly so clear, however, is what happens in the eye when it is adapted to much dimmer lights which do not bleach away significant fractions of the visual pigment. A simple consideration makes one aware that in dim lights, factors other than the visual pigment level must play a major role in adaptation. For example, mammalian rod cells contain on the order of 3×10^7 molecules of visual pigment (Wald, 1961; Cone, 1963), and it has been known for a long time that rod cells are single quantum detectors (Hecht, Shlaer, and Pirenne, 1941–42). That is, 1 quantum of light absorbed can excite a dark-adapted rod, and in the human eye, 1 quantum absorbed per 1,000 rods in a large visual field can result in a visual sensation (Stiles, 1939; Brindley, 1960). A simple calculation indicates, therefore, that adapting lights 70,000,000 times above the visual threshold will not bleach away more than 2 per cent of the visual pigment in a rod in the course of a 5 second adaptation. If we consider only photochemical adaptation, this small amount of bleaching should (in the case of the human rods) raise the visual threshold only a small amount, less than 0.5 of a log unit. Yet we know that adapting lights of this luminance raise the human visual threshold 2 to 3 log units when measured at the beginning of dark adaptation (Rushton and Cohen, 1954; Wald, 1954). This has now been demonstrated several times and has been used as a strong argument against the idea that visual adaptation is primarily related to pigment concentration in the eye (Rushton and Cohen, 1954; Dodt and Echte, 1961). (See also Granit's (1947, pp. 244–251) discussion of the drastic reduction of b-wave potential of the ERG upon adaptation of the frog eye to lights which bleach no more than 1 to 2 per cent of the visual pigment.) This second type of adaptation has been frequently termed "neural" adaptation in distinction to the "photochemical" adaptation due to the bleaching and regeneration of the visual pigments.

It is the purpose of this paper to distinguish and compare the roles of neural and photochemical adaptation in the rat, using the ERG threshold as a measure of visual sensitivity. The rat eye is particularly suited for a study of this nature because it acts primarily like an all-rod eye. Although there are a few cones present in its retina (Walls, 1934) they apparently play a very small physiological role, and their responses are not obvious unless special efforts are made to detect them (Dodt and Echte, 1961; Dowling, unpublished observations). In many respects, the rat eye resembles that of a human rod monochromat. The few cones in both instances have a very high threshold and seem to play a much less consequential role than in the typical rod-cone retina.

METHODS AND MATERIALS

Electroretinography The animals used in this study were albino rats from the Harvard University colony. The animals were anesthetized with nembutal (5 mg per 100 gm) and fastened with adhesive tape to a board fitted with a clay head-holder. The eye, directed upwards, was maximally exposed by drawing the eyelids back with sutures. This causes the eye to extend well out of its socket, so that the entire eyeball is exposed. The responses were recorded by means of cotton wick electrodes soaked in Ringer's solution, one placed carefully on the edge of the cornea, the other on a shaved area on the cheek. The electrodes were connected with silver-silver chloride wires to a Grass AC P-5 preamplifier with a time-constant setting of 0.75 second. The responses were observed on a type 502 tektronix oscilloscope and photographed when desired with a Dumont-polaroid oscilloscope camera.

The stimulating light was a 100 watt zirconium arc lamp, whose intensity could be continuously controlled with a pair of circular neutral wedges. The duration of the test stimuli (ordinarily $\frac{1}{50}$ second) was controlled with a camera shutter. The adapting light was a ribbon filament lamp whose intensity was controlled with neutral filters. The two light beams were superimposed by means of a half-silvered mirror and adjusted so that they both evenly illuminated the top third of a ping-pong ball which was placed over the exposed eye and acted as a light diffuser. This technique assures even illumination over the entire retina (Cone, 1963).

Throughout these experiments, the criterion of a threshold response, although termed the ERG threshold, was in fact a somewhat higher response than that of the absolute ERG threshold (Cone, 1963). Depending on the noise of the particular preparation, the response selected was a potential between 10 and 20 μv, just large enough to be easily distinguished from the noise.

Rhodopsin Analyses To estimate the amount of rhodopsin in the retina after light adaptation, the eyes were removed from a dark-adapted animal and placed pupil upward on cotton soaked in Ringer's solution, in a small Petri dish. The Petri dish was placed in the adapting light beam so that the eyes were at the same height as when in the animal. The top third of a ping-pong ball was placed over the eyes as when adapting the living eye, so that the retinas were evenly illuminated. After the bleaching period, the cornea and lens were quickly dissected away, the back of the eye plunged into cold 4 per cent alum solution, and quickly frozen by placing the test tubes in crushed dry ice. When all the eyes had been collected in this way, the tissues were thawed and crushed with a stirring rod. After hardening in the alum for 15 minutes, the eyes were washed twice with distilled water, once with buffer, and extracted overnight with 0.25 ml of digitonin. The next day, the digitonin solutions were centrifuged at 20,000 RPM in a Spinco preparative ultracentrifuge for 10 minutes, 0.01 ml of 1 M hydroxylamine added to each extract, and the absorption spectrum of the solutions measured in a Cary recording spectrophotometer. The solutions were bleached for 10 minutes with white light and the absorption spectrum remeasured. The change in density at 494 mμ measured the amount of rhodopsin present in the solution. The first extract removed about 80 per cent of the rhodopsin from the eyes, and a second extract was made as routine.

RESULTS

Fig. 1 illustrates the principal results of this study. The open circles show the rise of log increment threshold as a function of the adapting luminance (log I). The absolute dark-adapted threshold (elicited with a $\frac{1}{50}$ second

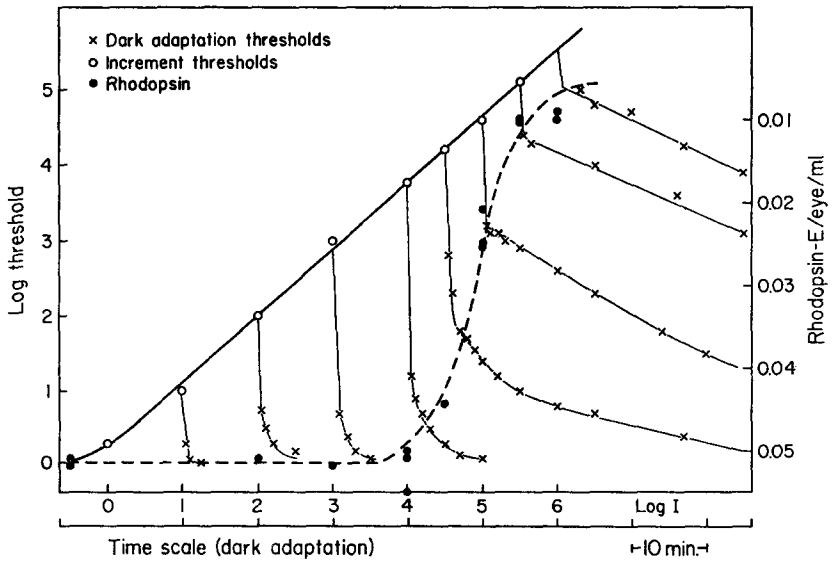

FIGURE 1. The effect of 5 minutes' light adaptation on the rhodopsin content (filled circles; dotted line), increment threshold (open circles; thick line), and dark adaptation (crosses; thin lines). The rhodopsin content (measured as extinction/eye/milliliter extract) is not significantly reduced until the eye is adapted to intensities greater than log $I = 4$. The log increment threshold rises linearly with intensities greater than log $I = 0.5$. Dark adaptation (crosses) is rapid until the eye is adapted to lights that bleach a significant fraction of rhodopsin (*i.e.* greater than log $I = 4$). After brighter light adaptations, dark adaptation breaks into two components, an initial rapid phase, and a slower component. The extent of the slow component of adaptation depends on amount of rhodopsin bleached.

flash) lies at about log $I = -1$. With adapting luminances greater than about 1.5 log units above threshold (*i.e.* log $I = 0.5$), the log increment threshold rises linearly with increase of log-adapting luminance (log I). However, the slope is slightly less than 45°, the value it would have if Weber's law ($\Delta I/I = C$) were obeyed exactly. For this experiment, the adaptation period was 5 minutes, and the plotted increment thresholds were measured 4.5 minutes after the lights were turned on. However, when the adapting light is first turned on, the final increment threshold level is established within a few seconds, faster than could be accurately measured with this equipment, so that increment threshold measurements made at 15 to 30 seconds after the

adapting lights were put on are similar to the 4.5 minute points plotted here. Thus the ERG increment threshold depends primarily on the luminance of the adapting light, not upon the duration of light adaptation (after a few seconds) or visual pigment concentration[1] (Rushton, 1961; Dodt and Echte, 1961).

Fig. 1 also shows the approximate concentration of rhodopsin left in the eye after the 5 minute adaptation to the various adapting luminances (filled circles). Until the adapting luminance is about 5 log units above the ERG threshold, the concentration of rhodopsin in the eye is not significantly reduced during a 5 minute adaptation period. With further increase of adapting luminance, however, the fraction of rhodopsin bleached away increases very rapidly, until at luminances of 6.5 to 7 log units above the ERG threshold, almost all the rhodopsin bleaches during the 5 minute adaptation period. (At the brightest adapting luminance, ($\log I = 6$), we still find a small fraction of rhodopsin remaining in the eye, which should have been bleached away at this intensity. Possible reasons for this small discrepancy are discussed by Cone (1963) in the preceding paper.) The dotted line drawn in the figure is a predicted curve relating fraction of rhodopsin with intensity of adapting field, as calculated by Cone (1963) from an absolute measure of quanta incident on the rat eye. The measured points were on excised eyes in which little regeneration probably occurred during the adaptation period, and the calculation was also based on a non-regenerating system. In the living eye, the points and curve are probably pushed a little to the right, although probably not by more than 0.1 to 0.2 log unit because rhodopsin regenerates so very slowly in the living rat eye (see below).

Finally (crosses) Fig. 1 shows the course of dark adaptation after 5 minute adaptations to the various adapting luminances. In these experiments dark adaptation is measured from the increment threshold level, which Blanchard (1918; see also Baker, 1953) has shown is the equivalent of the instantaneous threshold (*i.e.* the initial threshold when the adapting lights are extinguished). We see that until the adapting luminances reach intensities strong enough to bleach away a significant fraction of the visual pigment, dark adaptation appears to be a single process and is relatively fast, taking a maximum of about 10 minutes for completion. At higher light luminances, which bleach away a measurable fraction of the rhodopsin, dark adaptation is broken into two parts. An initial rapid fall of threshold is followed by a much slower rate of adaptation. At still higher light luminances the slow phase of adaptation is more prominent, so that when almost all the rhodopsin is bleached away ($\log I = 6$), the subsequent recovery of the eye takes about 2 to 3 hours, the

[1] Rushton (1960) has shown that bleaching 70 per cent of the foveal cone pigments in man causes a slight increase (0.2 log unit) in the cone increment threshold level. A small change of increment threshold of this order is within experimental variation here and would not be detected.

same time as it takes for rhodopsin to regenerate completely in the rat eye. The slow adaptation thus appears related to rhodopsin bleaching and resynthesis, and the fast adaptation to factors other than photochemical.

Figs. 2 and 3 show typical electroretinograms recorded before, during, and after adaptations to two different intensities of lights. In Fig. 2, the adapting intensity (log I = 3.5) was too weak to reduce significantly the rhodopsin

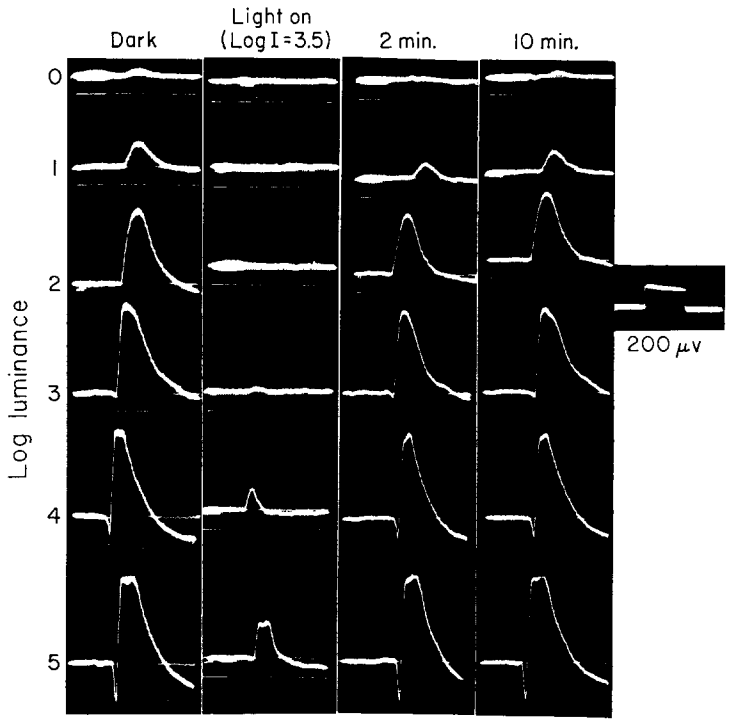

FIGURE 2. Typical electroretinograms recorded before, during, and after 5 minutes' adaptation to light too weak to significantly reduce the rhodopsin content of the eye. When the adapting light was extinguished, the ERG quickly recovered to its dark-adapted level. The duration of the 200 μv calibrating pulse is 0.15 second.

concentration in the eye during the 5 minute adaptation period, and consequently, when the light was turned off, the eye returned to the dark-adapted level very quickly. Within 2 minutes, the threshold had fallen about 2.5 log units and was within 0.3 log unit of the completely dark-adapted level. At 10 minutes the responses were identical with those of the dark-adapted control.

In Fig. 3, the adapting intensity (log I = 5) was sufficiently bright to bleach away about half the visual pigment in the 5 minute light adaptation period. When the light was extinguished, the threshold rapidly dropped in the first 2 minutes from the increment threshold level of about 4.5 log units

to just over 3 log units, but thereafter recovery was quite slow. At 10 minutes the threshold was about 2.5 log units above the dark-adapted level, while at 30 minutes the threshold was still 1.5 log units above the dark-adapted level. By 90 minutes the threshold was within 0.5 log unit of the dark-adapted level, but the eye still had not completely recovered its initial sensitivity. At

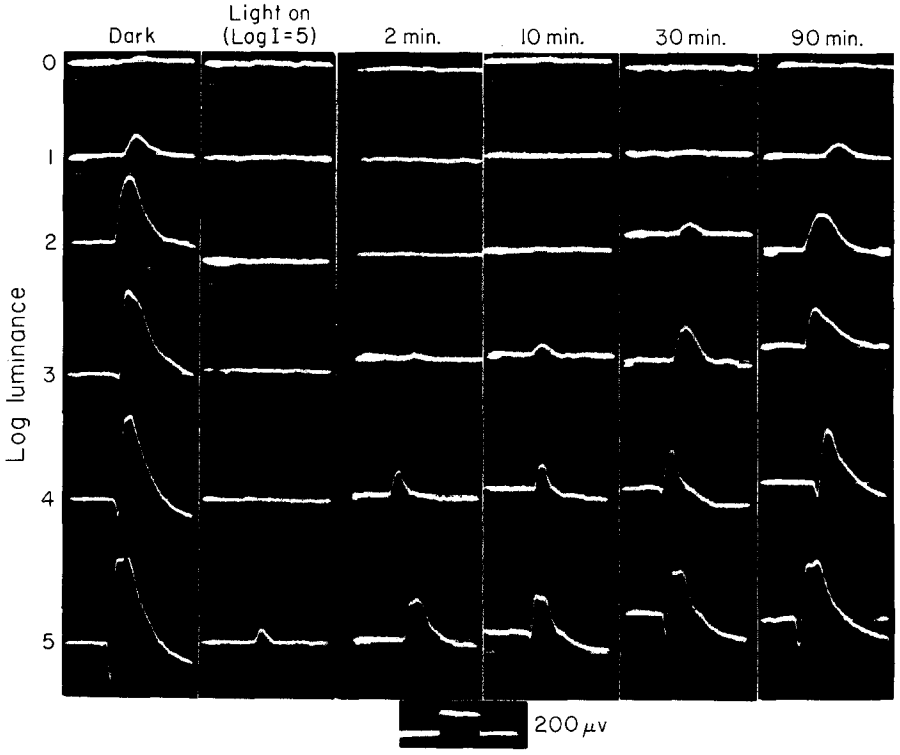

FIGURE 3. Electroretinograms recorded before, during, and after 5 minutes' adaptation to light that bleached about half the rhodopsin in the eye. When the adapting light was extinguished, the ERG threshold quickly fell to about 3 log units above the dark-adapted level, but thereafter recovery was quite slow. Even after 90 minutes the eye had still not regained its initial sensitivity. The duration of the 200 μv calibrating pulse is 0.15 second.

90 minutes the suprathreshold ERG responses were also somewhat smaller than the dark control responses. This is not entirely due to the effects of light adaptation. We find that in the course of a long experiment there is a gradual decline of ERG potential, so that after about 2 hours, as here, the maximal potentials we record are only 60 to 70 per cent of what they were initially. The sensitivity of the preparation during a long experiment, however, remains essentially the same. The decrease in amplitude is probably due to slight drying of the electrode wicks. Ordinarily we run an experiment with a

single animal for no more than an hour, obtaining as many measurements as possible in the first 30 to 40 minutes during which there is not a significant change in the potentials recorded. When we wish to measure the complete course of dark adaptation, we use unanesthetized animals (see below).

Fig. 4 shows the effect of varying the length of the adaptation period on the subsequent dark adaptaton. At low light intensities (*i.e.* log $I = 3$) which do not bleach significant amounts of rhodopsin, varying the length of adaptation has relatively little effect on the rate of dark adaptation. At higher

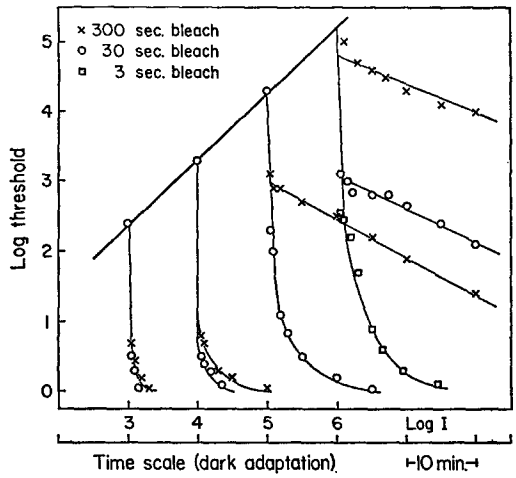

FIGURE 4. The effect of varying durations of light adaptation on the subsequent dark adaptation. At low light intensities, varying the length of adaptation has relatively little effect on the time course of dark adaptation. At higher intensities, the course of dark adaptation is very different depending on length of adaptation. Short adaptation periods do not bleach much rhodopsin and recovery is rapid. After longer adaptation, both fast and slow components of dark adaptation are seen. The extent of slow adaptation is dependent on the amount of light put into the eye (*i.e.* rhodopsin-bleached).

adapting luminances the course of dark adaptation is very different depending on the amount of light put into the eye. If short adaptation periods are used (*i.e.*, log $I = 5$ for 30 seconds or log $I = 6$ for 3 seconds) which do not bleach more than a few per cent of the visual pigment, then recovery is rapid, taking about 10 to 15 minutes. With longer light adaptations, we observe both rapid and slow phases of dark adaptation. The amount of the slow adaptation depends on the product of $I \times t$, a further indication that the slow adaptation is linked to the amount of rhodopsin bleached. We see, therefore, about the same amount of slow adaptation when we bleach at log intensity = 5 for 300 seconds or at log intensity = 6 for 30 seconds; in both cases we have put in sufficient light to bleach about 50 per cent of the visual pigment. Rhodopsin regenerates very slowly in the rat eye, and the Bunsen-Roscoe law ($I \times t = C$)

holds roughly for adaptation periods up to 300 seconds. In the human eye, on the other hand, rhodopsin regenerates very much faster and the Bunsen-Roscoe law holds only for adaptation periods of less than 50 seconds (Campbell and Rushton, 1955).

The amount of the rapid adaptation, on the other hand, is not necessarily dependent on the product of $I \times t$ but is related simply to the adapting luminance which determines the increment threshold level, the starting point of dark adaptation. So, for example, after adapting for 30 seconds at a log intensity of 5, dark adaptation is rapid, the threshold falling about 4 log units in roughly 10 minutes. After adapting with the same amount of light, but 10

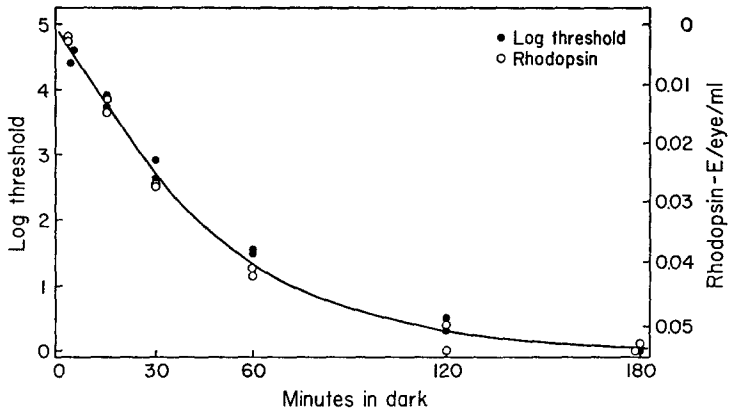

FIGURE 5. The course of the slow component of dark adaptation measured in unanesthetized rats. The fall of threshold occurs in parallel with increase of rhodopsin content of the eye.

times brighter for $\frac{1}{10}$ as long (log I = 6 for 3 seconds), dark adaptation is again rapid but the threshold now falls about 5 log units in about 10 minutes.

To demonstrate that the course of slow adaptation is dependent on the rhodopsin regeneration, a comparison was made between fall of threshold and increase of rhodopsin concentration during dark adaptation. Two groups of 6 albino rats were light-adapted for 30 minutes in a white porcelain pan illuminated with 3 photoflood lamps which were sufficiently bright to bleach all the rhodopsin (comparable to log I = 5.5 in Figs. 1 and 4). At designated times after the adapting lights were extinguished, the ERG threshold was determined. For each measurement a fresh animal was used, yielding therefore the rate of dark adaptation in the unanesthetized animal. This method of determining the rate of dark adaptation was used because it was found that the results were more consistent and the rate of adaptation somewhat faster in unanesthetized animals than in anesthetized animals. As already noted, it is difficult to keep a rat stable under anesthesia for periods of more than an

hour and to measure complete dark adaptation in a rat takes almost 3 hours. (Anesthetic was given to an animal whose ERG threshold was to be determined about 7 minutes before the measurement was made. It took the anesthetic 2 to 3 minutes to take hold, then the animal was prepared and positioned for the measurement in the next 3 to 4 minutes.)

Subsequently, two groups of animals were similarly light-adapted and sacrificed after designated times in the dark. Their eyes were enucleated and analyzed for rhodopsin. One animal (2 eyes) sufficed for a measurement. Fig. 5 shows the results of the experiment, and it is clearly seen that the log of the ERG threshold falls in parallel with the regeneration of rhodopsin.

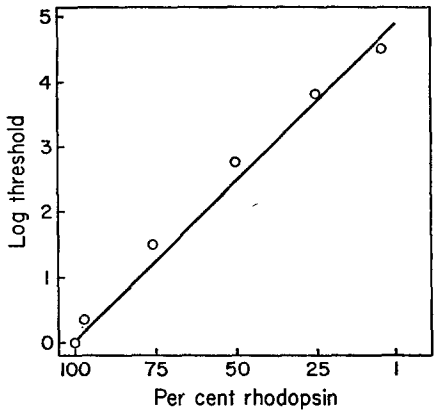

FIGURE 6. The linear relation between log ERG threshold and rhodopsin content in the rat eye.

This result is similar to those of our previous experiments except that the present experiments cover a wider range of thresholds.

Fig. 6 demonstrates the linear relation between concentration of rhodopsin and log threshold. This result is similar to those of our previous experiments except that the total change of ERG threshold per change of fraction of rhodopsin is larger than we found before. In the present experiments, in which a ping-pong ball was used to diffuse the test light, we were uniformly stimulating a much larger portion of the retina than in the earlier experiments which employed a small stimulus beam, and this may be the reason why the present adaptation range is larger. In the human eye, there is a restricted fall of the psychophysical threshold when the course of dark adaptation is measured with a small field, but this effect is seen only when the test field is very small (*i.e.* 10 minutes) (Craik and Vernon, 1941).

DISCUSSION

These experiments show that we can distinguish two mechanisms that operate during dark adaptation, one rapid, the other slow. If the eye is adapted to dim

lights which do not significantly alter the visual pigment levels, dark adaptation is entirely rapid. If during light adaptation a significant fraction of rhodopsin is bleached, dark adaptation is broken into two phases, an initial rapid fall of threshold, followed by a much slower recovery of threshold. The amount of slow recovery observed is dependent on the amount of rhodopsin bleached during the adaptation period, so that if all the rhodopsin in the retina is bleached, the slow fall of threshold extends about 5 log units. The fall of log threshold during the slow recovery of the eye occurs in parallel with the regeneration of rhodospin. Presumably the entire rapid mechanism of adaptation occurs after every light adaptation, but after bright adaptations, its full extent is hidden by the slower process.

The slow component of dark adaptation is clearly related to the rhodopsin concentration in the eye. What the fast component of dark adaptation is related to is not so clear. Adaptation in the eye not related to the visual pigment level is usually referred to as neural adaptation, and we use this term here. This, however, is not a specific term and does not clarify what such mechanisms might be. Suggestions have been put forth as to what may be involved in neural adaptation, such as restorative processes and neural network reorganization, but it will be up to future work to decide among the possibilities. We can, however, limit such mechanisms to those that operate either in the receptor or bipolar cell layers because the ERG arises only from these layers of the retina.

Another point to be emphasized is that the total rise of threshold upon light adaptation (*i.e.* increment or instantaneous threshold) is not related to the amount of rhodopsin bleached, but depends almost entirely upon the luminance of the adapting light. Thus, the extent of dark adaptation one observes also depends primarily on the adaptive luminance. Fig. 4 shows this clearly. With any adapting luminance, the total fall of threshold during dark adaptation is about the same regardless of the duration of light adaptation or amount of rhodopsin bleached. What the bleaching of rhodopsin affects is the *course* of dark adaptation; and specifically the extent of the slow component of dark adaptation. Often, though, dark adaptation is not measured from the increment or instantaneous threshold level, but from some point in time after the light has been extinguished. As the bulk of the rapid fall of threshold during dark adaptation occurs in the first few seconds after the light has been extinguished, we then only measure the slow component of dark adaptation, especially after bright light adaptations (*i.e.*, as in Fig. 5).

We might inquire whether the present experimental results have application to psychophysical adaptation measured in man. In these experiments, visual sensitivity is estimated by means of the ERG threshold, which, of course, is not a perfect analogue of the perceptual threshold. It is generally agreed, however, that adaptation measured as here, by evaluating the brightness of

light necessary to evoke a small constant electroretinogram response does approximate psychophysical adaptation (Johnson and Riggs, 1951; Best and Bohnen, 1956). Cone's finding (1963) that the absolute ERG threshold in the rat is less than 10 times higher than the human visual threshold for large fields goes further to support the idea that the ERG threshold is comparable to the perceptual threshold.

Furthermore, the linear relation between log threshold and visual pigment concentration holds for both the psychophysical and ERG thresholds. Finally, rapid neural adaptation has been observed during the initial stages of dark adaptation in man when dark adaptation is measured from the increment or instantaneous threshold level (Blanchard, 1918; Crawford, 1947; Baker, 1953; Baker *et al.*, 1959) and this rapid adaptation has been distinguished from a slower, presumably photochemical, phase of adaptation (Wald, 1957; Baker, 1963).

In some respects, the rat eye is more ideal than the human eye for the present type of experiment. As already noted it behaves like an all-rod retina, and it is possible to study adaptation in a rat eye without cone responses complicating the measurements. In a typical rod-cone eye the two photochemical phases of dark adaptation make it difficult to distinguish and sort out rapid neural changes in adaptation.

The rat eye has another advantage in that the regeneration of rhodopsin is very slow, taking 2 to 3 hours to complete. In man, complete rhodopsin regeneration takes only 30 minutes (Rushton *et al.*, 1955). The slow photochemical adaptation in the rat, therefore, can easily and clearly be differentiated from the fast neural adaptation. Also, the regeneration of rhodopsin in the rat eye may be initially linear rather than exponential. Although in the present experiments not enough measurements were made during dark adaptation to demonstrate this point unequivocally, Lewis' (1957) measurements show this clearly.[2] In other animals, frog (Zewi, 1939), cat (Weale, 1953), and rabbit (Rushton *et al.*, 1955), rhodopsin regeneration is also initially linear, although in other cases, including man, rhodopsin regeneration is exponential (Rushton *et al.*, 1955). By assuming an initial linear regeneration of rhodopsin in the rat, we can calculate roughly the amount of regeneration occurring in the eye during a light adaptation period, provided that the same mechanism for regeneration operates, both in the light and

[2] Lewis, measuring rhodopsin by retinal densitometry, found regeneration in his anesthetized animals to be very slow, taking over 5 hours to complete. As noted above, we have found that anesthesia slows down dark adaptation in the rat. Recently Dodt and Echte (1961) measured dark adaptation in anesthetized rats and also found that it was exceedingly slow. After 5 hours in the dark, the ERG thresholds of their animals were still raised about 2 log units. Tansley (1931) long ago measured regeneration in unanesthetized rats and found it was 80 to 90 per cent completed in 2 hours. This rate of regeneration is similar to our results with unanesthetized animals.

dark (Lewis, 1957). During the initial stages of dark adaptation, rhodopsin regenerates in the rat eye at the rate of about 1.5 per cent per minute, and on this basis we estimate that less than 7 per cent of the total rhodopsin regenerates during 5 minutes of light adaptation.

This study was supported in part by grant AM-05553-02 from the United States Public Health Service.

I should like to thank Professor George Wald for many stimulating and fruitful discussions concerning this study.

Received for publication, February 4, 1963

BIBLIOGRAPHY

BAKER, H. D., 1953, The instantaneous threshold and early dark adaptation, *J. Opt. Soc. America*, **43**, 798.

BAKER, H. D., 1963, Initial stages of dark and light adaptation, *J. Opt. Soc. America*, **53**, 98.

BAKER, H. D., DORAN, M. D., and MILLER, K. E., 1959, Early dark adaptation to dim luminances, *J. Opt. Soc. America*, **49**, 1065.

BEST, W., and BOHNEN, K., 1956, Vergleichende Untersuchungen über den Knick in der Dunkeladaptationskurve bei Verwendung des Electroretinograms und der subjektiven Schwellenreizleuchtdicte, *Docum. Ophthalmol.*, **10**, 351.

BLANCHARD, J., 1918, The brightness sensibility of the retina, *Physic. Rev.*, **11**, 81.

BRINDLEY, G. S., 1960, Physiology of the Retina and Visual Pathway, London, Edward Arnold Ltd.

CAMPBELL, F. W., and RUSHTON, W. A. H., 1955, Measurement of the scotopic pigment in the living human eye, *J. Physiol.*, **130**, 131.

CONE, R. A., 1963, Quantum relations of the rat electroretinogram, *J. Gen. Physiol.*, **46**, 1267.

CRAIK, K. J. W., and VERNON, M. D., 1941, The nature of dark adaptation, *Brit. J. Psychol.*, **32**, 62.

CRAWFORD, B. H., 1947, Visual adaptation in relation to brief conditioning stimuli, *Proc. Roy. Soc. London, Series B*, **134**, 283.

DODT, E., and ECHTE, K., 1961, Dark and light adaptation in pigmented and white rat as measured by electroretinogram threshold, *J. Neurophysiol.*, **24**, 472.

DOWLING, J. E., 1960, The chemistry of visual adaptation in the rat, *Nature*, **188**, 114.

DOWLING, J. E., and WALD, G., 1958, Vitamin A deficiency and night blindness, *Proc. Nat. Acad. Sc.*, **44**, 648.

GRANIT, R., 1947, Sensory Mechanisms of the Retina, London, Oxford University Press.

GRANIT, R., MUNSTERHJELM, A., and ZEWI, M., 1939, The relation between concentration of visual purple and retinal sensitivity to light during dark adaptation, *J. Physiol.*, **96**, 31.

HECHT, S., 1942, The chemistry of visual substances, *Ann. Rev. Biochem.*, **11**, 465.

HECHT, S., and MANDELBAUM, J., 1940, Dark adaptation and experimental human vitamin A deficiency, *Am. J. Physiol.*, **130**, 651.

HECHT, S., SHLAER, S., and PIRENNE, M. H., 1941–42, Energy, quanta, and vision, *J. Gen. Physiol.*, **25**, 819.

JOHNSON, E. P., and RIGGS, L. A., 1951, Electroretinal and psychophysical dark adaptation curves, *J. Exp. Psychol.*, **41**, 139.

LEWIS, D. M., 1957, Regeneration of rhodopsin in the albino rat, *J. Physiol.*, **136**, 624.

RUSHTON, W. A. H., 1960, The intensity factor in vision, *National Academy of Sciences-National Research Council Publ. No. 835*, 104.

RUSHTON, W. A. H., 1961, Rhodopsin measurement and dark-adaptation in a subject deficient in cone vision, *J. Physiol.*, **156**, 193.

RUSHTON, W. A. H., 1962, Visual pigments in man, *Scient. Am.*, **207**, 120.

RUSHTON, W. A. H., CAMPBELL, F. W., HAGINS, W. A., and BRINDLEY, G. S., 1955, The bleaching and regeneration of rhodopsin in the living eye of the albino rabbit and of man, *Optica Acta*, **1**, 183.

RUSHTON, W. A. H., and COHEN, R. D., 1954, Visual purple level and the course of dark adaptation, *Nature*, **173**, 301.

STILES, W. S., 1939, The directional sensitivity of the retina and the spectral sensitivities of the rods and cones, *Proc. Roy. Soc. London, Series B*, **127**, 64.

TANSLEY, K., 1931, The regeneration of visual purple: its relation to dark adaptation and night blindness, *J. Physiol.*, **71**, 442.

WALD, G., 1954, On the mechanism of the visual threshold and visual adaptation, *Science*, **119**, 887.

WALD, G., 1957, Retinal chemistry and the physiology of vision, *in* Visual Problems of Colour, National Physical Laboratory Symposium No. 8, London, Her Majesty's Stationery Office, **1**, 7.

WALD, G., 1961, The molecular organization of visual systems, *in* Light and Life, (W. D. McElroy and B. Glass, editors), Baltimore, The Johns Hopkins Press, 724.

WALD, G., BROWN P. K., and SMITH, P. H., 1955, Iodopsin, *J. Gen. Physiol.*, **38**, 623.

WALD, G., JEGHERS, H., and ARMINIO, J., 1938, An experiment in human dietary night blindness, *Am. J. Physiol.*, **123**, 732.

WALLS, G., 1934, The visual cells of the white rat, *J. Comp. Psychol.*, **18**, 363.

WEALE, R. A., 1953, Photochemical reactions in the living cat's retina, *J. Physiol.*, **122**, 322.

ZEWI, M., 1939, On the regeneration of visual purple, *Acta Soc. Sc. Fenn.*, **42**, No. 4.