The Rat Electroretinogram

I. *Contrasting effects of adaptation on the amplitude and latency of the b-wave*

RICHARD A. CONE

From the Department of Physics, The University of Chicago

ABSTRACT Characteristics of the electroretinogram (ERG) produced by the essentially all rod eye of the rat are presented as functions of the number of quanta absorbed by each rod per stimulus flash. The ERG's were obtained with 1.5 msec. stimulus flashes and uniform illumination of the entire retina. Under these conditions, distortions in the ERG due to stray light are minimized, and the ERG more accurately reflects the activity of its retinal sources. The effects of background light and two forms of dark adaptation were studied and compared. The results, especially for the b-wave, permit an interpretation in terms of two distinct processes. One process appears to determine the b-wave latency. This process is almost independent of the state of adaptation of the retina. The other process does not affect the latency, but determines the b-wave threshold and amplitude. This process strongly depends upon the state of adaptation. Moreover, the effects of dark adaptation on this amplitude-determining process are almost identical with the effects of background light.

I. INTRODUCTION

The experiments reported here are directed at finding out how the adaptation mechanisms in the retina affect the processes which precede and underlie the production of the b-wave of the electroretinogram (ERG). Numerous previous studies have been made to determine the effects of adaptation on the ERG (1–4) but most of these studies have concentrated only on a single form of adaptation for any given recording condition. It appeared that an additional and powerful method for analyzing a complex response such as the ERG would be to make quantitative comparisons between ERG's obtained under a wide variety of adaptation conditions with all other recording conditions held constant. In such a study, if any feature of the ERG is found to be quantitatively independent of the state of adaptation, it is reasonable to as-

sume that this feature must reflect a process which in some way is not affected by the adaptation mechanisms in the retina. Furthermore, if all the adaptation conditions produce identical alterations in some other feature of the ERG, it is reasonable to assume that these adaptation conditions all have some equivalent effect on the retinal process associated with this feature of the ERG. By making such comparisons, then, the quantitative information which is readily supplied by the ERG can be put to good use without requiring more knowledge about the exact origin and interplay of the various ERG components than is now known.

In the present experiments, ERG's were obtained under three quite different adaptation conditions: (a) light adaptation, (b) dark adaptation following a moderate adapting flash, and (c) dark adaptation following an extended exposure to a strong adapting light. The b-wave latencies and amplitudes of these ERG's were measured with high precision and then compared to determine (a) whether any features of the latency or amplitude were independent of these adaptation conditions, and (b) whether any features were altered in the same way by each adaptation condition.

There are two important conditions which must be met if comparisons of such measurements are to be meaningful. First, the stimulation and the state of adaptation of the entire retina must be controlled as reliably as possible. Otherwise, since the ERG is a summation of activity over the entire retina (5), the ERG becomes an uncertain mixture of a variety of responses. Second, because of the known differences in the characteristics of rod and cone receptor systems (6, 7), it is most desirable to obtain the ERG's from an eye dominated by only a single type of receptor system. In this way, systematic changes occurring in one receptor system will not be obscured by changes occurring in the other.

When the ERG is obtained from the rat, both of these conditions can be satisfied fairly well because the rat eye contains almost exclusively rods (100 to 400 or more rods per cone (8, 9)), and because the rat retina is relatively uniform. Lashley has shown (8) that the inner and outer nuclear layers vary less than ± 10 per cent in thickness over most of the rat retina. Also, there is no fovea and no macular pigment. With the rat, therefore, the first condition can be satisfied simply by uniformly illuminating the entire retina. In this case the entire retina will be uniformly stimulated and uniformly adapted, and the sources which contribute to the ERG should be almost as homogeneous as the small group of sources which contribute to the local ERG recorded from an electrode inserted in the retina (10).

In the following experiments, reasonably uniform illumination of the entire retina was obtained by a technique frequently used by psychologists. A small circular section of a ping-pong ball was placed over the eye and then illumi-

nated with a broad beam of light.[1] A calculation of the diffused light distribution indicates that the resulting illumination of the entire retina is almost as uniform as the retina itself. Of even greater importance, however, is that this technique should essentially eliminate the large distortions in the ERG that are caused by stray light when the entire retina is not illuminated directly. It was found earlier (12, 13) that if these adaptation experiments are performed with a Maxwellian view the distortion from stray light responses can invalidate many of the results, even if the Maxwellian view is as large as 110°, a field of view which illuminates almost half of the retina.

II. APPARATUS AND PROCEDURES

Apparatus   To obtain the desired stimulus and adaptation conditions, a stimulator with two light beams was constructed as depicted in Fig. 1. One beam of this stimulator was used for the stimulus flash and the other was used for steady or flashed adapting lights. Both light beams were obtained from a single Sylvania 300 watt zirconium arc. Two images of the small circular arc were focused on two focal plane shutters. These images were then refocused onto a one-half inch diameter section of a ping-pong ball, forming concentric magnified images of the arc. Both of these magnified images were greater than 1 inch in diameter. Therefore only the uniform central region of each image illuminated the ping-pong ball. A water cell for absorbing heat, interference filters for obtaining green light, and Wratten neutral density filters for adjusting the light intensity were placed in each beam as shown. The interference filters had peak transmissions near 500 mμ. The neutral density filters were carefully calibrated on a Cary model 14 recording spectrophotometer and then mounted so that they could be easily combined to cover a range of 8 log units in steps of 0.1 log unit.

The high-speed shutters were constructed by mounting vanes as shown in Fig. 1 onto Offner recording-pen galvanometers. For each stimulus flash, a vane was driven once across the focused beam, thereby delivering equal amounts of light to all areas of the ping-pong ball. The duration of the resulting flash could be made less than 3 msec. in the upper beam shown in Fig. 1, and less than 1.5 msec. in the lower beam, which had the smaller of the two focused images. The shutters were accurately controlled by a Tektronix series 160 waveform generator and two pulse generators. The ERG was amplified by a Tektronix 122 preamplifier with a low-frequency coupling time constant of 0.2 sec. This was long enough to avoid significant distortion in the ERG amplitudes here reported. The signal was displayed and photographed on a Tektronix 502 oscilloscope which could be triggered either by the pulse generators, or by a photocell placed to catch stray light from the stimulus beam.

Procedure   Mature (200 to 300 gm) albino rats (Sprague-Dawley) dark-adapted more than 12 hrs. were used in all experiments. The rats were anesthetized with nembutal (50 mg/kg) injected intraperitoneally, and were then maintained at a level

---

[1] An essentially equivalent method in which a contact-lens electrode is given a matte finish has been employed by Heck and Rendahl (11).

of weak tail reflex by small additional injections. The rats were mounted on their sides with their heads taped into a molded head-rest. By drawing back the eyelids with sutures, the eye was eased up from the socket exposing most of the eyeball, but exerting no undue pressure upon it. In this exposed condition, an electrode consisting of a loop of chlorided silver wire could be lightly but securely placed around the periphery of the cornea, and the eyelids would not short out the ERG current. Also, the section of the ping-pong ball could be placed so that it filled the entire visual field. The indifferent electrode was inserted in a small cut in the cheek. Depending on the level of anesthesia, the peak-to-peak noise level of this preparation was between 5 and 25 microvolts, the noise level decreasing as the depth of anesthesia increased.

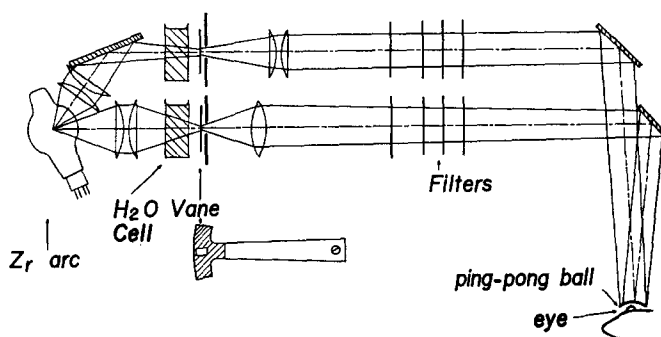Since a light level of anesthesia was used, the animals remained in good condition

FIGURE 1. Diagram of two beam stimulator. Approximate envelope of each beam is indicated. Light-tight baffles which isolate the two beams and prevent stray light from passing around filters are not shown. The section of the ping-pong ball used for a visual field was smaller than shown and intercepted only the central region of the magnified images of the arc.

for times considerably longer than the longest experiment here reported (3 hrs.). In all experiments, stimulus flashes were spaced far enough apart to make the effects of previous flashes negligible. To determine the minimum spacing required, the interval between representative stimulus presentations was increased until stable responses were observed. Several seconds were required at low intensities, several minutes at high intensities. Substantially longer intervals were then employed when obtaining data.

Because both the retina and the retinal illumination used here were uniform, the ERG characteristics could be related to the number of quanta *absorbed* by the average rod. This appears to be the intensity scale most fundamentally related to the responses observed. Therefore, the data are given in terms of an intensity, I, which is the number of quanta absorbed by the average rod per stimulus flash. With this definition, the intensity I could also be called the effective energy per flash. Unless otherwise indicated, the intensity will be abbreviated to read quanta/rod. The measurements needed to calibrate this absolute intensity scale were reported earlier (12). From careful consideration of all the measurements involved, it is estimated that the maximum absolute error is ± 0.4 log unit for the data reported here. This error limit is small compared to the range of intensity studied, and the absolute accuracy has been

confirmed by an independent test in which the rate of pigment bleaching was observed and found to be in accord with the scale (12).

The size of each symbol in most of the following figures indicates the estimated maximum error encountered in measuring the corresponding ERG characteristic. Near threshold, the noise level produced most of the uncertainty. At high intensities, the error limit was either estimated from how well the characteristic was defined, or, if well defined, from its reproducibility throughout the experiment and the accuracy with which it was measured from the oscilloscope photographs.

III. CHARACTERISTICS OF THE RAT ERG

An essential step in this investigation was to determine which features of the ERG were suitable for making quantitative comparisons between the effects
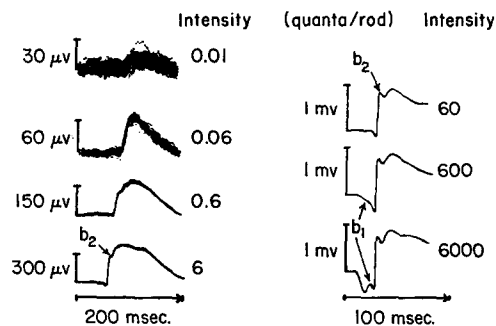

Figure 2. Photographs of the dark-adapted ERG of the rat obtained with a 1.5 msec. stimulus duration and uniform illumination of the entire retina with white light. The stimulus intensity for each record is the number of quanta absorbed by the average rod per stimulus flash. The oscilloscope sweep was triggered by the flash, and multiple traces were superimposed at low intensities to help smooth out the noise. Data in Figs. 3 and 4 were obtained in part from these records.

of the different adaptation conditions. It was also necessary to firmly establish characteristics of the dark-adapted ERG in order to form a basis for comparison. Fig. 2 shows a selection from a series of photographs of ERG's obtained from a fully dark-adapted rat. Measurements from this same series of photographs were used in constructing Figs. 3 and 4 which show some of the features of the rat ERG that were considered for use in this investigation. Data from this animal were chosen because they approximate the average of data obtained under these same conditions from more than twenty rats.

Certain features of the ERG were found to be inappropriate because they were not entirely consistent from rat to rat. For example, the four or five subsidiary peaks which appear in the b-wave (the first two are labeled b_1 and b_2 in Fig. 2) are distributed differently in different rats. This is readily seen by comparing the ERG's shown in Fig. 2 with those shown in Fig. 5. Other features were considered inappropriate because they were not clearly defined.

For example, as the intensity is increased from about 6 to 600 quanta/rod, the b_1-peak begins to emerge (see Fig. 2), and in part of this region it is not clear whether the a-wave appears before or after the b_1-peak. Thus the latencies of the a- and the b_1-waves are ambiguous in the intensity region shown by the dashed lines in Fig. 3.

In contrast with the rather poorly defined starting points of the a- and b_1-waves, it can be seen in Fig. 2 that the leading edge of the b_2-wave makes a sharp intersection with the base line, or with the a- and b_1-waves when they are present. The sharpness of this intersection increases as the retinal illumina-
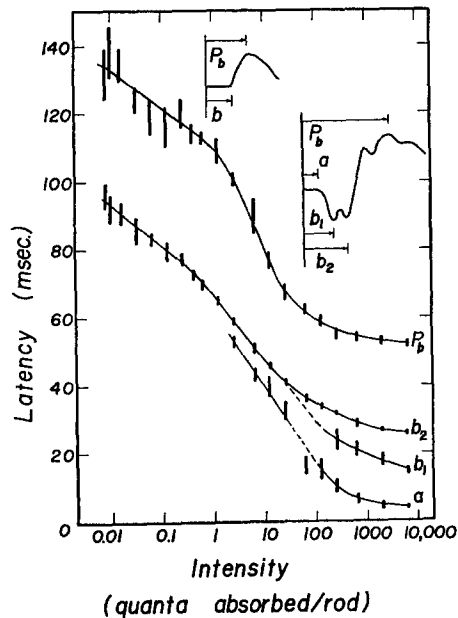

FIGURE 3. Latencies of the ERG obtained from a dark-adapted rat using a 1.5 msec. flash, ping-pong ball visual field, and white light. The data are taken from a complete series of photographs from one rat, some of which appear in Fig. 2. The measurements are made as depicted in the diagrams. Measuring uncertainties are indicated by the length of the vertical bars. The intensity scale, I, is the number of quanta absorbed by the average rod per stimulus flash. On this scale, $I = 1$ corresponds to about 12 trolands for a 1.5 msec. flash duration.

tion is made more uniform. With the present recording conditions this intersection can be used for a precise measure of the b-wave latency (note measuring uncertainties for the b_2 latency in Fig. 3). Of course, this is not the true b-wave latency, but for the experiments considered here, the true latency need not be known. Instead, any feature of the b-wave may be chosen which can be measured precisely and unambiguously and which yields a relatively pure indication of changes occurring only in the latency of the b-wave. In Fig. 3 it can be seen that the b_2 latency changes nearly in parallel with the latency of the peak of the b-wave, P_b. The b_2 latency is therefore closely associated with the latency of the main body of the b-wave. In addition, the b_2 latency can be measured precisely and unambiguously under all adaptation conditions studied here. For these reasons the b_2 latency was chosen for investigating changes in latency of the b-wave, and is referred to simply as the b-wave latency in the following discussion.

Another feature which could be measured unambiguously and with precision under all adaptation conditions was the b-wave amplitude. This amplitude is conventionally measured from the bottom of the a-wave to partially correct for the reduction in the b-wave (Granit's PII) amplitude caused by the opposing a-wave (Granit's PIII; see Fig. 4) (14). However, because the amplitudes of both the PII and PIII components increase rapidly in time at the point where the observable a-wave reaches its maximum size, slight changes in the latencies of these two ERG components would apparently lead
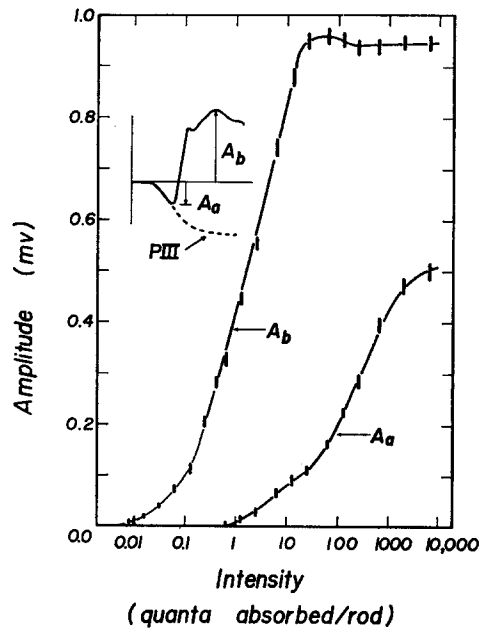

FIGURE 4. Amplitudes of the ERG obtained as described in the caption of Fig. 3 and taken from the same series of photographs.

to large changes in a-wave amplitude. Furthermore, as mentioned above, non-uniform illumination of the retina tends to round off the intersection of the b-wave with the a-wave, again causing relatively large variations in the a-wave amplitude which have little to do with the true variations in the PIII amplitude. For these reasons the a-wave amplitude was considered a less reliable indicator of the retinal processes than the b-wave amplitude. To help make the measurements of the b-wave amplitude independent of these undesired variations in the a-wave amplitude, the b-wave amplitude was measured from the base line as shown in Fig. 4. This gives essentially the net displacement of PIII and PII combined (PIII + PII) because the c-wave (PI) is rarely observed in albino rats. In addition, when measured in this fashion, the b-wave amplitude reaches a plateau value for all intensities above about 20 quanta/rod (see Fig. 4). This interesting characteristic of the ERG is ob-

scured if the b-wave amplitude is measured from the bottom of the a-wave. Apparently the plateau forms because both PII and PIII increase with intensity at the same rate when the intensity is greater than about 20 quanta/rod.

To partially determine the extent of the small contribution of a red-sensitive (possibly cone) system which has been detected in the rat by previous investigators (4, 6), all the following experiments were carried out using both
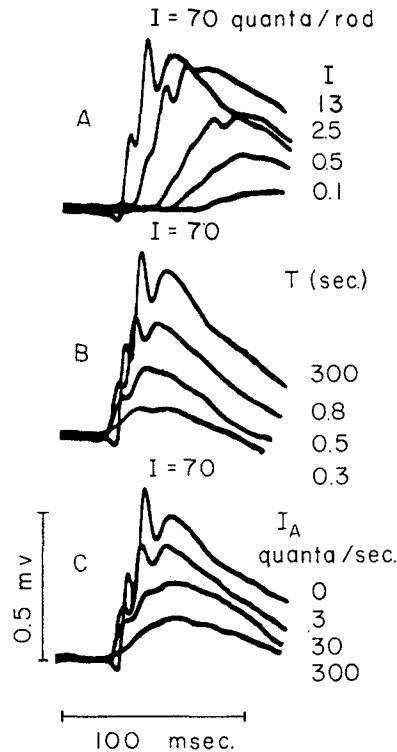

FIGURE 5. Effects of adaptation on the ERG. Superimposed photographs of ERG's obtained from one rat with a 1.5 msec. flash duration, ping-pong ball visual field, and green light. In (A) the stimulus intensity was varied with the rat dark-adapted. In (B) an adapting flash (I = 80 quanta/rod) was presented T seconds before the stimulus flash (I = 70 quanta/rod) to show the effects of rapid dark adaptation. In (C) the stimulus was kept at I = 70 quanta/rod and a steady adapting light was introduced to show the effects of light adaptation. The level of the adapting light, I_A, is given by the number of quanta absorbed per second by the average rod. 1 troland would correspond to about 55 quanta/sec. on this scale.

green (λ_{max} about 500 mμ) and white light. No significant differences were noted. It is therefore believed that the red-sensitive system does not have any important effect on the ERG characteristics primarily considered here, and that these characteristics are most likely produced by the rods alone.

IV. EFFECTS OF ADAPTATION

The effects of three different states of adaptation on the latency and amplitude of the b-wave are shown in Figs. 5 and 6. The three adaptation conditions studied are: (a) Dark adaptation following an exposure to a strong adapting light which bleached a large fraction of the visual pigment. The slow rate at which the eye dark adapts after such an exposure to light appears to be re-

lated to the slow rate of visual pigment regeneration (15, 16). In the following discussion, this adaptation condition will be called photochemical dark adaptation to emphasize this relationship even though the mechanism responsible for the relationship is unknown. (*b*) Dark adaptation following a moderate adapting flash. In what follows, this will be called rapid dark adaptation. It is sometimes called neural dark adaptation because it does not appear to be related to pigment regeneration (15). (*c*) Light adaptation produced by the constant presence of a moderate to weak adapting light.

To illustrate the effects of these adaptation conditions, superimposed photographs are shown in Fig. 5B and C which were taken during the various stages of adaptation. For comparison, ERG's from the fully dark-adapted rat for five different stimulus intensities are shown in Fig. 5A. The resulting ERG's show the simultaneous decrease in latency and increase in amplitude which occur when the flash intensity is increased. By contrast with Fig. 5A, Fig. 5B shows the effects of rapid dark adaptation following a moderate adapting flash. The superimposed responses in Fig. 5B were obtained from the same dark-adapted rat by flashing the stimulus (I = 70 quanta/rod) T seconds after an adapting flash (I = 80 quanta/rod). The eye was allowed to fully dark-adapt between each presentation of this pair of flashes. The amplitude of the b-wave from the stimulus flash decreased as the flash separation, T, decreased, but the latency for the response hardly changed. In fact, the change in latency which did occur was a small decrease from 34 to 29 msec. instead of the marked increase which occurs when the amplitude is reduced by lowering the stimulus intensity as shown in Fig. 5A.

Continuing with the same rat, the responses in Fig. 5C were obtained in the presence of a steady adapting light, I_A. The stimulus intensity was kept at I = 70 quanta/rod. As the level of the background light increased, the amplitude of the b-wave decreased, but the latency hardly changed, again decreasing only from 34 to 29 msec. Thus the effects of these adaptation conditions appear to distinguish at least two processes which are otherwise lumped together when only dark-adapted ERG's are considered. One process evidently determines the latency of the b-wave and is relatively unaffected by these adaptation conditions. Another process evidently determines the amplitude and threshold of the b-wave. This process is strongly affected by these adaptation conditions.

Changes in the ERG similar to those in Fig. 5B and C occur during photochemical dark adaptation, but because of the slow rate of adaptation, it was not convenient to obtain superimposed photographs. Instead, experiments were performed in which a complete series of photographs was obtained for a wide range of stimulus intensities throughout the course of photochemical dark adaptation. The data so obtained were compared with data from similar experiments in which complete series of photographs were obtained
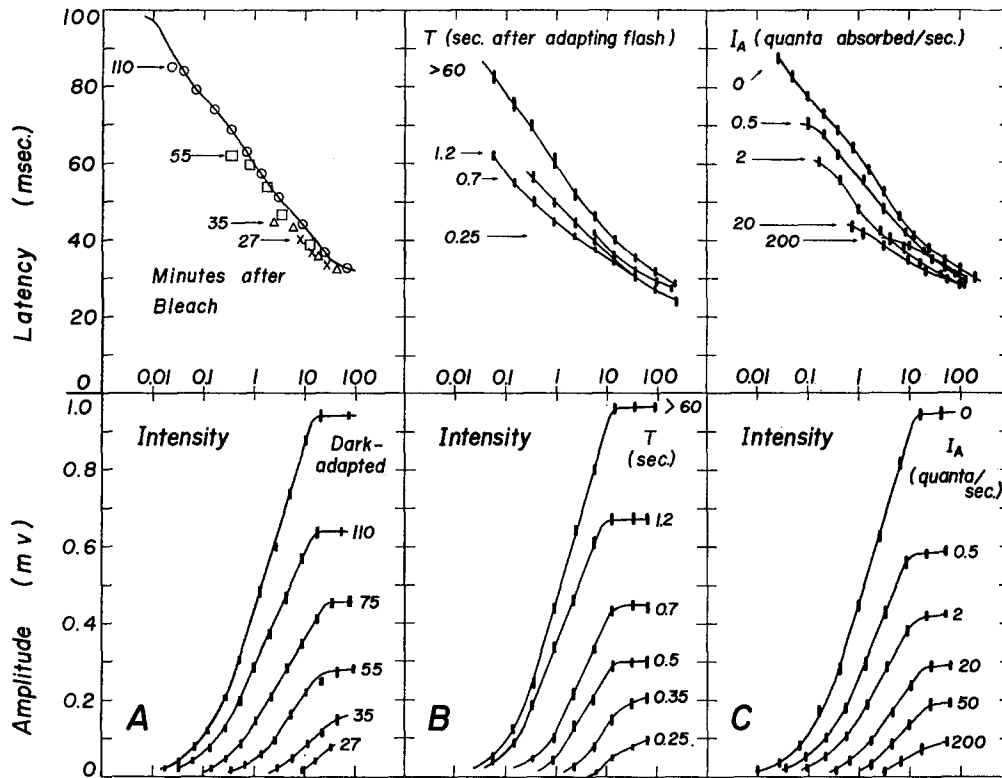
FIGURE 6.   Effects of adaptation on the ERG. (A) Photochemical dark adaptation, (B) rapid dark adaptation, (C) light adaptation. All data obtained from one rat using 1.5 msec. stimulus durations, ping-pong ball visual field, and green stimulus light. In (A), the incident intensities have been corrected for the loss of pigment after the bleach by adding $\Delta \log I = -0.5$ at 27 min., -0.3 at 35 min., and -0.05 at 55 min. The solid latency curve was obtained from the animal when fully dark-adapted. In (B), an adapting flash ($I = 80$ quanta/rod) preceded each stimulus flash by T seconds in the otherwise dark-adapted eye. In (C), an adapting light was introduced. The level of the adapting light, I_A, is given by the number of quanta absorbed per second by the average rod.

under the other two adaptation conditions. In Fig. 6 the effects of all three adaptation conditions on the latency and amplitude of the b-wave are shown as measured from three such series of photographs. To obtain the highest degree of self-consistency, all the data in Fig. 6 were taken from one rat. However, after performing such experiments on more than four rats for each of the three adaptation conditions, it became apparent that the data from all the rats closely approached the same level of consistency.

Photochemical Dark Adaptation   To obtain the data on photochemical dark adaptation shown in Fig. 6A, the b-wave latency and the b-wave amplitude were observed using various stimulus intensities on a fully dark-adapted

rat. Then a strong adapting light was turned on for 1 min. The fraction of pigment bleached by the adapting light could be roughly determined by observing the b-wave threshold because Dowling (15, 17) has shown that the logarithm of the b-wave threshold is linearly proportional to the fraction of pigment bleached. Also, a second, independent measurement of the pigment bleached was available because the level of the adapting light was known in terms of the number of quanta absorbed per second. Both these methods indicated that the adapting light bleached about three-fourths of the visual pigment. During the next 2 hrs., ERG's were obtained with stimulus intensities low enough to avoid retarding the slow regeneration of the pigment. As in the previous figures, the intensity scale in Fig. 6A is a scale of the intensity in terms of the quanta absorbed/rod. To be placed on this scale the incident intensity values must be corrected for the loss of pigment after the bleach. The threshold of the b-wave was used to indicate the fraction of pigment present at the time the data were obtained. Since the largest correction was relatively small, −0.5 log unit at 27 min., this method was considered adequate. The use of this corrected scale makes possible a direct comparison of the effects of photochemical dark adaptation with those of other adaptation conditions because it presents these data consistently in terms of the effective stimulus; *i.e.*, the number of quanta absorbed, not the number incident.

Fig. 6A shows that the latency of the b-wave for a given stimulus is almost unchanged during photochemical dark adaptation even if the threshold of the b-wave changes by 3 log units, and even if the amplitude for any given intensity falls by more than a factor of 10. The family of amplitude curves obtained here is similar to the families obtained by previous investigators (1, 3, 4). However, a new characteristic of the b-wave amplitude is brought out by the present recording conditions and the method used for measuring the b-wave amplitude. The plateau in the amplitude curve which occurs in the dark-adapted eye at an intensity near 20 quanta/rod also occurs throughout most of photochemical dark adaptation, and it always occurs at about the same intensity (Fig. 6A, bottom). Thus, the onset of this plateau seems to be a feature of the ERG which does not depend on the level of adaptation.

Rapid Dark Adaptation   Fig. 6B shows the effects of dark adaptation following a moderate adapting flash. An adapting flash with an intensity of 80 quanta/rod was presented, and then the ERG for the stimulus flash was recorded as in Fig. 5B. In this case, however, responses for a wide range of stimulus intensities were also observed in order to obtain curves like those in Fig. 6A. If the time T between the adapting flash and the stimulus flash was longer than 60 sec., the latency and amplitude were the same as in the fully dark-adapted state. However, as T was reduced to 1.2 sec., the latency for any given stimulus intensity decreased to the level shown and the amplitude also decreased. When T was further reduced, the amplitude continued to

decrease, but the latency shifted back towards the dark-adapted value. The data in Fig. 6B for $I = 70$ quanta/rod may be compared with the superimposed ERG's shown in Fig. 5B which were obtained with about the same stimulus and adapting intensities on a different rat.

From Fig. 6B it can be seen that the b-wave latency for a given stimulus intensity first decreases after a moderate adapting flash, and then returns to the dark-adapted value within a few seconds as the eye becomes dark-adapted again. The maximum reduction in the latency is never more than about one-fourth of the dark-adapted value. However, the threshold of the b-wave may be raised several log units by the adapting flash. It seems especially important to note that the family of amplitude curves produced during rapid dark adaptation is quite similar to the family produced during photochemical dark adaptation. In particular, the amplitude plateau occurs at about the same intensity of $I = 20$ quanta/rod throughout most of the rapid dark adaptation, and for a given rise in threshold, the slopes and curvatures of the amplitude curves are the same. (One curve was obtained at a flash separation of 1.2 sec. because the latency went through a minimum at that time. A somewhat shorter time, about 1.1 sec., would have generated a curve which closely matched the 110 min. curve in Fig. 6A.) Thus by choosing appropriate times, the same amplitude curves can apparently be obtained from either of these two dark adaptation conditions.

Light Adaptation   Fig. 6C shows the effects of light adaptation. After the b-wave latency and amplitude curves were obtained from the same dark-adapted rat, a steady adapting light was introduced and the latency and amplitude curves were again obtained. This was repeated for different levels of the adapting light. The adapting light was on for about 10 min. at each level, and, for the moderate light levels used here, all the responses were stable throughout this period except during the first few seconds after each change in the level. Increasing the level of the adapting light raised the b-wave threshold and reduced the amplitude. In fact, it is seen that the moderate to low light adaptation brought about by the adapting light produced essentially the same family of amplitude curves as those produced by photochemical and by rapid dark adaptation. Again, for a given rise in b-wave threshold, the slopes and curvatures of the amplitude curves were the same, and again, the amplitude plateau occurred at an intensity of about 20 quanta/rod for most levels of light adaptation. Thus, all three of these adaptation conditions have equivalent effects on the b-wave amplitude. Of course, this equivalence in the effects of three quite different states of adaptation would not be so evident unless the ERG were obtained under circumstances which closely satisfy the conditions prescribed in section I. In the case of light adaptation, for example, if the family of amplitude curves is obtained using a small-angle Maxwellian view (2), it only loosely resembles the family of curves obtained here.

The b-wave latency differs from the b-wave amplitude in that it is not affected equivalently by these three states of adaptation. In light adaptation, it is reduced by increasing the level of the adapting light, and then, as the adapting light level is further increased, the latency begins to return to the dark-adapted value (see I_A = 200 quanta/sec. in Fig. 6C). The maximum decrease, as in rapid dark adaptation, is usually less than one-fourth the dark-adapted value. Thus light adaptation and rapid dark adaptation have somewhat similar effects on the latency, but both of these adaptation conditions differ from photochemical dark adaptation in which the latency is hardly altered. To determine whether this difference was permanent and reproducible and not due to some transient adaptation effect, the latency was observed during extended light adaptations. In one experiment, within a few seconds after turning on an adapting light of about 2 quanta/sec., the latency curve shifted approximately as shown in Fig. 6C for this adapting level, and remained unchanged during the next 40 min. Then the adapting light was turned off, and the latency curve rapidly returned to its previous dark-adapted value. Thus the shift in latency caused by a moderate adapting light is not due to a transient adapting effect.

V. DISCUSSION

Latency Characteristics

Since Gotch's work in 1903, it has been known that the latency of the b-wave decreases with increasing stimulus intensity (18). It might be supposed that such a decrease in latency with intensity could occur merely because the amplitude of the b-wave increases, thereby allowing the b-wave to rise above the experimental noise level after a shorter time. But it is clear from the photographs in Figs. 2 and 5, and from data in Figs. 3 and 4 that this is not the case. With the present recording conditions, the leading edge of the b-wave rises so rapidly from the base line that decreasing the noise level of the preparation apparently would not lead to appreciably decreased b-wave latencies. Also, the latency of every subsidiary peak of the b-wave decreases as the intensity increases (Figs. 2, 3, and 5). Moreover, when the amplitude of the b-wave is reduced by changing the adaptation condition, the latency decreases instead of increasing as this supposition would suggest (Fig. 5B and C compared to Fig. 5A). Therefore, the observed latency of the b-wave appears to be caused by a true latent period during which the sources of the b-wave are not active.

The results of the adaptation experiments reveal the following characteristics of the b-wave latency: (a) It is almost unchanged throughout photochemical dark adaptation even though large changes occur in the amplitude and threshold. (b) It is slightly reduced by light adaptation and by rapid dark adaptation, but the slight reduction does not appear to be related to the large

changes which occur in the amplitude and threshold. (c) The slight reduction in latency which occurs during light adaptation persists unchanged the entire time that background light is present.

The first result implies that, throughout the course of photochemical dark adaptation, the process which determines the latency does not depend on the level of adaptation and therefore must depend only on the number of quanta absorbed in the rods per stimulus flash. Evidently, in the other two forms of adaptation studied here, there are small changes in this latency-determining process, but these changes do not appear to be related to the changes in the amplitude of the b-wave. Taken together, these results strongly imply that the process which determines the latency of the b-wave is independent of the process which determines the amplitude of the b-wave. Furthermore, because changes in the amplitude do not affect the latency, the latency-determining process probably occurs earlier in the sequence of visual excitation than the processes which determine the amplitude of the b-wave. In fact, as suggested by several recent experiments (19–21), there is good reason to suppose that the amplitude and threshold of the b-wave are determined in part by the state of adaptation of the b-wave sources in the bipolar cell layer. Therefore, if the latency is primarily determined by a process located in the rods or rod-bipolar synapses, and if this process is little affected by changes in adaptation, the characteristics of the latent period would be as found here. Other characteristics of the latent period are considered in the following paper (22).

Amplitude Characteristics

Two important characteristics of the b-wave amplitude are evident in the data shown in Fig. 6. First, the plateau in the amplitude curve begins at an intensity of about 20 quanta/rod throughout most of the range of each of the adaptation conditions. Therefore this feature of the ERG, like the b-wave latency, is nearly independent of the state of adaptation. This implies that at least one characteristic of the retinal processes which determine the b-wave amplitude, (the sum of PII and PIII, when the amplitude is measured from the base line as in the present experiments), is not affected by these adaptation conditions, and is a function only of the number of quanta absorbed in the rods.

Second, the families of the b-wave amplitude curves obtained under each of the three adaptation conditions are identical within experimental accuracy. Therefore, each of these adaptation conditions must have an equivalent effect on the amplitude-determining mechanisms. This equivalence can be stated more precisely by saying that all the curves found in each of the three adaptation conditions form a single "one parameter" family of curves. Such an equivalence is surprising because of the great differences among the three adaptation conditions. These differences are discussed below.

Photochemical dark adaptation occurs in the rat, as shown by Dowling (15), only if more than about 1 per cent of the visual pigment (rhodopsin) has been converted to the bleached state (retinal + opsin). If a strong adapting light has bleached more than this fraction of the pigment, the logarithm of the b-wave threshold is proportional to the fraction of bleached molecules still present. Rushton has found a similar relation between the fraction of bleached pigment and human visual threshold (16). In experiments with vitamin A–deficient rats (17), Dowling has further demonstrated that it is probably the presence of pigment in the bleached state, and not the process of pigment regeneration, that controls the level of photochemical dark adaptation. In these rats, not all of the pigment could regenerate because insufficient quantities of the chromophore retinal (formed from vitamin A) were available. Nevertheless, the relation between b-wave threshold and the fraction of bleached pigment was the same in these rats as in normal rats. Therefore it is reasonable to assume that the level of adaptation during photochemical dark adaptation is controlled by the fraction of pigment molecules which are in the bleached state.

In each rod of the rat eye there are about 3×10^7 rhodopsin molecules (12). Therefore, during most of photochemical dark adaptation there are about 10^6 to 10^7 pigment molecules in the bleached state in each rod. These relatively enormous numbers may be compared with the small number of molecules which were bleached in producing the light adaptation and rapid dark adaptation studied here. In the case of rapid dark adaptation shown in Fig. 6B, an adapting flash which bleached no more than about 80 pigment molecules/rod was capable of producing a family of curves equivalent to those found during photochemical dark adaptation. Therefore, rapid dark adaptation is distinguished from photochemical dark adaptation not only by a much more rapid decrease in the level of adaptation (seconds as compared to hours in Fig. 6) but also by the considerably smaller number of pigment molecules per rod which need be involved. On the other hand, rapid dark adaptation appears to be closely related to light adaptation because approximately the same small number of molecules are involved and similar reductions occur in the latency. With background light (Fig. 6C), about 200 quanta absorbed per second by each rod produce effects approximately equivalent to those of an adapting flash of 80 quanta/rod when the time after the adapting flash is 0.25 sec. Therefore, the primary distinction between these two adaptation conditions is not that different numbers of bleached molecules are involved, but that in one the light is on, while in the other it is off and has been off for a time longer than the duration of the transient components of the ERG.

It is clear from this discussion of the adaptation conditions studied here that the level of adaptation is strongly altered soon after a small number of pigment molecules absorb quanta (about 1 to 100 per rod). However, this

strong alteration is transient because throughout most of the time in which these molecules remain in the bleached state (hours), the level of adaptation is only slightly altered, unless more than about 10^6 molecules per rod are bleached. Nevertheless, it is clear from the equivalent adaptation effects of the above three adaptation conditions that there is some fundamental equivalence between the strong adapting effect per molecule produced for a short while after the molecule is stimulated by light and the progressively weaker adapting effect per molecule produced from then on until the molecule is regenerated. In this respect, these experiments corroborate the experiments by Rushton (23, 24) in which he finds that for the human visual threshold there is an equivalence between the effects of photochemical dark adaptation and background light. Moreover, these ERG experiments extend this equivalence to include not only the effects on threshold responses but also the effects on responses for all stimulus intensities up to 1 to 4 log units above threshold.

However, it must be pointed out that a complete equivalence does not exist because the b-wave latency is not reduced by photochemical dark adaptation, but it is reduced by background light. Further study should help to clarify the relationship between the mechanism which determines the latency of the b-wave and the mechanisms which are affected equivalently by these adaptation conditions.

REFERENCES

1. Johnson, E. P., *Arch. Ophth.*, 1958, 60, 565.
2. Riggs, L. A., and Johnson, E. P., *J. Exp. Psychol.*, 1949, 39, 415.
3. Johnson, E. P., *J. Exp. Psychol.*, 1949, 39, 597.
4. Dodt, E., and Echte, K., *J. Neurophysiol.*, 1961, 24, 427.
5. Brindley, G. S., Physiology of the Retina and Visual Pathway, London, Edward Arnold, Ltd., 1960, 47, 186.
6. Granit, R., Sensory Mechanisms of the Retina, London, Oxford University Press, 1947, 148.
7. Auerbach, E., and Burian, H. M., *Am. J. Ophth.*, 1955, 40, pt. 2, 42.
8. Lashley, K. S., *J. Comp. Psychol.*, 1932, 13, 173.
9. Walls, G. L., *J. Comp. Psychol.*, 1934, 18, 363.
10. Brown, K. T., and Wiesel, T. N., *J. Physiol.*, 1961, 158, 229.
11. Heck, J., and Rendahl, I., *Acta Physiol. Scand.*, 1957, 39, 167.

12. Cone, R. A., *J. Gen. Physiol.*, 1963, **46**, 1267.
13. Unpublished observations by the author.
14. Granit, R., *J. Physiol.*, 1933, **77**, 207.
15. Dowling, J. E., *J. Gen. Physiol.*, 1963, **46**, 1287.
16. Rushton, W. A. H., *J. Physiol.*, 1961, **156**, 193.
17. Dowling, J. E., *Am. J. Ophth.*, 1960, **50**, pt. 2, 875/205.
18. Gotch, F., *J. Physiol.*, 1903, **29**, 388.
19. Rushton, W. A. H., and Westheimer, G., *J. Physiol.*, 1962, **164**, 318.
20. Mitarai, G., *et al.*, *in* The Visual System: Neurophysiology and Psychophysics, (R. Jung and H. Kornhuber, editors), Berlin, Springer Verlag, 1961, 463.
21. Lipetz, L., *Science*, 1961, **133**, 639.
22. Cone, R. A., *J. Gen. Physiol.*, 1964, **47**, 1107.
23. Rushton, W. A. H., *J. Opt. Soc. America* 1963, **53**, 104.
24. Rushton, W. A. H., personal communication.