

## THE EFFECT OF STIMULUS SIZE AND RETINAL ILLUMINATION ON THE HUMAN ELECTRORETINOGRAM\*

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### INTRODUCTION

When a light stimulus is presented to an eye, a characteristic electrical potential is generated. This potential, called the illumination or action potential of the retina, may be picked up by electrodes, and recorded on an oscillograph. The recording is called an electroretinogram (ERG).

The ERG has, classically, three waves: a small, quick, negative one followed by a large positive one which in turn is followed by a relatively slow positive wave. These have been termed by Einthoven and Jolly<sup>1</sup> *a*, *b* and *c* respectively. A small positive wave sometimes appears after cessation of the light stimulus and is called the *d*-wave. This off-effect is never found in the human ERG.

Granit<sup>2</sup> has investigated three apparently basic underlying processes in the illumination potential which sum to yield the ERG. Recent investigations<sup>3</sup> have confirmed Granit's view that these processes

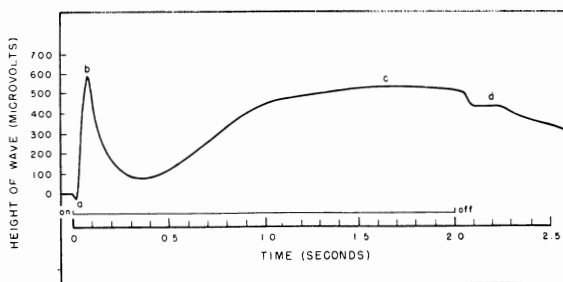


Fig. 1. A classical electroretinogram of the type found in man. (Redrawn from Granit.) The *d*-wave or off-effect is never found in man.

are an oversimplification of the mass electrical phenomena of the retina, but it seems that the processes are still fundamentally sound. Figure 1

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is a classical representation of a mammalian ERG of the type found in human beings, redrawn from Granit.<sup>2</sup> Process (P) I contributes essentially to the *c*-wave; P II, to the *b*-wave, and P III to the *a*-wave. These processes have been separated by the use of various drugs and hypoxia.

The human ERG has often been investigated. Holmgren in 1865 and Dewar & McKendrick in 1873 independently discovered it. While there is much older work in the field, only in recent years, has the human ERG been extensively investigated by Riggs<sup>4</sup> and his collaborators and, clinically, by Karpe<sup>5</sup> and his co-workers. Others have written about the human ERG to a lesser extent.

Germane to the present study, Boynton and Riggs<sup>4f</sup> investigated the effect of stimulus area and retinal illuminance on the ERG. They found that the light scattered by the ocular media was strong enough to dominate the ERG. Variation in the size of the stimulus up to a maximum diameter of  $12^\circ$ , but with a constant total luminous flux caused no obvious changes in the ERG.

The purpose of this paper is to describe the changes in amplitude of the human ERG with varying retinal illuminance and stimulus size (up to  $41^\circ$ ). The data will be viewed with particular reference to the effect of area of the stimulus on the ERG when the total luminous flux is constant.

#### APPARATUS

*Recording Apparatus:* Two systems were used, a direct current (D.C.) amplifier and an alternating current (A.C.) amplifier. The D.C. system, diagramed in Figure 2, consisted of a battery box (zero set) to nullify constant potentials from the electrodes and the subject. The circuit then led to a pair of Brown Converters (choppers) which

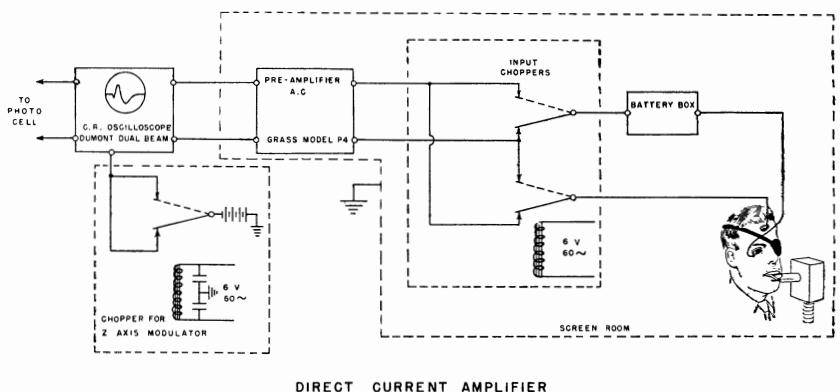


Fig. 2. For explanation see text.

modulated the D.C. input signal with 60 cycle rectangular waves; consequently, the signal could be amplified by the 2 megohm input pre-amplifier. This pre-amplifier was a Grass P4, 4-stage capacity coupled and battery operated. The pre-amplifier, which had a time constant of  $4/5$  second,\* controlled one beam of a cathode ray oscilloscope. A chopper system used for very high gain amplifiers has the serious disadvantage of producing large transient noise signals from the mechanical contacts of the choppers. Because of these, the system while more stable than a direct coupled D.C. amplifier does not necessarily yield as good sensitivity. These transients can be suppressed, however, by modulating the cathode ray oscilloscope (z-axis modulation) so that the beam is suppressed when the noise would appear. This is accomplished by connecting another similar chopper to the z-axis modulator and adjusting it so that the beam is not suppressed during the noiseless central portion of each rectangular wave. Hence, the D.C. amplifier system had the stability and sensitivity (stable at the noise level of  $<3\mu V.$ ) of an A.C. amplifier. However, there was an upper frequency limitation of about 20 cycles which is characteristic of the 60 cycle carrier system. Also there were two traces of the beam, one the mirror image of the other. One of them was partially suppressed by adjustment of the z-axis chopper. An amplification was used such that a one millivolt signal deflected the oscilloscope beam 50 cm.

A Fairchild Oscillo-Record camera with an  $f$  1.5 lens was used.

Eastman Linagraph Ortho 35 mm. film recorded from the P-11 screen which presents short-persistence blue tracer with high photographic efficiency for the film used. The film was run at a constant speed of  $1/2$  inch/second and a built-in neon timer light registered on the film every  $1/5$  second.

The second beam of the oscilloscope was connected to a Weston Photronic cell which was mounted on the stimulus apparatus. It was used as a stimulus marker. Tests indicated that the lag in the barrier type photoelectric cell was not significant for measuring the times involved. However, it was found that the differential drift of the oscilloscope amplifiers and hence that of the beams was too great in relation to film speed (film motion was used as a time base) to yield time measurements of the ERG relative to stimulus time (latent periods).

The tracings on the film were measured with a millimeter rule after projection by a Spencer Microfilm Reader; the reader provided an easily measured image of the ERG 15 times the original size.

\*Time constant based on time required for the output of a constant input signal to fall to  $1/e$  or 37% of its maximum value.

The pre-amplifier provided calibration which was checked by a Calidyne "Calivolt" Model 3 (accurate to  $\frac{3}{4}$  %). This check demonstrated that the Grass calibration was correct within  $2\frac{1}{2}$  %. The amplifier system gave a linear response over the part of the screen used.

The electrodes were of silver-silver chloride. They were made by electrolysis in sodium chloride solution with about +3 volts on the silver wire electrodes and the negative lead on a platinum one. Approximately 5 milliamperes per silver electrode were drawn for less than a minute and stopped when adequate chloridization was completed.

The specific or local electrode was mounted in a contact lens, a technique originated by Riggs.<sup>4a</sup> The contact lens had a special plastic

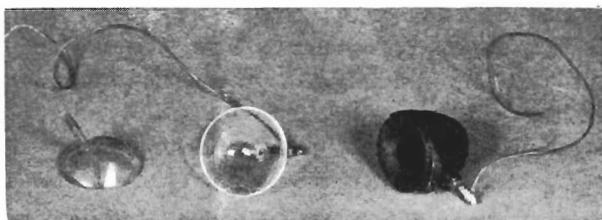


Fig. 3. Left: contact lens with plastic tube receptacle for an electrode. Center: similar contact lens with silver-silver chloride electrode in place. Right: rubber suction cup with remote electrode inserted.

tube to receive the electrode to facilitate its removal for recoating. This may be seen in Figure 3. The indifferent or remote electrode was mounted in a specially constructed rubber electrode cup, also shown in Figure 3,\* which was filled with the same solution used in the contact lens and held on the forehead by suction. Light, flexible wires from both electrodes were held by alligator clips which were mounted through insulation on a leather head band. Shielded leads from the amplifier system plugged into the clips. Figure 4 shows the electrode arrangement. The subject's head was maintained in position firmly by a dental impression bite. The contact lens with electrode was mounted on the right eye and an occluding patch (not pictured) was placed over the left.

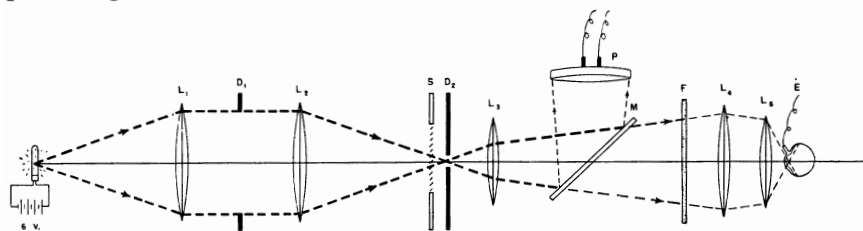
*Stimulus Apparatus:* The stimulus apparatus (Figure 5) consisted of the following. A projection type incandescent lamp with a small coil filament (G.E. 6v., 17 amp.) was energized by lead-acid batteries to avoid introducing electrical noise from without since the whole apparatus was within the screen room. A voltmeter across the battery allowed a monitor of the maintenance of the illuminance level during experimental sessions. Iris diaphragm  $D_1$  controlled the

\*Special care was taken to avoid bubbles which could interfere with the electrode.



Fig. 4. Subject fixed in position by a dental impression bite, with corneal and remote electrodes in place.

angular size of the stimulus ultimately entering the eye from a maximum of  $41^\circ$  to a minimum of  $5^\circ$ . The steps used were chosen arbitrarily by the convenient markings on the diaphragm. Compur shutter S controlled the duration of the stimulus. Diaphragm  $D_2$  was imaged at the nodal point of the eye through lenses  $L_3$ ,  $L_4$  and  $L_5$ . Half-reflecting mirror M diverted part of the light beam to photoelectric cell P which provided the stimulus marker. Filter F consisted of Wratten neutral density filters. Lenses  $L_4$  and  $L_5$  formed a Ramsden eyepiece. An image of  $D_1$  was focused in the plane of  $L_4$  to provide sharp edges for the stimulus spot. The eye was placed at E so that the stimulus bundle entered the pupil (or nodal point) at the focal point of the optical system, thus providing a "Maxwellian view." At maximum field size, the bundle



OPTICAL SYSTEM

Fig. 5. For explanation see text.

was 5 mm. wide which was smaller than the dark adapted pupil.

A Macbeth Illuminometer was used to measure the luminance of the stimulus. It was found that without filters there was a stimulus luminance of 2,600 millilamberts. Stimulus luminance was directly proportional to retinal illuminance and could be considered as equal to it in milliphots assuming no absorption, reflection or scattering by the ocular media.

#### RESULTS

There is a marked change in the shape of the human ERG, when the size of the stimulus field is varied and retinal illuminance is kept constant. As the diameter of the stimulus is increased from  $5^{\circ}$  to  $41^{\circ}$ , the well-known *a*-wave becomes larger, that is, more and more negative. The much studied *b*-wave, after quickly increasing to a maximum becomes smaller as the stimulus increases in size. Immediately after the *b*-wave, there appears a negative wave which is larger than the *a*-wave, but on the average tends to vary with it. This negative after-*b*-wave may be called the *b'*-wave. The course of the change in shape of the ERG with stimulus size is presented in Figure 6 for subject R. H. with the A.C. amplifier and in Figure 7 for subject R. K. with the D.C.

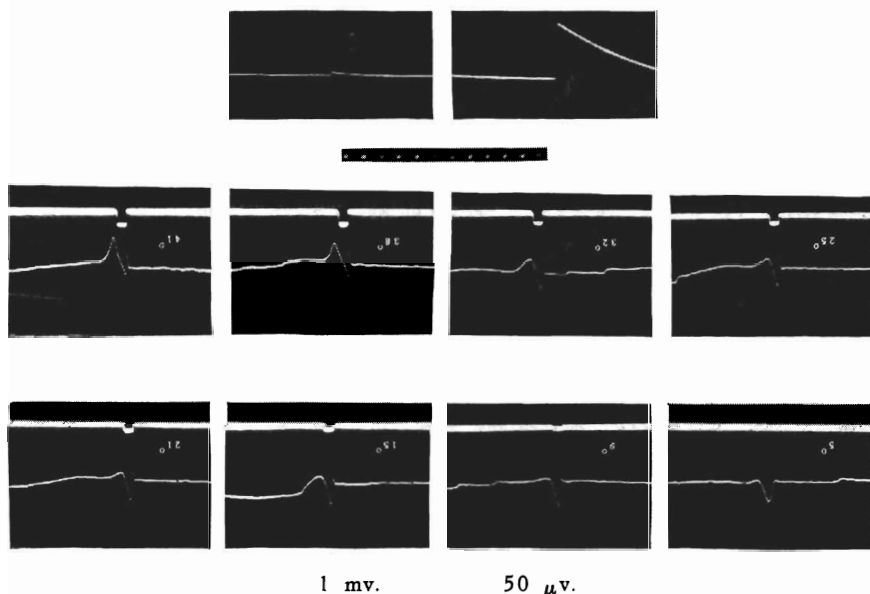


Fig. 6. Series of electroretinograms taken with the A.C. amplifier with increasing stimulus field size from  $5^{\circ}$  to  $41^{\circ}$  diameter as marked. Time scale is 5 dots per second. Subject R. H.

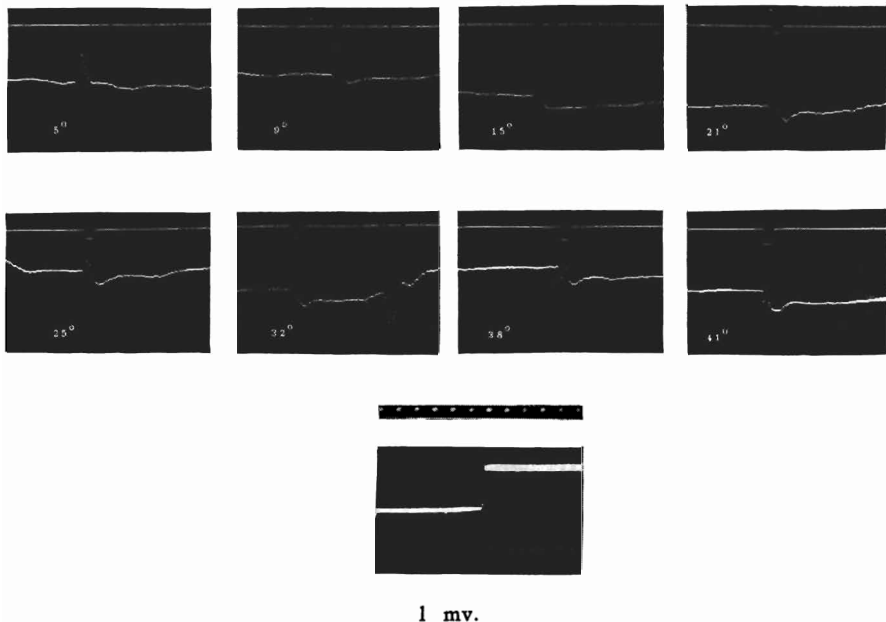


Fig. 7. Same as previous figure except that the records are taken with the D.C. amplifier and the subject is R. K.

amplifier. These are two series of ERG's from the original records which illustrate how the *a*-, *b*-, and *b'*-waves change strikingly with increasing area and constant illuminance.

The height of each wave was measured in microvolts for the two subjects with four runs each. The ERG was determined 10 times for each stimulus size in each run. ERG's for the same stimulus size were averaged. Hence, each point on the curves drawn in Figure 8 is the mean of 80 separate measurements. The course of the negative *a*-wave height with the diameter of the stimulus is seen to be linear except, perhaps, at the extreme ends. The scatter of the points seems to be notably small. The *b*-wave amplitude in this figure exhibits a maximum at about a  $9^\circ$  field and then falls rapidly as the stimulus size is increased.

It is obvious from the graph that the negative after-*b*-wave, or *b'*-wave has a greater scatter than the *b*-wave, and a much greater one than the *a*-wave. However, it is clear that this *b'*-wave, although larger in magnitude, essentially parallels the course of the *a*-wave.

When the same data are plotted as a function of area of stimulus rather than diameter, the curves are somewhat changed. Figure 9 shows

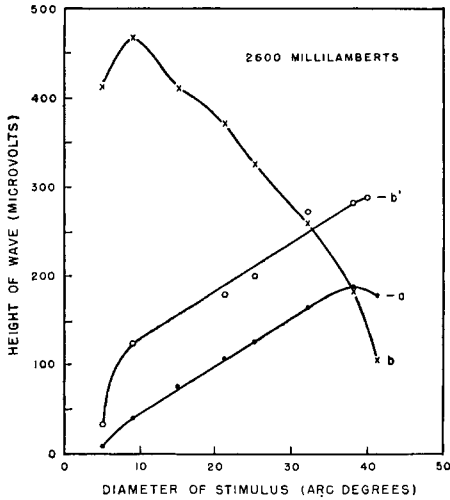


Fig. 8. Mean values of experimental sessions. Note that  $a$ - and  $b'$ -waves tend to be parallel linear functions. These two waves are plotted negatively in order to make a more compact graph.

that the  $a$ -wave plot is no longer linear. Surprisingly, the  $b$ -wave curve, except for the extreme ends, becomes virtually a straight line. If the data are drawn as a function of the logarithm of the area, both  $a$ - and  $b$ -wave plots lose their linearity while the  $b'$ -wave appears to maintain it, probably because of the greater scatter of the points. In plotting the log area, both  $a$ - and  $b$ -waves lose their linear sections as seen in Figure 10.

Let us examine the differences between the two subjects and the

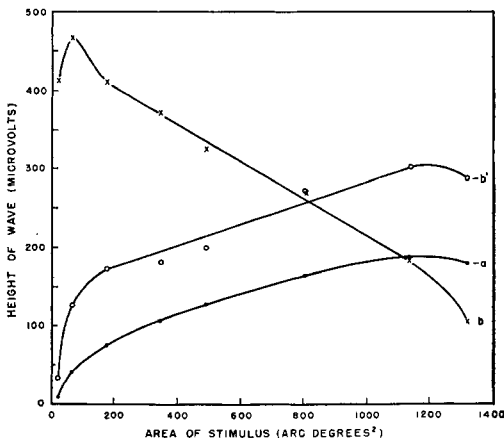


Fig. 9. Replotted data from previous figure. Note that the greater part of the  $b$ -wave curve appears essentially linear.

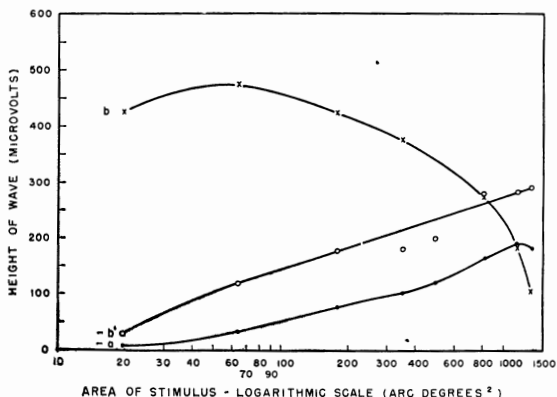


Fig. 10. Replotted data from previous figure.

difference between the electrical methods of obtaining the data. Figures 11, 12 and 13 are plots of the *a*-, *b*- and *b'*-waves respectively of each subject taken with both A.C. and D.C. amplifiers. Each point on the graph represents an average of ten waves. One run was taken with a physiological sodium chloride solution in the contact lens and remote electrode cup instead of the 1½ per cent sodium bicarbonate solution used throughout this experiment.

The D.C. amplifier may limit slightly the fast *a*-wave response because of its limited frequency response. In measuring the *a*-wave amplitudes with the 60 cycle chopper D.C. amplifier the actual peak

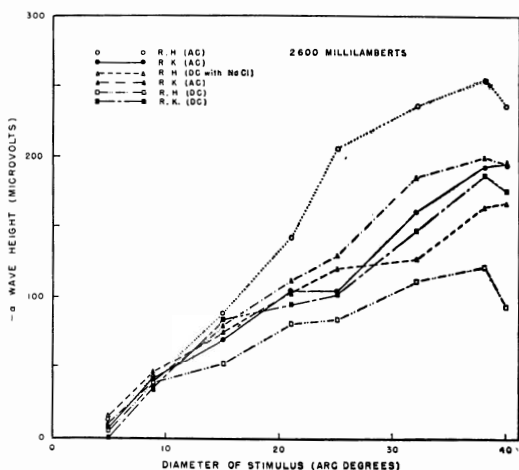


Fig. 11. Plots of individual *a*-wave curves to illustrate possible effects of differences of amplifying system, subjects and electrode fluid. Except where otherwise noted, a 1½ % solution of sodium bicarbonate was used. Each point is the mean of 10 electroretinograms.

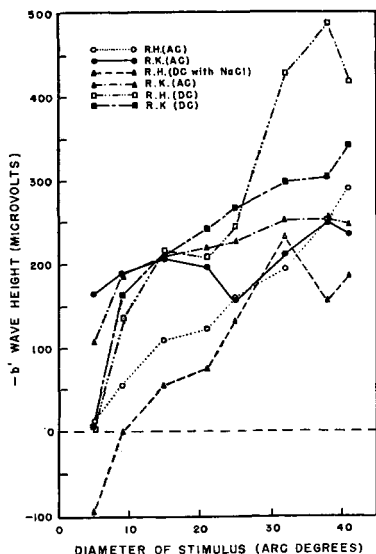
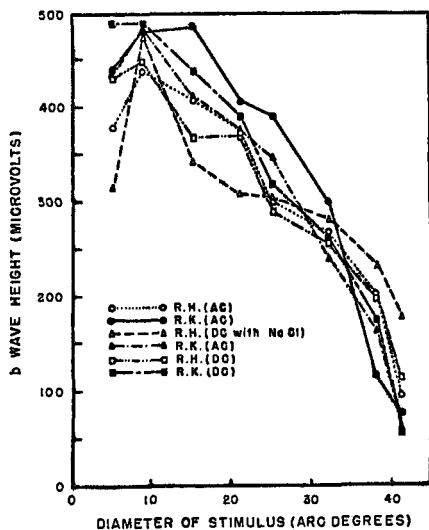


Fig. 12. Plots of  $b$ -waves, similar to previous figure. (Left.)

Fig. 13. Plots of  $b'$ -waves, similar to previous figure. (Right.)

of the wave may be located between the dots that trace the wave form. Measurement of the height of a dot then may give a value less than the true one. This is reflected in Figure 11 where it may be seen that the curves taken with the D.C. amplifier are generally somewhat smaller in potential than the curves taken with the A.C. amplifier. This slight reduction of the amplitude of the  $a$ -wave due to the frequency limitation of the chopper D.C. amplifier does not appear to occur with the slower  $b$ - and  $b'$ -waves.

With the exception noted above, there appear to be no obvious differences attributable to the subjects or the type of electrical amplification. The variation appears to be primarily scatter and day to day changes. For example, the two A.C. curves of subject R. K. were recorded in the same experimental run, separated only by the recording of another similar experiment. These two curves tend to be similar throughout the graphs of the three waves.

The stimuli for each curve were presented in order of increasing size with the exception of those used for R. H. (D.C.) which was from large to small.

The 2,600 millilambert stimulus was reduced in logarithmic steps to 260, 26, 2.6 and 0.26 ml. by the filters. Measurements were taken with three stimulus sizes, 15°, 25° and 41°. One run was taken on each subject and the two were averaged to produce the curves shown in

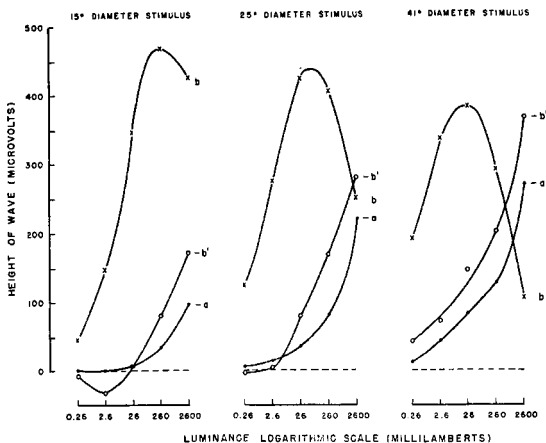


Fig. 14. The effect of stimulus size and luminance on the electroretinogram. Mean values for two subjects. Note that the peak of the *b*-wave curves reduces with larger stimulus diameters. Total luminous flux (area  $\times$  luminance) at the abscissa under each *b*-wave curve maximum is the same.

Figure 14. Each point, then, is an average of 20 waves. It may be worth noting that the position of the maxima of the *b*-wave curves shifts from about 260 ml. at a  $15^\circ$  stimulus to about 26 ml. at  $41^\circ$ . Within limits of error of measurement, the luminous flux appears to be constant for the position of the maximum of the *b*-wave plot regardless of the size and illuminance of the stimulus. However, the maximum height of the *b*-wave appears to depend on the size of the stimulus.

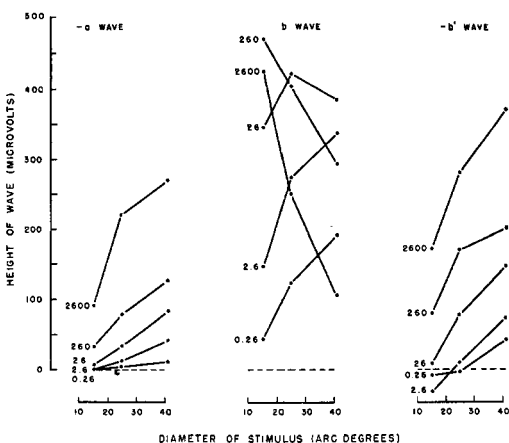


Fig. 15. Replotted data from previous figure illustrating the change of slope of the electroretinogram curves with increasing stimulus luminance. Numerals to the left of each curve indicate the luminance in millilamberts.

The dip of the *b*-wave curve below the base line in Figure 14 is sometimes seen in published curves of the ERG. Often the *b*-wave appears to merge into a *c*-wave without having returned to the base line as seen in Figure 1. In order to relate apparent mergence with the *b*-wave, the after-*b*-wave above the base line was considered as a positive *b'*-wave and so plotted. It appears to show a continuity with the negative *b'*-wave.

The data from Figure 14 were replotted in Figure 15 to illustrate the change of slope of the height of waves and diameter of stimulus curves (Figure 8) by varying the luminance. The *a*-wave plot at 2,600 ml. has a steep, positive slope which decreases with the luminance. The *b'*-wave plot is similar to that of the *a*-wave. The *b*-wave plot has a steep, negative slope at 2,600 ml. It decreases at 260 ml. and is more or less zero in the neighborhood of 26 ml. As the luminance is further reduced, the slope becomes more positive.

#### CONTROL EXPERIMENTS

Karpe<sup>5a</sup> has shown that a wave resembling the *b'*-wave is produced when the corneal electrode is touched by an air bubble. Extreme care was taken to avoid air bubbles and in spite of this the *b'*-wave could always be obtained.

A stimulus duration of  $1/10$  second was used throughout the above experiments. The choice of the duration was based on a desire to have a sufficiently long duration in order to obtain a large electrical response and at the same time a concern not to have the duration long enough to interfere seriously with recovery of the retina in the time allowed before the next stimulus was presented. Figure 16 shows the electrical responses as functions of stimulus duration. All responses obviously fall off at durations of less than  $1/10$  second. The only advantage of longer duration would be the appearance of a strong *c*-wave. However, it was decided not to study this wave at the present time.

With a  $1/10$  second duration and a maximum stimulus size and retinal illuminance, there must be a certain time or critical duration between stimuli which, if reduced, will not allow recovery of adaptation and associated processes which provide the ERG. It was found that each stimulus presented at a three second interval from the previous one did not appear to be affected; however, the interference was obvious in a reduction of the ERG when the interval was reduced to  $1\frac{1}{2}$  seconds. Therefore, the time between stimuli was maintained at about  $\frac{3}{4}$  second and varied between 2 and 5 seconds.

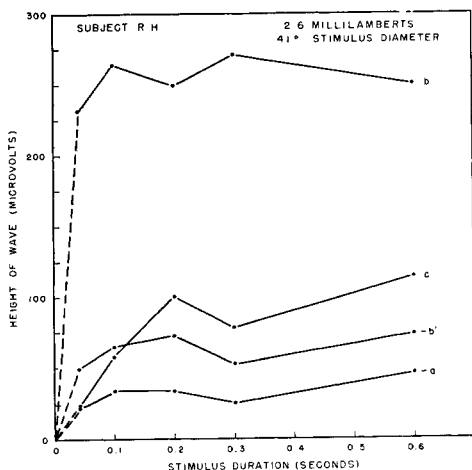


Fig. 16. The effect of stimulus duration on the electroretinogram. The *a*- and *b*-waves are completed within 1/10 second and hence longer stimulus durations would not be expected to affect them. Only the *c*-wave is effectively increased by a duration of more than 1/10 second.

It has often been reported and equally often denied in the literature that light will produce a potential across silver-silver chloride electrodes in a sodium chloride solution. This was tested for the present experiment by mounting the contact lens with its electrode filled with physiological saline at the secondary focus of the stimulus apparatus. The remote electrode was dipped in the same saline and the maximum "stimulus" light was focused on one electrode. No indication of a potential whatever was recorded from this light.

It has been claimed in the literature that there is an electric wave produced, perhaps, as a result of the intraocular musculature. Karpe<sup>5a</sup> has shown that such a wave can be demonstrated with a scleral electrode but is minimized with a corneal electrode, that is, an electrode mounted in a contact lens. Furthermore, this wave which can be recorded from the eye which is not being stimulated, disappears with mydriasis following the application of homatropine. In order to be certain that the electrical response was not being influenced by the pupillo-accommodative mechanism, the left eye was stimulated with the maximum stimulus illuminance and size, and the right eye, shielded from all light, was recorded. There was no response to the stimulus in subject R. K. In subject R. H., however, there was a slow response, amounting to an average of  $-62 \mu\text{V.}$  and a mean starting, peak and cessation time of 250, 430 and 750 msec. respectively (Figure 17). The *b*-wave was completed in about 150 msec. with the small stimulus and in

approximately 60 msec. with the large. If the time from the end of the *b*-wave to the peak of the *b'*-wave (50 to 100 msec.) is added to this, one finds that the peak of the *b'*-wave from the start of the ERG is about 110 to 250 msec. Hence, the contralateral effect found in this subject apparently was temporally distinct and, therefore, did not interfere with the measurements of the ERG including the *b'*-wave.

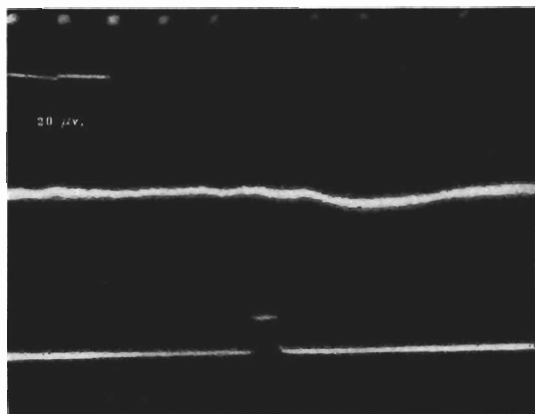


Fig. 17. Small, slow electrical response of subject R. H. to the maximum stimulation of the contralateral eye. Subject R. K. exhibited no response at all under the same conditions.

A control experiment was made with subject R. H. after instilling homatropine and awaiting the mydriasis and the paralysis of accommodation. Substantially the same results were obtained. This is further evidence that neither possible action of the ciliary muscle nor movement of the iris appear to affect the curves previously measured. The pupil, conceivably, could have momentarily constricted to less than the 5 mm. diameter of the beam of light entering the eye. The results with the pupil widely dilated by homatropine do not indicate any noticeable effect from this potential source of error.

The most difficult of all control experiments concerned the holding of the lids to try to determine whether lid motion was responsible for the *b'*-wave. Quantitative measurements were not practicable because fingers on the lids disturbed the base line of the recording. Furthermore there was scarcely enough room for the fingers between the eye and the eyepiece of the stimulus apparatus. The *b*-wave was obtained while the lids were held which indicates that lid movement is not responsible, but these movements may not have been completely eliminated. The *b'*-wave has the same polarity as the electrical pulse resulting from a blink as may be seen in the 32° field record of Figure 7.

It might be thought that the relatively frequent flashes cause enough light adaptation to play a part in the production of the  $b'$ -wave. It was found, however, that the  $b'$ -wave occurred as a result of the first stimulus presented after 25 minutes of dark adaptation. Because of the multiple stimuli presented, the state of dark adaptation was certainly not maximal during the course of the experiment. However, it does not appear that the results were affected by the level of adaptation of the eye.

#### DISCUSSION AND CONCLUSIONS

One of the reasons for using the less convenient D.C. amplifier was to learn if electrode polarization had distorted the records obtained with the A.C. amplifier. It has been shown that polarization is of no consequence if the current density of electrodes does not exceed one microampere per square centimeter at the electrode surfaces in physiological saline solution.<sup>6</sup> The D.C. amplifier was operated from a zero point of no current flow. The actual flow during the ERG was negligible and polarization was not a likely problem. With the A.C. amplifier, the battery box was not used to provide a null reference and the limiting current density mentioned above was sometimes exceeded. However, no difference was found in the records from the two systems of amplifiers which indicated that polarization was no more serious with the A.C. than with the D.C. system even when sodium bicarbonate was used for the electrode fluid.

Fry and Bartley<sup>7</sup> have shown that the ERG as commonly taken with a small field is a nonfocal ERG, rather than focal. In other words, scattered light within the eye over the large retinal area is the cause of the ERG rather than the focused light over a small circumscribed retinal area of a few degrees. Boynton and Riggs<sup>11</sup> and also Asher<sup>8</sup> have confirmed this finding by showing that the ERG produced by a beam of light whose focus is confined to the optic nerve head gives the same ERG as the same beam does on the retina. Fry and Bartley theorize that there are actually two separate ERG's, the focal  $b$ -wave being larger with a shorter latent period than the nonfocal  $b$ -wave. They believe that with a large enough focal area, the two  $b$ -waves may be resolved into a bimodal  $b$ -wave. No bimodal curve was found under the conditions of this experiment. However, evidence was found for the change of relative contributions of the focal and nonfocal  $b$ -waves in Figure 14. As already mentioned, the position of the maxima appears to depend on a constant luminous flux regardless of the size of the field. However, the height of the maxima clearly depends on

the size of the stimulus. The effect of area on the ERG with a constant flux and therefore presumably constant scattering appears to be demonstrated. The fact that the height of the maxima of the *b*-wave curves decreases with increasing area may be attributed to the inhibition of the *b*-wave or the stimulation of the *a*-wave with area.

It has been hypothesized in terms of processes proposed by Einthoven and Jolly<sup>1</sup> and exhaustively investigated by Granit<sup>2</sup> and others, that as P III, which essentially causes the *a*-wave, increases negatively with increasing stimulus, the effect of P II, which essentially causes the *b*-wave, is progressively reduced. One could further hypothesize that as the stimulus is increased, P III becomes more negative and its termination is no longer masked by P II but extends beyond the *b*-wave. This would provide a negative after-*b*-wave which has been demonstrated here and called *b'*. The fact that the *b'*-wave tends to parallel the *a*-wave is evidence for this hypothesis.

Parry, Tansley and Thomson<sup>9</sup> have reported a negative after-*b*-wave in the dog which persisted after severing the optic nerve. The light reflex pathway to the pupil was interrupted and therefore this reflex could not have generated the wave. This after-*b*-wave appears to be the same phenomenon demonstrated here in man.

#### SUMMARY

The human electroretinogram (ERG) was measured with the aid of an electrode mounted in a contact lens. Measurements were made with an A.C. amplifier and a cathode ray oscilloscope and camera. Possible distortions of the A.C. amplifier were evaluated by remeasuring the potentials with a unique, sensitive and stable D.C. chopper (converter) amplifier system.

The human ERG was produced by a stimulus of one-tenth second duration, provided by a "Maxwellian view" of the optical system. Stimulus sizes were from 5° to 41° and luminance up to 2,600 millilamberts. Curves illustrate the course of the *a*-wave and *b*-wave. Of interest is the emergence of a negative wave immediately after the *b*-wave which may be a part of the retinal illumination potential.

Evidence is presented for a change in the relative contributions of the nonfocal and focal ERG's as the stimulus is varied in size and luminance.

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