

## THE ELECTRICAL RESPONSES OF LIGHT-ADAPTED FROGS' EYES TO MONOCHROMATIC STIMULI

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AFTER adaptation to bright sunlight the frog's retina is still capable of responding to strong stimuli with a definite electroretinogram. This, no doubt, must be set up by an initial reaction in the cones which we shall define as active elements lacking visual purple [Granit, 1936]. Two facts support this conclusion: (i) the practically pure rod eye of the white rat gives no electrical response in the light-adapted state [Charpentier, 1936], and (ii) the "mixed" eye of the frog with an approximately equal number of rods and cones shows an electrically measurable Purkinje shift [Himstedt & Nagel, 1901, confirmed by others]. After light adaptation the maximum of the initial *b*-wave of the frog's electroretinogram shifts towards the red end of the spectrum. But the extent of this shift in sensitivity and the precise nature of the curves relating size of *b*-wave to wave-length for an equal energy spectrum has not yet been determined.

Continuing the work of Granit & Munsterhjelm [1937] in which the *b*-waves were measured in the dark-adapted frog's eye, we have now with the same apparatus obtained a similar body of data for the light-adapted preparation. The measurements have also been planned to elucidate the interesting question concerning a possible "specific effect of wave-length" on the electrical response.

Now there can be no doubt about the fact that the frog's eye shows a specific effect of wave-length. This was described by Gotch [1904], by Brossa & Kohlrausch [1913], and more recently by Smit [1934] and Granit & Munsterhjelm [1937]. But since rods and cones separately give rise to *b*-waves, the specific effect of wave-length can be due (i) either to different types of cones being selectively sensitive to different spectral regions, or (ii) to rods and cones giving somewhat different types of responses overlapping in certain parts of the spectrum. Only in the former

case does the specific effect of wave-length assume any significance for colour theory. In the frog's eye the rods alone do not react specifically to wave-length [Granit & Munsterhjelm, 1937; for work on other eyes see Kohlrausch, 1931; Graham & Riggs, 1935].

It is necessary at the outset to realize how a specific effect of wave-length might be demonstrated. Kohlrausch and Brossa have devoted several papers to this question [Brossa & Kohlrausch, 1913; Kohlrausch & Brossa, 1914; Kohlrausch, 1918] and with the pigeon representing one type of eye find that, no matter how intensity of the stimulus is varied, it is impossible to obtain a match between responses elicited by long and short wave-lengths. These experiments have recently been repeated by Graham *et al.* [1935] who have come to quite a different conclusion that, merely by adjusting the intensity of the stimulus, responses from the two ends of the visible spectrum can be made identical.

It is also possible to approach the problem in a slightly different and more satisfactory manner, applied by Brossa & Kohlrausch [1913] in their first contribution to this problem. This is to use equipotential responses from the red and blue side of the spectrum and find out whether in every respect they match. If they do, the method is not applicable to a study of the hypothetical selective sensitivity to different wave-lengths, as identical responses may well be given by, say, photochemical substances with different absorption curves. But if the electroretinograms obtained do not match, except in height of potential, then the equipotential responses certainly are given by different elements or combinations of elements. The chief experimental difficulty is to avoid mixed rod and cone responses due to dark adaptation. Thus in the frog's eye equipotential responses do not always match, but extensive measurements led Granit & Munsterhjelm [1937] to the conclusion that, at least in the dark-adapted eye, such cases are due to rods and cones overlapping. In our opinion the other "true" type of specific effect of wave-length has not yet been convincingly demonstrated for any eye (cf. Graham *et al.* [1935] criticism of the best supported claim in the case of the pigeon).

#### TECHNIQUE

A detailed description of the technique has been given by Granit & Munsterhjelm [1937]. The same string galvanometer, amplifier and Tutton Monochromator were used, the experiments with light-adapted frogs being carried out on sunny days in April, May and June with twenty-seven frogs (*R. esculenta*) obtained from Hungary in March. Frogs from the same group were used in work with dark-adapted eyes

when the sky was cloudy. The animals to be light adapted were first dark adapted for not less than 12 hours. Apart from minor differences of procedure, to be mentioned below, the high intensity curve of Granit & Munsterhjelm [1937]—their Fig. 4—and our standard curve for light-adapted eyes (Fig. 4) have been obtained in the same manner. In order to obtain the latter curve it was only necessary to raise the intensity of the spectral light by 1.3 logarithmic units (removal of the “auxiliary density”, mentioned in the work of Granit & Munsterhjelm to which all our comparisons with scotopic eyes refer, unless otherwise stated). Wave-length  $0.560\ \mu$  was used as a control of the general level of sensitivity of the preparation. The other readings are given in percentage of the value at  $0.560\ \mu$ .

Light adaptation was secured by leaving the glass jar containing the frog for an hour in a sunny window. Then the eyes were removed, padded in cotton soaked with Ringer, and left for a further 10–15 min. in the window. The excised eye was moistened every now and then. The pupil of the light-adapted, intact frog is relatively wide, though, of course, smaller than in the scotopic state. It contracts during the operation, and again dilates when the eye is left undisturbed in the sunshine. Further dilatation takes place in the dark box. In some experiments atropine was tried, but it was soon found to be of little use, as was to be expected.

A great number of experiments were carried out with opened bulbs, and in these cases the bulbs, filled with Ringer, were left for only 5–10 min. in the window after the standard exposure of 1 hour of the intact frog. Several eyes were repeatedly light adapted so that in most cases a fairly large number of observations was available for an eye in good condition.

The readings were taken at a rapid pace, usually with one, sometimes with half a minute between exposures lasting 1 or 3 sec. With good preparations ten exposures of a given wave-length at this rate could be identical within the errors of measurement. The onset of dark adaptation was recognized by several signs, to be mentioned separately below. Sometimes dark adaptation was allowed to proceed, sometimes the eye was readapted to light and the experiment repeated. According to Kühne [1879] visual purple may be detected after some 20 min. of dark adaptation. Actually this is roughly the time within which the responses are free from an admixture of rod reactions (rods being defined as active elements possessing visual purple). But in our case this presupposes that the eye is repeatedly stimulated with a bright light. The well-known optograms of Kühne demonstrate that illumination of a given area leads to local bleaching of visual purple.

Often the retinae were removed after the experiment and inspected. Generally the colour was a pale yellow or red, but then most experiments were carried well beyond the point when signs of dark adaptation occurred. When immediately after light adaptation the second eye was removed and its retina inspected it was always found to be colourless. No oxygen was used in these experiments [cf. Granit & Munsterhjelm, 1937] as it was suspected of furthering regeneration of visual purple.

## RESULTS

### *General observations*

As long as the bulb is intact, the values for size of *b*-wave against wave-length fall on a very definite and regular curve. Diphasic *b*-waves have been noted with the smaller responses towards the end of the spectrum but they have been exceptions, and not the rule, as in dark-adapted eyes. With opened bulbs we have sometimes had indications of a switchboard effect of the type encountered with the scotopic preparation, but though responses to a constant stimulus have changed in size, they have not differed in type as definitely as in the scotopic eye. As diphasic responses have also been seen by Meservey & Chaffee [1927] in the cone eyes of the horned toad and the chameleon, we have every reason to assume the *b*-wave to be multiple in cone eyes. The difference in this respect between the lability of the scotopic eye and the stability of the photopic preparation merely indicates that in the latter case we worked near the threshold, whereas with the light-adapted eye we used relatively strong stimuli. As will be shown below, weak stimuli could not very well have been used.

Another difference between light- and dark-adapted eyes probably has some physiological significance. This is the fact that with the scotopic retina the distribution of sensitivity to the spectrum varied a great deal from eye to eye, whereas the light-adapted retina is a fairly stable preparation until dark adaptation sets in and alters the picture.

### *Recognition of dark adaptation*

Dark adaptation is heralded by several signs to which a great deal of attention must be devoted. Unless it can be regarded as demonstrated beyond doubt that a given type of eye is lacking rods containing visual purple, the question as to whether or not some regeneration of visual purple has taken place must be settled with the aid of definite criteria before a given result can be attributed to the cones alone. The stimuli are

supermaximal for the visual purple system which probably is activated as soon as traces of visual purple appear.

In the dark-adapted eye the *b*-wave is of maximal size between  $0.500$  and  $0.510\mu$  and the response at  $0.560\mu$  is at about 68 p.c. of the maximum. We shall see below that after light adaptation the maximum is at about  $0.560\mu$  [cf. Smit, 1934], and the response at  $0.510\mu$  is about 72 p.c. of the maximum. In accordance with this fact, the general change indicating the onset of dark adaptation is a shift in sensitivity making the short wave-lengths relatively more effective.

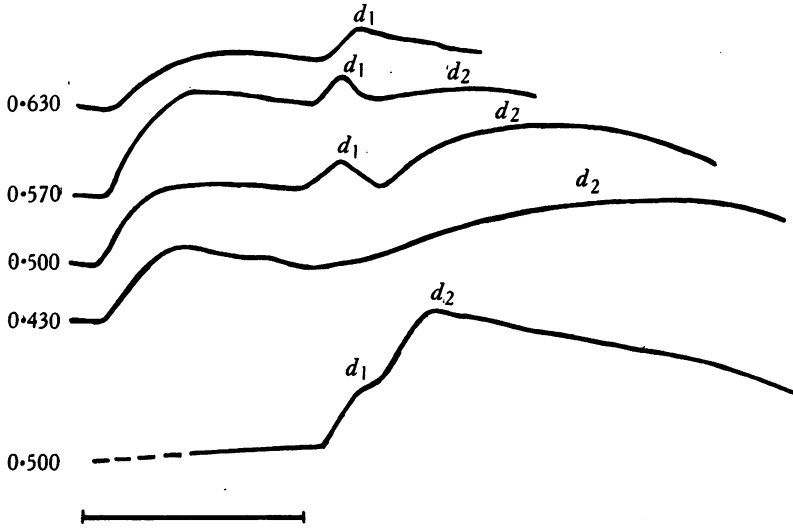


Fig. 1. Series of four responses to 1 sec. flashes at the standard intensity, illustrating diphasic off-effects. The lowest curve shows end of 3 sec. stimulus. Note: Only first phase of off-effect at  $0.630\mu$  (uppermost curve), second phase already present but small at  $0.570$ , a great deal larger at  $0.500$ , only second phase visible at  $0.430$ . The lowest response at  $0.500$  also shows diphasic off-effect, but owing to the long exposure the off-effect larger than in the other curves. Time below 1 sec.

During the course of the experiment the following five criteria are the most useful ones: (i) the appearance of a secondary rise (*c*-wave); (ii) the responses around  $0.500$ – $0.510\mu$  become relatively too large; (iii) the off-effect from having been rapid and monophasic often becomes diphasic, an initial, small and rapid phase, and a second slow rise (see Fig. 1), part of which may be a *c*-wave; (iv) when the responses at  $0.620$  and  $0.470\mu$  are compared the secondary slow rise of the *c*-wave and the similarly slow second phase of the off-effect are found to be absent at  $0.630\mu$  and large at  $0.470\mu$  at a time when the difference in the *b*-waves has not yet clearly

developed; (v) the changes mentioned above are progressive. In the best eyes all these changes are to be seen simultaneously.

The secondary rise and the second phase of the off-effect are identified as rod phenomena by following the standard curves for size of *b*-wave against wave-length which characterize the rods [Granit & Munsterhjelm, 1937] and also with the aid of the very convenient criterion (iv). Long ago v. Brücke & Garten [1907] showed that in the frog's eye the *c*-wave disappears after light adaptation. Diphasic off-effects have also been seen before, e.g. by Smit [1934], but the second slow phase does not seem to have been identified as a rod property. It may have some significance that the differentiation of the off-effect into two phases is not easily detected in a completely dark-adapted eye.

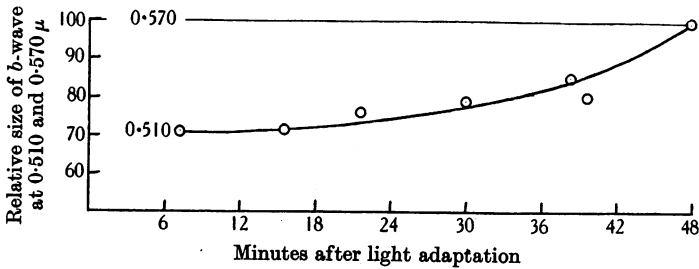


Fig. 2. Wave-length 0.570 is given the value of 100 and 0.510 gradually rises to the same value as dark adaptation takes place. Zero abscissa is at first exposure of the eye immediately after light adaptation. Opened bulb.

When the responses are measured further opportunities of identifying rod activity arise. Thus the rate of rise of the *b*-wave becomes relatively faster towards the short wave-lengths, as is found in the completely dark-adapted eye at high intensities of stimulation. The maximum of the curve for the *b*-waves (see Fig. 4) expands, particularly towards the blue. The values in the red then become relatively too small. All these effects can be predicted from the known properties of the rod system of this eye on the assumption that rod responses are superimposed upon cone responses [see Granit & Munsterhjelm, 1937] and need not be further discussed.

The time course of the changes indicating dark adaptation have raised some interesting points. The usual expansion of the maximum is shown in Fig. 2. This is from an experiment in which the eye had been readapted to light, having already served once for a series of measurements at different wave-lengths. The wave-lengths 0.570 and 0.510 μ were taken alternately, and after some ten or twenty readings the time was noted and the string calibrated. The observations preceding each calibration were

averaged and have been marked in the diagram as referring to the moment of calibration. The *b*-wave at 0.570 μ has been given a constant value of 100 and the diagram shows how the *b*-wave at 0.510 μ rises to become identical with the reading at 0.570 μ after 48 min. During the first 20 min. their mutual relationship remains practically unchanged. These time intervals are typical of most experiments, but there have been exceptions. With one eye we succeeded in taking ninety-five observations, all of which fell on much the same curve for size of *b*-wave against wave-length. The averages differed by a mean variation of only 2.8 p.c. from the final averages for the light-adapted eye shown in Fig. 4.

Whereas observations of this type show that, despite the presence of rods, it is possible to isolate another element with a fairly stationary distribution of sensitivity to spectral light, they hold good only for size of *b*-wave as an index of this sensitivity. They require the further proviso that the intensity be relatively high.

If size of *b*-wave, off-effect and rate of rise of the *b*-wave all be measured in the same experiment it is found that the three indexes do not show signs of dark adaptation at the same moment. The off-effects first increase towards the short wave-lengths. Next in order of sensitivity to dark adaptation comes the rate of rise of the *b*-wave. The process least sensitive to dark adaptation is the one determining the size of the *b*-wave. This is shown in Table I where the initial values are given to the left and

TABLE I

Wave-length μ	Initial values						Later values					
	0.450	0.490	0.500	0.530	0.580	0.650	0.440	0.470	0.500	0.540	0.600	0.640
Order of observation	5	8	2	6	9	10	16	24	13	25	15	19
Rate of rise of <i>b</i> -wave	28	47	70	92	74	33	28	53	63	114	—	—
Size of <i>b</i> -wave	20	38	65	87	92	32	20	41	63	89	70	33
Size of off-effect	39	82	76	89	85	32	73	105	112	116	48	33

All values are given in percentage of reading at 0.560 μ.

later values to the right with the figure for the order of the observation in the upper horizontal row. The later *b*-waves do not differ very much from the earlier ones, but “rate of rise” and “size of off-effect” have increased a great deal in the short wave-lengths, the latter values already being distributed as in a dark-adapted eye. All values are given in percentage of the reading at 0.560 μ.

The danger of using too low an intensity is illustrated by Fig. 3, representing the result of an experiment in which alternating observations at a high and at a low intensity were obtained by removing and replacing a filter. The low intensity (circles) was the one used by Granit &

Munsterhjelm [1937] for their high intensity scotopic curve. The high intensity is the standard stimulus used in the experiments presented in Fig. 4. It is obvious that the readings at the low intensity (circles) show an effect of dark adaptation which is much less marked at the higher intensity (dots). Incidentally the results demonstrate that the maximal intensity, used by Granit & Munsterhjelm [1937], actually was above the cone threshold, as concluded by them from other evidence.

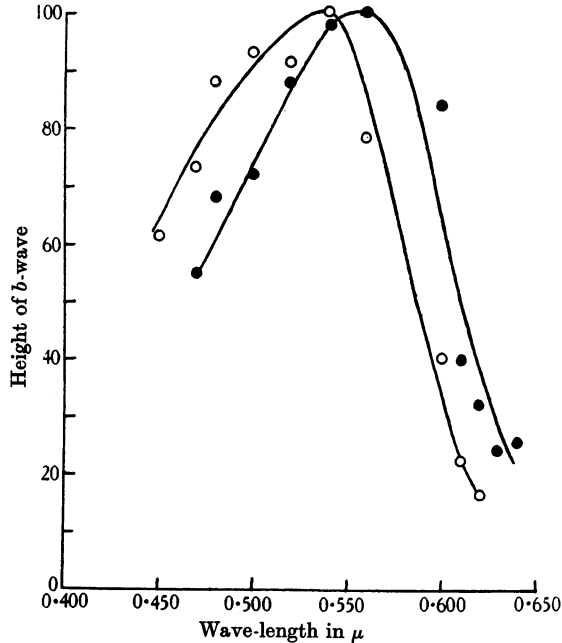


Fig. 3. Size of *b*-wave against wave-length. Circles, low intensity; dots, standard (high) intensity (see text). *Note:* Low intensity curve shifted towards the short wave-lengths. Dark adaptation just beginning.

Relative to an index of sensitivity or "visibility" such as size of *b*-wave dark adaptation may be latent or manifest. Apparently dark adaptation takes place very rapidly, but for some reason or other high intensity *b*-waves do not register this change until a certain limiting value has been reached. Thus size of *b*-wave is a more reliable index of pure cone vision than its rate of rise or the size of the off-effect. Why the off-effect, when present, should be so sensitive to dark adaptation is still a matter of conjecture.



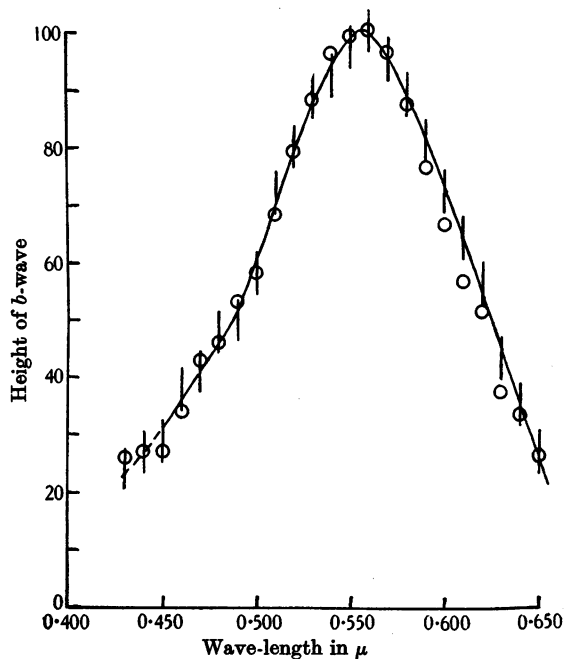


Fig. 4. Average results of 295 observations (six series) showing height of *b*-wave, plotted as lines (mean variation doubled), and its rate of rise (circles) against wave-length. Only the former curve drawn. Average potential of *b*-wave at 0.560  $\mu$  is 0.230 mV.

TABLE II

Wave-length $\mu$	Size of <i>b</i> -wave in percentage of 0.560	Mean variation
0.420	18	1.5
0.430	24	2.8
0.440	27	4.5
0.450	29	4.3
0.460	38	3.2
0.470	41	3.8
0.480	48	3.8
0.490	50	7.0
0.500	58	5.5
0.510	72	5.2
0.520	80	4.0
0.530	89	1.9
0.540	92	2.4
0.550	97	3.0
0.560	100	—
0.570	95	3.8
0.580	89	3.2
0.590	81	2.8
0.600	72	2.3
0.610	64	3.5
0.620	56	2.5
0.630	43	5.8
0.640	35	4.5
0.650	27	4.3

*The cone response*

The results presented in Fig. 4 and Table II show the averages of 295 readings obtained from six series, all interrupted when some influence of dark adaptation on the *b*-wave was detected. The series were averaged separately and the mean variation between them is shown in the table.

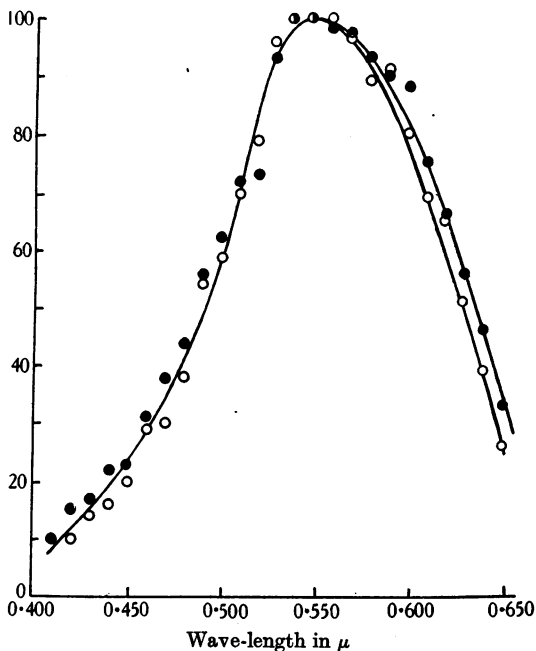


Fig. 5. Dots, size; circles, rate of rise of *b*-wave against wave-length at maximal intensity. Single experiment.

The latter figures indicate maximal lability in the region around the maximum of the rod curve between 0.490 and 0.530 μ. The circles in Fig. 4 refer to rate of rise of the *b*-wave. The curve for size of *b*-wave, drawn through the lines, is symmetrical between 0.490 and 0.650 μ around a maximum at 0.560 μ. From 0.480 μ farther towards the blue and violet the values are relatively too high to fit a symmetrical curve.

The curve for rate of rise of the *b*-wave has not been drawn, but obviously it is identical with the curve for size of *b*-wave except towards the red end beyond about 0.590 μ. Equipotential responses in the red and in the blue therefore differ with respect to  $dp/dt$ , the red responses being relatively slower.

Size of  $b$ -wave and its rate of rise are plotted in Fig. 5 for a still higher intensity than the one used above. This was obtained by trebling the width of the collimator slit. The results are from a single experiment in which the eye was light adapted twice, giving a total of seventy-two observations to be measured and plotted. The maximum is expanded and the values towards the red side somewhat larger than those of Fig. 4. The same intensity was used in the experiments with excessive light adaptation to be mentioned below.

*The specific effect of wave-length and the off-effect*

The curves of Figs. 4 and 5 show a specific effect of wave-length, inasmuch as the responses in the short wave-lengths reach a given potential more rapidly than those in the long wave-lengths. Is this effect a pure cone property or does it depend upon rods and cones overlapping in this part of the spectrum?

We have seen that dark adaptation is characterized by the  $b$ -waves becoming relatively faster towards the short wave-lengths and that this change may be noticed before dark adaptation has influenced the size of the  $b$ -wave as a function of wave-length. If the cone  $b$ -waves actually are relatively faster towards the short wave-lengths, then this effect should be noticeable in all experiments, and also in the earliest values taken in each series. As a matter of fact this is not the case. There have been individual experiments in which rate of rise and size of  $b$ -wave are perfectly symmetrical with respect to one another, and the slight specific effect of wave-length in the final averages undoubtedly is due to dark adaptation, latent with respect to size of  $b$ -wave, but manifest in its rate of rise. The specific effect of wave-length thus belongs to the second of the two types mentioned in the introduction. The section on recognition of dark adaptation shows other instances of the same type of effect, in fact, most of the criteria used for diagnosing dark adaptation depend upon the appearance of a specific effect of wave-length.

If two potentials be otherwise identical but different with respect to the size of the off-effect, this also would represent a specific effect of wave-length and we would have to raise the same questions as above with respect to  $dp/dt$ . As a matter of fact all off-effects are relatively too large between about 0.460 and 0.520  $\mu$ . Let us as an illustration choose an experiment in which the size of the  $b$ -wave and its rate of rise were symmetrical with respect to one another and latent dark adaptation thus at a minimum. Even in this case, as shown by Fig. 6, the off-effects are

relatively larger in the green and blue part of the spectrum, and this phenomenon is also present in the earliest observations of this series.

Are we then to assume that we have not succeeded in light adapting so as to remove the influence of dark adaptation on the off-effect, or have

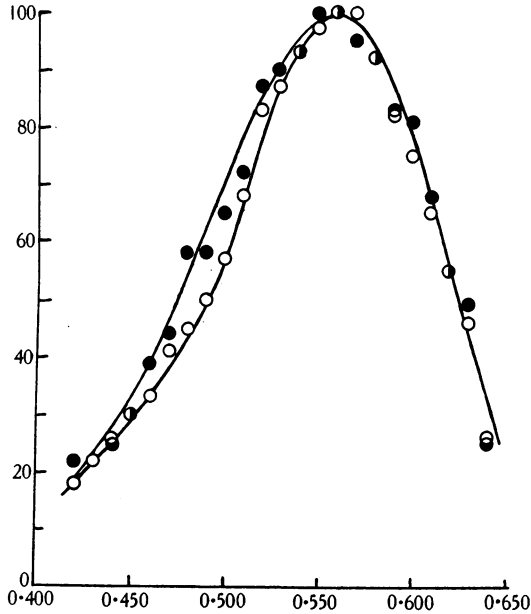


Fig. 6. Size of *b*-wave (circles) and off-effect (dots) against wave-length. Single experiment at standard intensity.

we discovered a true specific effect of wave-length in an isolated cone system? The present results favour the latter explanation, but a final decision in this issue is better postponed till we have more information.

#### *Excessive light adaptation*

We have found the initial phases of equipotential cone responses to be identical in every respect independently of wave-length. Rate of rise is the only function carefully measured, but inspection of the curves, the method hitherto considered satisfactory, has not brought to light any other differences deserving mention. Let us assume this to mean that a red and a green of the same potential are elicited by the same group of elements, or, in other words, that the retina—inasmuch as its properties are expressed by its action potential—has no means of sending afferent impulses which are different for “red” and “green”. Then, no matter

how the eyes were treated, the red and green responses had a chance of remaining identical, provided that they could still be measured with a reasonable degree of accuracy. From this point of view it would be interesting to try various methods of interfering with the responses. With the experiments on excessive light adaptation we are making a beginning along this line of approach.

The excised, opened bulbs were left in the sunshine for some 5–10 min. Some retinæ did not stand this exceedingly strong stimulus, but with twelve preparations the experiments gave definite and measurable results. The eyes recovered later to the extent of dark adapting. As it was impossible to test the immediate effect of excessive light adaptation on the whole spectrum several readings were taken at some wave-lengths and occasional readings at others. Readings were always taken at 0.560, 0.600 and 0.510  $\mu$ .

The averaged results for wave-lengths at which the greatest number of observations were made are shown in Table III. Readings from among the first ten following light adaptation are shown in the upper row, whilst

TABLE III

Wave-length $\mu$ ...	0.450	0.510	0.530	0.560	0.580	0.600	0.610
Size of initial <i>b</i> -waves ...	30	66	81	100	95	88	70
Size of later <i>b</i> -waves ...	33	74	86	100	87	73	65

All values in percentage of reading at 0.560  $\mu$ .

later readings obtained before there were any signs of dark adaptation are shown in the lower row. The *b*-waves between 0.560 and 0.610  $\mu$  are relatively larger immediately after light adaptation, those around 0.500–0.530  $\mu$  relatively smaller. At 0.450  $\mu$  the difference between the two sets of values is less definite.

The increase in the red and the diminution in the green relative to 0.560  $\mu$  have not been equally definite in all experiments. However, no initial value for 0.510  $\mu$  has been above 74 p.c., but several readings at 0.600  $\mu$  have been between 100 and 90 p.c. Later in the experiment 0.510 and 0.600  $\mu$  give approximately equipotential responses, as in the standard curve of Fig. 3, but obviously they are selectively sensitive to excessive light adaptation.

The results throw doubt on the assumption that equipotential red and green responses are elicited by the same elements or combination of elements, and suggest that at least two elements go to make up the symmetrical part of the cone curve.

*Blue and violet*

The region between  $0.480$  and  $0.400\mu$  occupies a special place in this as well as in the work with the dark-adapted eye [Granit & Munsterhjelm, 1937]. Quite often the responses were relatively too large in this region in the dark-adapted eye, and in Fig. 4 the same part of the spectrum appears as a kind of an "appendix" to what otherwise would have been

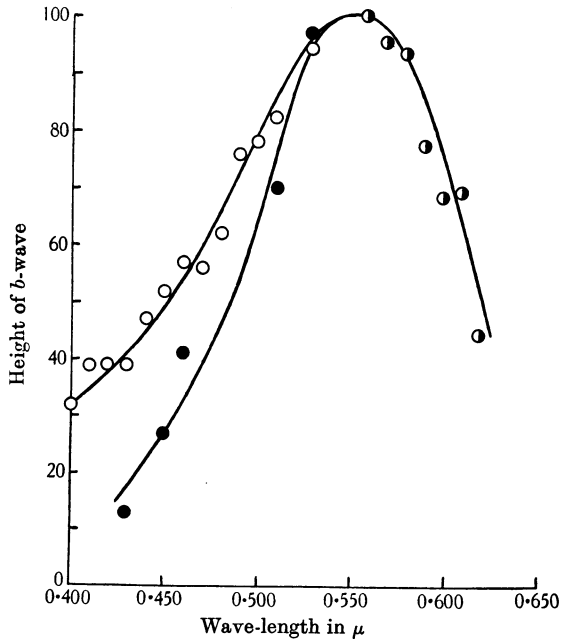


Fig. 7. Height of *b*-wave in two experiments averaged and plotted against wave-length. Dots, initial values; circles, later values, before dark adaptation has set in. Opened bulbs and maximal intensity.

a perfectly symmetrical curve, reaching a zero value around  $0.440\mu$ . If this had been the case we would have had a curve corresponding roughly to the human curve of the photopic eye similarly plotted. As it is, the responses are relatively too big in this region.

In two experiments with excised opened bulbs the same part of the spectrum reacted in a manner suggesting a common explanation of the irregularities in the two states of adaptation. Some dark adaptation was allowed to take place and the curves, instead of rising considerably around  $0.500\mu$ , as they generally do, rose chiefly towards the blue and violet end as seen in Fig. 7. The narrow curve shows the initial values, the curve

expanding towards the short wave-lengths the later results. At  $0.400\mu$  the response is about 33 p.c. of the maximum. In both these cases the retinae were inspected after the experiment. One retina was yellow, the other one showed a pale reddish colour. It should be noted that the energy of the spectrum between  $0.450$  and  $0.400\mu$  is uncorrected. Between these wave-lengths the stimuli are relatively too weak and yet the responses are relatively too big.

Some recent observations from work in progress are of interest in this connexion. We have been comparing dark-adapted frogs stored cold (at about  $5^\circ$ ) and warm (at about  $22^\circ$ ) using weak stimuli. Most of the cold frogs so far have given very large responses in the blue and violet, whereas the warm ones have behaved irregularly, some of them giving small responses in the blue and violet.

#### DISCUSSION

Our main task in this paper and in the corresponding work with dark-adapted eyes [Granit & Munsterhjelm, 1937] has been, as it were, to survey the ground in order to learn something about the limitations and possibilities of this method as an aid to the study of colour vision. From this point of view we want to draw attention to complications such as the multiple nature of the *b*-wave, the "switchboard effect" [Granit & Munsterhjelm, 1937] and the difficulty of isolating rod and cone responses.

The rod and cone curves are compared in Fig. 8. In the section on recognition of dark adaptation other differences between the electrical properties of rod and cone responses are to be found. The lability of the rod curve is expressed by its double contour and the presence of asymmetrical curves combining inner right and outer left leg and vice versa.

Can we now assume that we have completely succeeded in isolating rod and cone responses? We believe the relatively stable, symmetrical part of the cone curve to represent the maximum degree of isolation obtainable with this eye. As to the rod curve of Granit & Munsterhjelm it is possible that some low threshold cones participate in the reactions towards the red end, owing to the fact that work very near the absolute threshold is made impossible by the switchboard effect. This again we have had reason to note when experimenting with cold and warm frogs. But there remains to be explained the fact that some eyes give very large responses in the blue and violet, i.e. a region in which the absorption curve for visual purple varies between 40 p.c. at  $0.450\mu$  and values near zero at  $0.400$  (see Fig. 8 of Granit & Munsterhjelm [1937], giving the

absorption curve for visual purple as determined by Dr R. J. Lythgoe). There is also the "appendix" to the otherwise symmetrical cone curve in the same region. A curve published by Chaffee & Hampson [1924] shows exceedingly large responses at  $0.400\mu$ , and Smit [1934] also has noted very large responses in this region in some experiments with dark-adapted eyes. Finally our results indicate a common cause in light

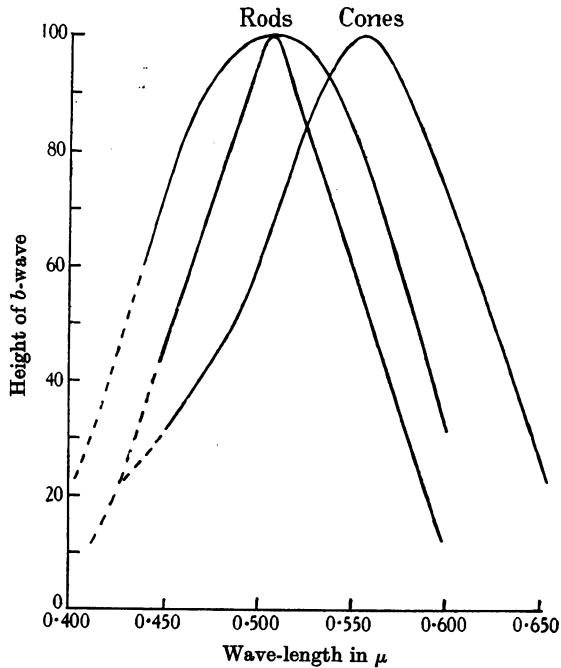


Fig. 8. Rod and cone curves for size of  $b$ -wave against wave-length. Rod curve with double contour and maximum at  $0.507\mu$  from Granit & Munsterhjelm [1937]. Cone curve with maximum at  $0.556\mu$  from Fig. 4. Broken lines in region where energy of spectrum uncorrected and stimuli thus relatively too weak.

and dark-adapted eyes, to the irregularities in this region which seems to occupy a special place in our results just by virtue of this property of the responses to blue and violet.

Recent work on the photochemistry of visual purple [referred to by Granit & Munsterhjelm, 1937; see also Hosoya & Saito, 1935; Chase, 1936] suggests that some intermediate product in the chain of bleaching of visual purple [Wald, 1935], as e.g. visual yellow or visual "white", has photochemical properties which would meet the case.



The rest of the cone spectrum is more closely knitted together in a homogeneous symmetrical curve, but the results with excessive light adaptation suggest that this curve is composite and at least contains two elements, a "red" and a "green" which, in order to give the cone curve, would have to overlap within the cone spectrum to a considerable degree.

Most interesting seems to us the fact that even immediately after light adaptation the curves for size of *b*-wave and size of off-effect did not coincide, the latter giving relatively larger responses in the green. "On" and "off" therefore have not been elicited by photochemically identical combinations of elements. It is difficult to believe that this result was due to latent dark adaptation, but the question is important enough to merit a reinvestigation with differently designed experiments. Hartline [1935] has shown that "on" and "off" are partly transmitted by different fibres so that there is nothing inherently improbable in our result, which is also supported by the experiments with excessive light adaptation throwing doubt on the apparent homogeneity of the cone spectrum.

Dr Hartline also has kindly informed one of us that he finds the different fibres of the optic nerve differ greatly in their reactions to monochromatic light. As the various types of *b*-waves are associated with a discharge of impulses through the optic nerve [Granit & Therman, 1935] our results together with those of Granit & Munsterhjelm [1937] demand a minimum of three types of fibres: fibres connected with (i) rods containing visual purple, (ii) rods (or cones) containing a substance absorbing in the blue and violet part of the spectrum ("visual yellow"), (iii) cones. In addition our results suggest that there are at least two types of cones, "green" and "red" cones over which "on" and "off" fibres may be asymmetrically distributed with respect to one another.

#### SUMMARY

The electroretinograms of light-adapted frogs' eyes in response to monochromatic stimulation with an equal energy spectrum (0.450–0.650 $\mu$ ) have been obtained with a string galvanometer and a directly coupled amplifier.

Curves showing size of the *b*-wave, its rate of rise and size of the off-effect as a function of wave-length are given in the paper, special precautions having been taken to avoid dark adaptation.

Dark adaptation may be latent or manifest with respect to size of *b*-wave. Its rate of rise and the off-effect are relatively more sensitive

to dark adaptation than the size of the *b*-wave. Dark adaptation often gives rise to a slow second phase of the off-effect, and generally to a *c*-wave.

The initial phases of the electroretinogram show a specific effect of wave-length only after dark adaptation. The isolated cone response does not show a specific effect of wave-length.

The off-effects in the green are relatively larger than the *b*-waves of the same region, even when care has been taken to avoid dark adaptation.

Excessive light adaptation depresses "red" less than "green".

This work supplements that of Granit & Munsterhjelm [1937] with the dark-adapted eye. Together the two sets of results show that there must be at least three substances, retinal elements and types of fibres in the eye: (i) rods containing visual purple, (ii) rods (or cones) containing a substance absorbing light in the blue and violet, (iii) cones. The results with excessive light adaptation and the relative asymmetry of *b*-wave and off-effect in the light-adapted eye suggest that there are at least two types of cones overlapping with respect to distribution of sensitivity to spectral light.

#### REFERENCES

- Brossa, A. & Kohlrausch, A. (1913). *Arch. Anat. Physiol.*, Lpz., p. 449.  
 v. Brücke, E. Th. & Garten, S. (1907). *Pflügers Arch.* **120**, 290.  
 Chaffee, E. L. & Hampson, A. (1924). *J. opt. Soc. Amer.* **9**, 1.  
 Charpentier, G. (1936). *Acta ophthal.*, Kbh., Suppl. ix.  
 Chase, A. M. (1936). *J. gen. Physiol.* **19**, 577.  
 Gotch, F. (1904). *J. Physiol.* **31**, 1.  
 Graham, C. H. & Riggs, L. A. (1935). *J. gen. Psychol.* **12**, 279.  
 Graham, C. H., Kemp, E. H. & Riggs, L. A. (1935). *Ibid.* **13**, 275.  
 Granit, R. (1936). Die Elektrophysiologie d. Netzhaut und d. Sehnerven, etc. *Acta ophthal.*, Kbh., Suppl. viii.  
 Granit, R. & Munsterhjelm, A. (1937). *J. Physiol.* **88**, 436.  
 Granit, R. & Therman, P. O. (1935). *Ibid.* **83**, 359.  
 Hartline, H. K. (1935). *Amer. J. Physiol.* **113**, 59.  
 Himstedt, F. & Nagel, W. A. (1901). *Ber. naturf. Ges. Freiburg i. B.* **9**. (Quoted from Kohlrausch, 1931.)  
 Hosoya, Y. & Saito, Z. (1935). *Tohoku J. exp. Med.* **27**, 172.  
 Kohlrausch, A. (1918). *Arch. Anat. Physiol.*, Lpz., p. 195.  
 Kohlrausch, A. (1931). *Handb. norm. path. Physiol.* 12/2, 2, 1393.  
 Kohlrausch, A. & Brossa, A. (1914). *Arch. Anat. Physiol.*, Lpz., p. 421.  
 Kühne, W. (1879). *Handb. Physiol. (Herrmann)*, 3/1, 235.  
 Meservey, A. B. & Chaffee, E. L. (1927). *J. opt. Soc. Amer.* **15**, 311.  
 Smit, J. A. (1934). Over den invloed van intensiteit en golfengte van licht op de elektrische verschijnselen van het oog. Thesis, Utrecht.  
 Wald, G. (1935). *J. gen. Physiol.* **19**, 351.