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

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With the aid of the slow d'Arsonval galvanometer and the dispersion spectrum of a gas lamp, Himstedt & Nagel [1901] showed that a phenomenon, corresponding to the well-known Purkinje shift, could be obtained with the "mixed" eye of the frog. In dark adaptation and with weak stimuli maximal electrical responses were elicited at $0{\cdot}544\mu$; after adaptation to bright sunlight the maximum shifted to $0{\cdot}590\mu$ and strong stimuli had to be used. The results were confirmed and extended to other eyes by Piper [1904, 1905] who used a similar instrument and the spectrum of a Nernst lamp. In the work of Brossa & Kohlrausch [1913], with the faster string galvanometer, the various phases of the electroretinogram were separated and the old results shown to hold good for the *b*-wave of the electrical response. The literature up to 1931 has been summarized by Kohlrausch [1931].

The recent results of Charpentier [1936] have served to simplify the problem. With the rod eye of the albino rat he found that the electrical responses disappear after complete bleaching of the visual purple in sunlight. During V.P. regeneration in darkness the *b*-wave returned and increased in size along the curve determined by Tansley [1931] for regeneration of visual purple in the rat, the height of the *b*-wave corresponding to the concentration of V.P. Now, in the "mixed" eye of the frog, electroretinograms may still be obtained after adaptation to bright sunlight. Thus the old work, referred to above, means that rods and cones separately give rise to *b*-waves so that in work with monochromatic stimuli little can be gained by using, for example, a high intensity and a dark-adapted "mixed" eye. In such cases the rod *b*-waves add themselves to the cone *b*-waves, and the broad band of maximal responses in the middle of the visible spectrum, which is thereby obtained, is uninterpretable in terms of photochemical events in the two types of receptors.

In the old work with the frog's eye the state of adaptation relative to intensity of stimulation was controlled, but the important condition of an equal energy spectrum was neglected. In more recent work, where the latter factor has been observed, either the two states of adaptation have not been kept rigidly separated [Chaffee & Hampson, 1924; Smit, 1934], or the *b*-waves have not been systematically measured [Smit, 1934]. With six spectral regions, obtained with filters and equalized with respect to energy, Graham & Riggs [1935] and Graham, Kemp and Riggs [1935] have studied the initial phase of the electroretinogram of the white rat and the pigeon.

Our original intention, in starting this work, was to collect and average for the two states of adaptation a great number of measurements of size and rate of rise of the *b*-wave, relative to an equal energy spectrum divided into narrow bands 0·010μ apart. This plan has been carried out, but since in our routine experiments, new facts turned up after some 1500 potentials had been recorded with the dark-adapted eye alone, we decided to publish separately the results with light- and with dark-adapted eyes.

TECHNIQUE

Apparatus and preparation

A straight filament quartz lamp, calibrated by the National Physical Laboratory for a mean colour temperature of 2800° K. was used as a light source for a Tutton monochromator. In this type of lamp (Phillips' tungsten) the filament is a single narrow band easily focused so as to fill the collimator slit. The lamp was calibrated in amperes, but adjusted, in its final position, for the corresponding voltage with the aid of an ammeter, kindly lent to us by the Physical Laboratory of this University, as in the range needed only a voltmeter was available in our own laboratory. Storage batteries were used to supply current to the lamp. The voltage was controlled between every exposure of the eye.

With the aid of Hg, Cd, and Cu spectra the dispersion curve of the monochromator was determined, and the exit slit finally adjusted so as to let through respectively 0·001μ at 0·580μ, 0·0006μ at 0·500μ, and 0·0013μ at 0·630μ. These adjustments were carried out through the eyepiece with the slit focused for 0·580μ. The size of the exit slit and the focus were thereafter kept constant throughout the experiments, but the eyepiece was removed and replaced by two apochromatic lenses.

Together with the Tutton monochromator and extra apochromatic lenses, a wedge and an auxiliary non-adjustable density were obtained from the makers (Adam Hilger, Ltd., London), both specially calibrated by them for selective absorption in the visible part of the spectrum. By setting the wedge (nonius readings) and, if necessary, inserting the auxiliary density, the intensity at any wave-length could be reduced by a known amount.

In order to obtain an equal energy spectrum the following procedure was adopted: using the tables computed by de Groot [1931] for shortening calculations with the well-known formula of Planck, the black body energy distribution at 2800° was obtained between 0·450 and 0·650μ. The factor for converting black body radiation to tungsten, a minor correction, was located in a graph given by Hulburt [1917]. A further correction was obtained from the dispersion curve, determined in the manner described above. The final relative energies could now be equalized with the minimum amount of energy in 0·450μ by adjusting the wedge which automatically brought in a correction for selective absorption in the standard wave-lengths used. The wedge settings were calculated in advance, and different levels of intensity obtained by putting in the auxiliary density or diminishing the collimator slit, for which certain standard apertures were used.

The bright lamp and the Tutton monochromator with its large prism and aperture ratio of $F/6$ combined to give strong stimuli, despite the narrow exit slit used, so that with dark-adapted eyes the collimator and exit slits were set at much the same values. The image of the exit slit, after removal of the ocular, was focused so as to fall on the retina of an excised frog's eye with its normal hypermetropia of about +6 D. Exposures of 1 sec., given by a Compur shutter, were used at higher intensities. At lower intensities 3 sec. were timed with a stopwatch. The longer duration at these intensities was chosen in order to ensure full development of the *b*-wave.

Values from the uncorrected region between 0·450 and 0·400μ have been included in our results. In this region the stimuli are relatively too weak, but it is doubtful whether towards the violet end this factor actually is a very important source of error.

In our laboratory winter frogs are kept at a low temperature in the basement. The preparations were found to be more satisfactory, if the animals had been living for some time in a basin at room temperature. The results to be reported were obtained with winter and spring specimens of *R. esculenta* and winter specimens of *R. temporaria* which had been kept for some days at room temperature and dark adapted for not less than 12 hours. No differences were observed between the two kinds of frog.

The eyes were exised in red light and left for some 15–20 min. in the dark box with the electrodes inserted and oxygen bubbling through a capillary alongside the bulb. Preliminary experiments had shown that the dark-adapted eyes gave bigger and more regular responses in an atmosphere of oxygen.

Procedure

More than one level of intensity was rarely used with a given preparation, but at this level it was our aim to collect as many readings as possible at intervals in the spectrum of $0{\cdot}010\mu$. At higher intensities exposures were taken every 3 min., at lower intensities every 2 min. Generally the order of the observations was determined in advance, so as to give the various wave-lengths and big and small responses an impartial distribution. This was particularly important in view of the fact that in most eyes the sensitivity first rose, then became steady, and finally began to fall. The interval of 20 min. in oxygen before the experiment began was calculated to cover the period of rapidly increasing responses, due partly to a slow dilatation of the pupil after the operation, but chiefly to after-effects of the operation itself and to the gradual oxygenation of the tissues.

At least at higher intensities the first stimuli seem to enhance the performance of the eye, a "staircase" effect noted previously by Brossa & Kohlrausch [1913] and by Smit [1934]. The pupil contracts during the operation independently of whether the eye has been stimulated with light or not. As is to be expected, atropine is of little use with the frog's eye, though in some experiments it has been tried. The pupil normally dilates in the dark box and is too slow to be influenced by short flashes of light some minutes apart.

The slow changes in the sensitivity as well as other factors, to be mentioned below, make it necessary to use one or two standard wave-lengths as controls and calibrate intervening observations in terms of the standards. Generally $0{\cdot}510\mu$ was used as control and given the value of 100. A systematic change in the control was distributed in equal steps over the interval, usually four or five observations. With observations as near as $0{\cdot}010\mu$ apart following in haphazard order we often have had opportunities of testing the correctness of the assumption made in this method of correcting the readings.

Most eyes, when they cease to respond normally, do so fairly rapidly. It is difficult to give any definite figures for the time a preparation may last, but it should last for at least an hour to be at all useful. We find that we have averaged thirty observations with each preparation, but then several experiments have been discontinued merely because we have been limited by the time at our disposal.

I. GENERAL OBSERVATIONS

Before describing the results of the measurements we want briefly to review a number of observations which, in many ways, have served to remodel our own conceptions both of the retinal responses and of the task undertaken in this work.

The multiple b-wave

A problem to which our results provide a definite answer was, we believe, first raised by Gotch [1903] and Einthoven and Jolly [1908], and concerns the origin of certain humps or extra waves sometimes to be seen on the retinal response to continuous stimuli. An important contribution to this question was made by Chaffee, Bovie & Hampson [1923] who observed that the initial *b*-wave of the frog's electroretinogram sometimes rises in two or more steps and that this phenomenon is more marked at lower intensities. Meservey & Chaffee [1927] with other types of eyes, Smit [1934], Granit & Riddell [1934] and others with the frog's eye, have described similar phenomena. But, when later Granit & Therman [1935] found that synchronized impulses in the optic nerve produce extra waves as artefacts on the retinal response, the existence of a multiple *b*-wave could not be held to be proved.

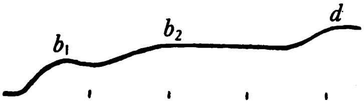

Fig. 1. Typical diphasic low intensity response showing b_1, b_2 and *d* (off-effect). Wave-length of stimulus 0·470 μ. Time in sec.

With weak stimuli, at or below the cone threshold (see section on measurements) we soon confirmed the fact that the *b*-wave often becomes polyphasic, usually diphasic. A typical diphasic low-intensity response is shown in Fig. 1. The *b*-wave rises in two phases, b_1 and b_2, and the response often ends in an off-effect (*d*). It is hardly ever possible to measure more than two phases though, at times, three steps may be seen.

It is easily shown that the slow b_2 actually is a *b*-wave and not identical with the well known *c*-wave, often termed "the slow secondary rise", which on the analysis of the retinal action potential [Granit, 1933] can be isolated as a specific component P I. At low intensities no typical *c*-waves can be seen, only b_1 and b_2, and sometimes the off-effect. But, as the intensity is increased, a very large slow secondary rise appears after a latency of 6–8 sec. Now the latent period of b_2 is about equal to but not more than a second, b_2 reaches its top value in 2–3 sec., long before the *c*-wave becomes visible, and it is best seen as a separate entity at intensities below the threshold of the *c*-wave. Further, it is often possible, by carefully increasing the strength of the stimulus, to see b_1, b_2 and the *c*-wave simultaneously, though, as a rule, at higher intensities b_1 and b_2 unite in forming a single homogeneous *b*-wave. Generally also the *c*-wave requires a longer exposure than the two phases of the *b*-wave.

It is probable that the retinal response is sometimes modified by leakage from the nerve, but it is possible to show that in general b_2 is part of the retinal reaction to light. The *b*-wave is associated with the discharge through the optic nerve [Granit & Therman, 1935] so that if b_2 were an artefact from the nerve, then the interval between b_1 and b_2 would correspond to intraretinal delay. The latter, however, is of the order of magnitude of a few milliseconds [Granit & Therman, 1935], whereas the interval between the maxima of b_1 and b_2 may be a matter of 1–3 sec. On repetition of the same stimulus after an interval of some 15–30 sec., b_2 is often found to be much smaller than during the first exposure, whilst b_1 is practically unchanged. This experiment, when it succeeds, indicates different rates of recovery of b_1 and b_2. Direct evidence for the existence of several *b*-waves differing in latent period, size, and rate of rise and decay is provided by the phenomenon which we have termed "the switchboard effect".

The switchboard effect

By starting with the false assumption that all irregularities indicated a bad preparation, we very nearly missed the chance of finding this interesting group of phenomena. But as some eyes gave non-repeatable readings without in the least being fatigued or otherwise in a bad condition, we finally decided in some cases of this type to use only a few wavelengths instead of samples from the whole spectrum. It then became evident that in some eyes different types of responses may be elicited by the same stimulus.

The switchboard effect is most easily seen at low intensities (at or below cone threshold, as shown in section on measurements). The maximal *b*-waves around $0{\cdot}500\mu$ are then about 0·100 mV. In such cases the regularly repeated control at $0{\cdot}510\mu$ often shows a number of stages between (i) b_1 and b_2 separated in two clearly defined phases, (ii) the two *b*-waves combining themselves and giving together a homogeneous big initial rise, or (iii) either of them, usually b_2, failing to appear altogether.

Some specimens are shown in Fig. 2. Why in such cases the one or the other type of response is "switched on" remains obscure, but it makes quantitative work near the threshold extremely difficult. The whole effect is most irregular; sometimes one type of response may be obtained for a long time, sometimes new combinations of b-waves are continuously appearing.

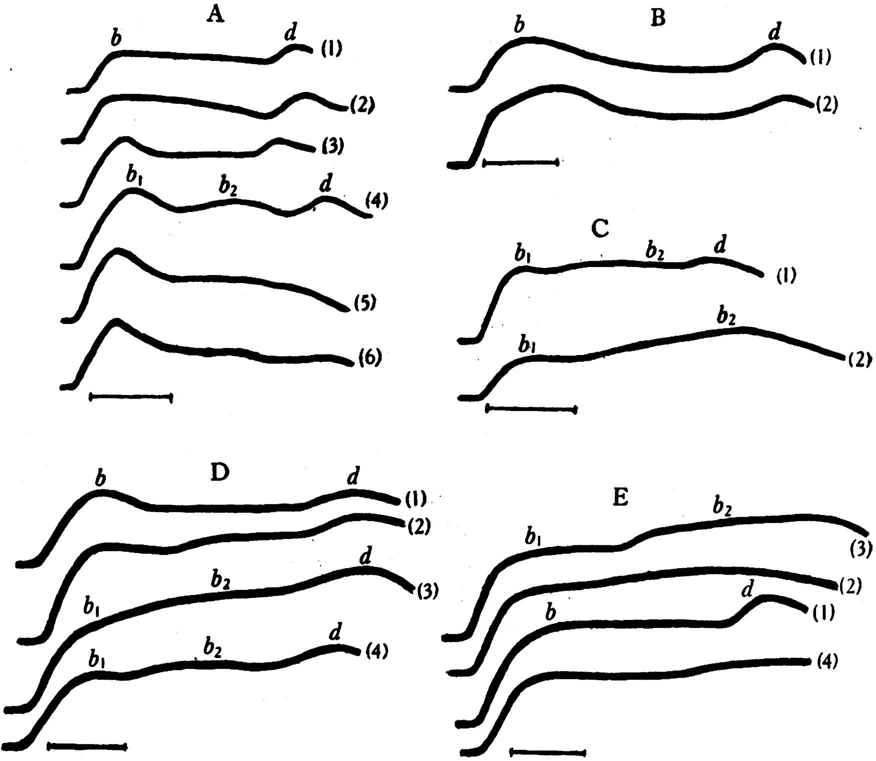

Fig. 2. Series of responses illustrating multiple b-wave and switchboard effect at low intensities. Figures against individual curves show order of the observations regardless of intervening exposures at other wave-lengths. Time in sec. A, Control at 0·510 μ showing various types of responses "switched on" during an experiment. Note b_2 appearing at (4), off-effect disappearing at (5). B, Two records at 0·510 μ. In (1) b-wave is 0·086 mV., in (2) the type of response suddenly has changed and b-wave now is 0·134 mV. Yet b_2 not clearly separable from b_1. C, Illustrates small b_2 at 0·510 μ, large b_2 at 0·570 μ. At 0·510 μ (upper response) b_1 is 0·200 mV., at 0·570 μ (lower response) b_1 is 0·105, b_2 0·152 mV. D, (1) and (2) are two responses at 0·510 μ, the latter one showing b_2: (3) and (4) at respectively 0·560 and 0·450 μ, the former one illustrating especially large b_2, not clearly separable from b_1. E, Four responses at 0·510 μ from experiment with typical switchboard effect changing b_2 and the off-effect. The initial phase, b_1, practically unchanged.

Only in one experiment did there seem to be a definite rule in the "switching" process. In this experiment a fast and a slow response alternated at both low and high intensities. The electroretinograms of Fig. 3 show results at the high intensity, used in the experiments collected in Fig. 4. A and B are two responses elicited by wave-length 0·420 μ, the pairs C and D, E and F were obtained with 0·600 and 0·510 μ respectively. In these three cases the stimuli were repeated at the standard interval of 3 min. Then, as two types of responses seemed to alternate, 0·450 μ was repeated six times at intervals of 3 min. The standard 1 sec. exposures were used. The two responses G and H alternated with perfect regularity and did so also at intervals from 1 to 4 min. It was further found that the slow response H at 0·450 μ also alternated with the fast response E at 0·510, but not so definitely with the fast response C at 0·600 μ. The eye seemed to be in excellent condition for several hours and there could be no question of fatigue.

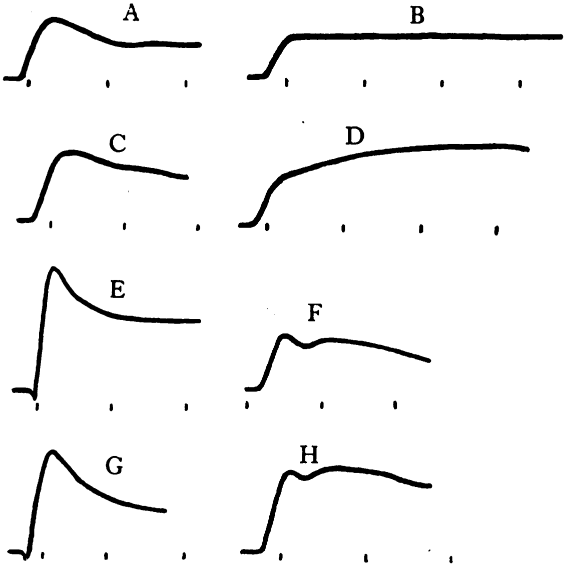

Fig. 3. Series of high intensity responses showing switchboard effect. A and B at 0·420 μ, C and D at 0·600 μ, E and F at 0·510 μ, G and H at 0·450 μ. Time in sec.

The off-effect and the c-wave

With exposures of 3 sec. the off-effect is fairly regularly seen, even at very low intensities. In some eyes, however, it is absent. Very often the off-effect is present at the beginning of the experiment and absent at the end of it. A switchboard effect like the one encountered with the *b*-waves also seems to be at work at "off". But as the off-effects are small it is difficult to be certain whether the "switching" process concerns different types of off-effects or not. "Off" and "on" often vary independently.

When light-adapted eyes have been allowed to dark-adapt and strong stimuli have been used, diphasic off-effects have often been observed [cf. also Smit, 1934], but these observations are better described in connexion with light adaptation [Granit & Wrede, 1936].

As on the analysis of the frog's retinal action potential the off-effect is connected with the negative component P III [Granit, 1933; Granit & Riddell, 1934], it is of some interest that at times purely negative responses, rising in two steps towards the base-line at "off", may be obtained at low intensities.

The relatively high threshold of the *c*-wave was mentioned above. Sometimes regular rhythmic waves may be seen on this phase of the electroretinogram [cf. Granit & Therman, 1935].

DISCUSSION

In terms of components of the retinal action potential the various *b*-waves of different latent period, size, and rate of rise and decay have been described as parts of the positive P II, associated with excitation [Granit & Therman, 1935], though previously it has not been recognized that the *b*-wave and, accordingly, P II is multiple. As changes in the *b*-wave have been found to be mirrored in terms of frequency of impulses in the optic nerve, the complex nature of the *b*-wave would seem to imply that the discharge in the nerve consists of the summed activity of elements in which the frequency varies in rate of rise and decay.

In this respect our results and deductions, supported also by the work of Chaffee, Bovie & Hampson [1923] referred to above, are in complete agreement with Hartline's [1935] interesting observations on the discharge in single fibres of the frog's eye. In some fibres the frequency of the impulses rises and falls quickly, in others the discharge rises slowly and is better maintained. The off-effect also is more marked in some fibres, less developed in others, and some fibres only give an off-effect just as others only react to the onset of the stimulus.

The switchboard effect shows that there are a number of alternative pathways or couplings of pathways from the entrance station in the receptor to the optic nerve. This conclusion is independent of whether the switchboard effect is a normal or a pathological process. The mechanism whereby sometimes one, sometimes another circuit is "switched on" can hardly be localized elsewhere than in the neurones between the receptor and the optic nerve. It is, of course, not necessary to represent the functional mosaic of different types of nerve responses or electrical component potentials as a pattern of stable units with different properties. The secret of the problem may lie in the switchboard effect being itself capable of providing different types of responses by suitably coupling units differing very little from one another. With inhibition also present in the retina [Granit & Therman, 1935; Hartline, 1935] the number of possibilities is legion.

The nearest analogy to the switchboard effect in a similar type of tissue seems to be the well-known "deviation of the response" in the central nervous system. In this case a constant electrical stimulus to the motor cortex may elicit various movement patterns. It is obvious that there are several visual phenomena, e.g. the various forms of contrast, which could be explained by a mechanism built on the principles of the switchboard effect. It may well be partly responsible for the lability of the visual threshold.

II. THE MEASUREMENTS

The foregoing section has shown that in measuring the size of the *b*-wave we are using an index sampling, as it were, the sum total of positive components at the moment when they all combine in producing a maximal deflexion of the galvanometer. At higher intensities the *b*-wave is superficially homogeneous, at lower intensities often diphasic. In the latter case b_1 and b_2 have to be measured separately.

The *b*-wave however does not split up into components at a given level of intensity. The latter factor is important merely because it determines the size of the deflexion at the onset of the stimulus. Thus, when the *b*-wave diminishes towards the ends of the visible spectrum, it often becomes diphasic, even though in the optimal region of about $0{\cdot}500\,\mu$ it is still homogeneous. Also, before the split is effected, b_1 and b_2 may combine so as to give relatively too big or too small responses in terms of p.c. of the value at $0{\cdot}510\,\mu$. Dependent upon the way in which b_1 and b_2 overlap the curves showing height of *b*-wave against wave-length could become too steep and narrow or too broad and flat. The switchboard effect, when present, may complicate matters, but it is generally recognizable and compensated for by a sufficient number of readings taken haphazard. A few series have been left out, as it proved to be impossible to decide what type of response to measure.

The curves plotted on size of *b*-wave against wave-length will be compared with the absorption curve for visual purple [Köttgen & Abelsdorff, 1896; Trendelenburg, 1904]. As V.P. standard will be used a curve, kindly sent to us by Dr R. J. Lythgoe and obtained by the method used by Bayliss, Lythgoe & Tansley [1936] in their recent work with eyes of various sea fishes. The curve is based on observations with V.P. of six *Esculentas*.

Separation of rods and cones

Fig. 4 shows the averaged results at the high intensity, based on about 500 observations with twenty frogs. As a measure of the strength of the stimulus we have used the average of all the maximal *b*-waves at 0·510 μ in each series. This value here is 0·686 mV. The outer curve shows height of *b*-wave calculated in p.c. of the value at 0·510 μ. The inner curve shows the average rate of rise in p.c. of the value at 0·510 μ. Rate of rise is defined by the height of *b*-wave divided by the time during which it has risen to its top value. The average maximal rate of rise at 0·510 μ is

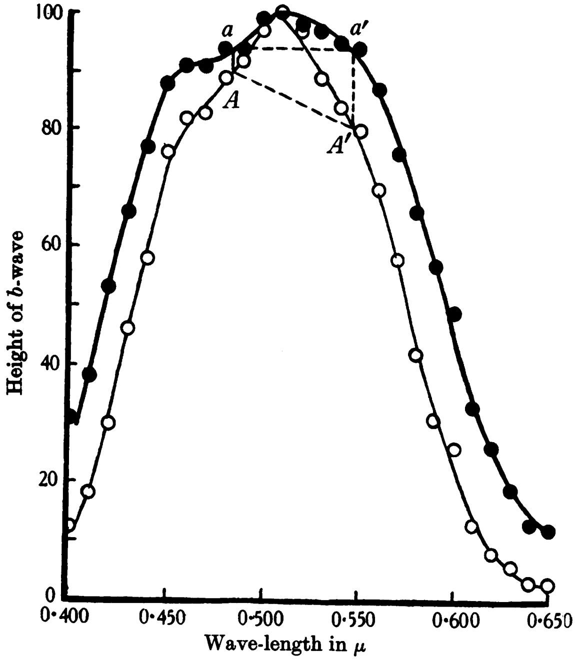

Fig. 4. Height and rate of rise of *b*-wave in p.c. of value at 0·510 μ, plotted against wave-length. Dots = height, circles = rate of rise. High intensity. Winter frogs.

$2{\cdot}96 \times 10^{-3}$ mV./msec. The band of maximal responses is very broad, values between 0·450 and 0·560 μ differing from one another by less than 12 p.c. A hump is found at 0·460 μ. In the individual curves its locus has varied between 0·450 and 0·470 μ.

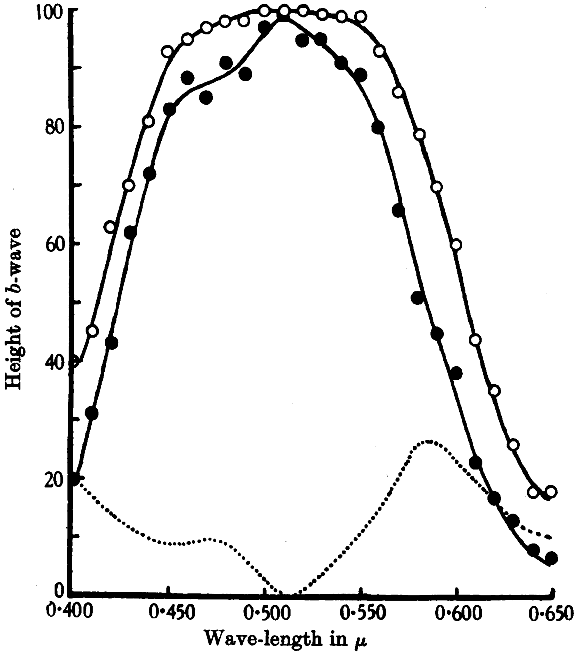

Fig. 5. Height of *b*-wave in percentage of value at 0·510 against wave-length. Dots = separately averaged values above the common averages shown in Fig. 4. Circles = averages of values below the common average.

The average curve for size of *b*-wave has been split up in Fig. 5, in which the values above and those below the average have been separately averaged. The dotted curve below shows the difference between them. The hump at 0·460 μ seems to be confined to the lower curve; the band of maximal responses in the upper curve shows that there have been eyes in which the response has been practically maximal between 0·450 and 0·560 μ. In contradistinction to the average curves obtained at lower intensities these are asymmetrical around 0·507 μ. The difference between low and high responses is maximal around 0·580 μ.

If in Fig. 4 equipotential points *a* and *a′* be chosen on either side of the maximum of the average curve, the line *AA′* shows the corresponding values on the inner curve for rate of rise. Obviously the *b*-wave on the red side rises more slowly to a given potential than does the *b*-wave on the blue side of the spectrum. Therefore the elements or the combination of elements giving potential *a* are not identical with those giving the same potential (a') on the other side of the maximum. There is a specific effect of wave-length on the response. This effect is not found at the base of the curve where, however, rates of rise are very difficult to measure. It has previously been seen, e.g. by Gotch [1904], Brossa & Kohlrausch [1913], Kohlrausch [1918] and Smit [1934].

At long exposures the qualitative differences between equipotential responses in the red and in the blue region become more marked owing to the appearance of the *c*-wave which the short wave-lengths seem to stimulate specifically (cf. also Brossa & Kohlrausch, 1913; Smit, 1934). That the latter belongs to the visual purple system was shown long ago by v. Brücke & Garten [1907] who found it to disappear after light adaptation.

After a further increase in the intensity of the stimulus the qualitative differences between equipotential responses to the left and to the right of the maximum become more pronounced, particularly with respect to rate of rise. The off-effect then seems to become faster in the blue region and the plateau of maximal responses expands. However, we have no systematic observations at very high intensities, as obviously such experiments are of little theoretical value (cf. the Introduction and last section).

More interesting and important are the experiments at lower intensities. In Fig. 6 the average maximal *b*-wave at $0{\cdot}510\mu$ is 0·272 mV. and the number of observations 392 (fourteen frogs). The spectrum is of feeble intensity but is still coloured to the dark adapted human eye. Below 50–60 p.c. of the maximum the *b*-wave is diphasic but b_1 alone has been plotted for the whole spectrum. In p.c. of the value at $0{\cdot}510\mu$, b_2, which is superimposed upon what remains of b_1 (cf. Fig. 1), is larger than b_1 so that a curve plotted on b_2 would broaden downwards as shown by the values for b_2 towards the red side. The inner curve shows rate of rise of the *b*-wave, the average maximal rate of rise being $4{\cdot}9 \times 10^{-4}$ mV./msec.

The curves tend to be symmetrical, and are considerably narrower and steeper than the high intensity curves. As the value for $0{\cdot}520\mu$ is below the value for $0{\cdot}500\mu$, the maximum probably is between 0·510 and $0{\cdot}500\mu$. There are no qualitative differences between equipotential responses in the various regions of the spectrum. All measurements at or below this intensity show that the rate of rise is a function of the size of the *b*-wave and is unaffected by wave-length as such. The eye now reacts like the rod eye of the rat [Graham & Riggs, 1935].

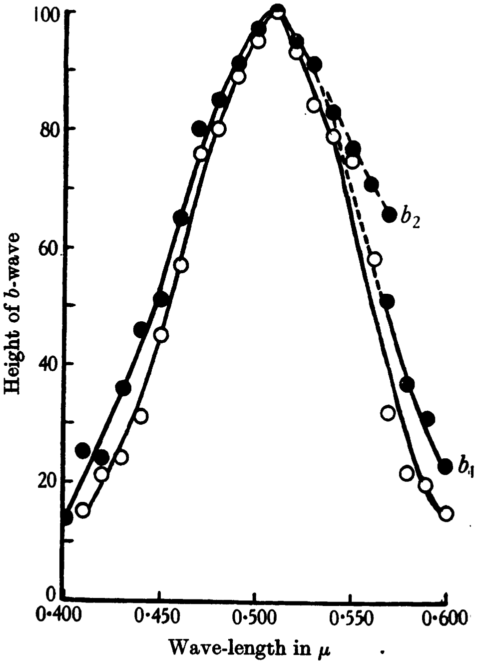

Fig. 6. Height and rate of rise plotted as in Fig. 4. Dots = height, circles = rate of rise. Low intensity. Winter frogs. Note: in this group of experiments b_2 appeared fairly regularly in the red, less regularly in the violet; in the red critical region where b_1 and b_2 separate. Further towards the red only b_1 plotted. In the violet only b_1 plotted.

The low intensity response

Though now we are dealing with a simple type of reaction in which a variation in wave-length influences size alone and not shape of the response, there are other complications left which deserve to be studied in detail. The difficulties encountered in exploring this region are clearly shown by Fig. 7 in which b_1 and b_2 have been plotted separately in terms of p.c. of the value at 0·510μ. As b_2 does not occur regularly, only 165 observations are available. The average curve for b_1 (801 observations) is shown in the same figure. The irregularities in the form of the curve for b_2 are due not only to the small number of observations, but also and, perhaps, chiefly to the variations in b_2 itself. As often is the case with a well-developed diphasic response at low intensities, the switchboard effect was a serious disturbance, occurring in four of the nine experiments concerned.

Apart from showing how difficult it is to obtain accurate results with b_2, the figure also illustrates the fact that curves plotted on b_2 tend to become broad and flat. The multiple *b*-wave therefore is an important source of error when comparisons are made with the absorption curve for visual purple. We have to reckon with the possibility that b_2 also influences the curve for b_1 in cases where two or several *b*-waves overlap so as *not* to be separable. But as this effect is independent of wave-length as such,[1] nothing but symmetrical curves, broader or narrower, can be obtained from a large body of data if the average symmetrical curve of Fig. 7 is transformed by disturbances arising from the multiple nature of the *b*-wave or the switchboard effect.

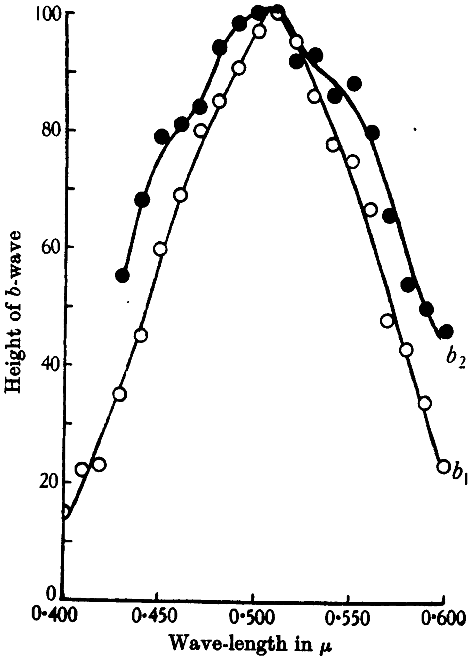

Fig. 7. Height of b_1 (circles) and b_2 (dots) plotted as in Fig. 4. Low intensity. The curve for b_1 average of 801 observations with twenty-seven frogs; the curve for b_2 165 observations unevenly distributed over the same material.

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[1] Actually we have had series in which b_2 has been much more definite *either* in the red (cf. Fig. 6) *or* in the violet. But such differences disappear with a large material of averaged results.

The average curve for b_1, drawn in Fig. 7, to some extent misrepresents our actual findings. In the individual curves behind the average some values may be too low or too high, simply because of the ordinary random distribution of errors. But, considering the nature of the switchboard effect, some values may also be too low or too high because they belong to different curves. In addition the average curve is a sum of individual curves differing typically among themselves. All these factors are taken account of, and, at the same time, the advantages of averaging preserved, if the values below and those above the average are averaged separately. This has been done for the results presented in Fig. 8 and Table I. In this final presentation of our measurements at low intensities two levels of intensities (slit openings) have been used, the one employed for the results of Fig. 6, and a still lower intensity eliciting maximal *b*-waves between 0·200 and 0·100 mV. The average maximal *b*-wave at 0·510μ of all the experiments at the low intensities is 0·20 mV. The number of readings is 801, obtained with 27 frogs.

The circles around the inner curve of Fig. 8 have been taken from Dr Lythgoe's curve for the absorption spectrum of visual purple. The absorption curve for V.P. has been measured in terms of densities for a solution of V.P. in digitonin, density being the logarithm of the fraction, light incident/light transmitted.

Through the two sets of values we have plotted two curves, symmetrical around 0·507 as a maximum. The inner curve is better fitted by our own data as well as by those of Dr Lythgoe with visual purple. Yet the two sets of data do not agree as well as might have been expected. The outer curve seems to be more influenced by factors tending to produce irregularities (see the mean variations, Table I). However, both types of curves are typically obtained with individual eyes. As we have averaged thirty observations with each preparation, there can be no doubt about the fact that some eyes give broad, others narrow, curves. But there is also a minor number of eyes which equally definitely give asymmetrical curves or, at least, curves shifted towards the red or the blue side of the spectrum, corresponding roughly to inner left, and outer right leg of the averaged curves combined, or vice versa. Thus four types of response curves may be obtained, exemplified by combining the four legs of the average curves in the four possible ways. In some cases the spectrum gradually has become shortened in the region of red towards the end of the experiment, in other cases the switchboard effect has produced a sudden change in the curve. But though such results indicate causes of lability, most eyes have been fairly stable. The mean variations show

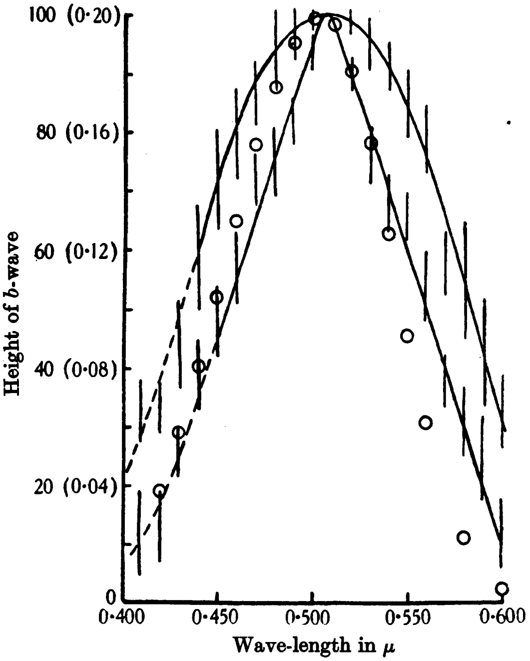

Fig. 8. Height of *b*-wave in mV. and in p.c. of value at 0·510 μ against wave-length. Inner and outer curve drawn symmetrically through respectively low and high values separately averaged as explained in text. Same material of twenty-seven winter and spring frogs as in Fig. 7 (see also Table I). Values marked by vertical lines giving mean variations doubled. The curves are drawn with broken lines through the region in which energy of spectrum not corrected. Circles = absorption of visual purple in digitonin; values obtained from Dr R. J. Lythgoe.

TABLE I

Wave-length in mμ	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600
Average height of large *b*-waves	22	33	33	44	59	72	80	87	94	97	100	100	99	95	88	85	79	58	55	43	33
Mean variation	7·0	5·0	3·6	7·6	8·9	9·1	7·8	5·7	7·0	4·3	1·1	—	2·5	4·8	4·4	5·8	5·8	4·9	10·3	9·1	6·2
Average height of small *b*-waves	8	12	13	26	39	48	57	72	75	82	94	100	90	76	68	66	54	38	31	25	12
Mean variation	6·7	7·0	6·1	4·2	6·6	6·2	5·7	4·4	6·1	4·0	2·9	—	3·3	5·0	5·0	3·9	5·8	4·4	5·5	3·6	5·6

the individual results to have been particularly labile between 0·440 and 0·460 and about 0·580 μ.

The off-effect and the c-wave

The off-effect at low intensities is rather too small to be accurately measured and often is very irregular. In three experiments it has been exceptionally well developed and regular and for them the off-effects have been averaged and plotted in Fig. 9 in terms of p.c. of the value at 0·510 μ. The corresponding *b*-waves are given in the same figure, similarly averaged. The mean values for the maximal *b*-waves and off-effects at 0·510 μ were respectively 0·174 and 0·068 mV. The number of observations was 50.

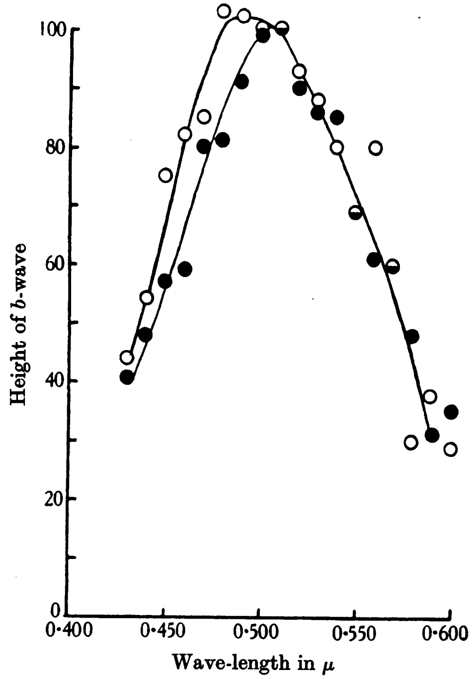

Fig. 9. Height of off-effect (circles) and *b*-wave (dots) plotted against wave-length as in Fig. 4. Low intensity.

The curve for the off-effect is markedly asymmetrical, compared with the curve for the *b*-wave. The large off-effects in the blue region were seen in all the three experiments. This does not necessarily mean that the curve for the off-effects generally is asymmetrical and maximal about 0·490μ. There have been cases in which the off-effect seems to have followed the curve of the corresponding *b*-waves. On the other hand, we have seen *b*-waves in other eyes asymmetrical and expanded towards the short wave-lengths. We can therefore only conclude that *b*-waves and off-effects show variations within the same limits but may do so independently of one another.

The *c*-wave has only been measured once, when at the higher intensity (see Fig. 4), despite the short exposure of 1 sec., it nevertheless was very large. Thus at 0·510μ the *b*-waves gave 0·520 mV., the *c*-wave 1·50 mV. In this case the curve for the *b*-waves was of the broad type always obtained at this intensity, whereas the curve for the *c*-waves was of the narrow low intensity type. At 0·430 and 0·590μ the *c*-wave hardly was measurable as a definite secondary rise, but the *b*-wave fell on the curve shown in Fig. 4. The maximum of the *c*-waves was between 0·500 and 0·510μ.

Size of b-wave and the absorption curve of visual purple

A discussion of our results from the photochemical point of view is better left to those engaged in working on the photochemistry of visual purple. Below we shall collect and briefly comment upon a number of factors tending to modify each set of data, and thereby to invalidate comparisons between the two lines of approach:

(i) The switchboard effect, and (ii) the multiple *b*-wave have already been given due consideration. These factors, with a sufficient number of data, should only influence the area under the curve, not the symmetry and the locus of the maximum, provided that the symmetrical absorption curve for visual purple alone is responsible for the initial reaction to light. When these effects have been measurable, they have tended to broaden the curve, suggesting that the narrow curve of Fig. 8, best fitted by Dr Lythgoe's values, represents a theoretical optimum among the possible combinations of *b*-waves.

(iii) The intensity of the stimulus likewise has been considered inasmuch as too strong stimuli may activate high threshold receptors lacking visual purple (cones). These receptors alone give a symmetrical curve with a maximum about 0·560μ and 50 p.c. of the maximum about 0·490 and 0·640μ [Granit & Wrede, 1936]. At 0·450μ values of about 25–30 p.c. are obtained. The cones no doubt account for the specific differences with respect to wave-length found at higher intensities. To prevent what seems to be a common misunderstanding, it is necessary to point out that in this case the specific differences with respect to wave-length prove nothing about the presence of receptors selectively sensitive to colour. They can be fully explained by the shift in the curve (the Purkinje shift), leading to various combinations of rod and cone *b*-waves, as well as by the absence of *c*-waves in light-adapted frogs' eyes. The faster rate of rise in the blue region may be due simply to rods and cones both adding to the rate of rise in this part of the spectrum, whereas towards the red and the violet ends one type of receptor alone predominates.

(iv) But intensity of the stimulus may also come in as a source of error tending to make the curve for the *b*-waves too broad, if, as seems probable [cf. Graham, Kemp & Riggs, 1935], there be a definite limit for the production of potential in the retina. Supermaximal stimuli around $0{\cdot}500\mu$ would then cause maximal potentials, but so would also stimuli at, say, 50 p.c. of the maximum. Dependent upon the amount of overlap of rod and cone *b*-waves the curves would then be symmetrical or asymmetrical. This factor could explain the large *b*-waves in the blue region of the spectrum at high intensities but not the fact that many eyes give similar curves at the lowest intensity.

(v) Stray light in the spectrum. This factor, by raising the low values, would tend to widen the area under the curves, but it cannot explain the four types of curves obtained.

(vi) The negative component of the retinal action potential, by neutralizing a certain amount of positive potential, may be an important source of error, difficult to estimate. Having seen that the off-effect and the *b*-wave may fall on different curves and knowing the former to be intimately associated with the negative P III [Granit & Riddell, 1934], we must reckon with the possibility of P III also being able to cause asymmetrical curves. Thus, with the negative P III falling on the curve for the off-effects in Fig. 9, the curve for the *b*-waves would shift towards the red end. The only negative deflexions measured actually had their maximum in $0{\cdot}490\mu$. But this explanation of the asymmetry does not remove the necessity of finding some substance other than visual purple or a modification of visual purple in order to explain the presence of asymmetrical curves. It merely means that this substance may activate the negative instead of the positive component and that instead of two substances only one substance need be evoked to account for the two asymmetrical curves.

(vii) Selective absorption in the media of the eye should be mentioned though in the few cases, when opened bulbs have been used, it has not been noted. This factor can hardly be of any significance compared with other sources of error.

(viii) The concentration of visual purple in living rods may be high, in which case the absorption of light would not be proportional to the density of the solutions at various wave-lengths. As Trendelenburg [1904] has pointed out, this would tend to make the effective absorption curve for visual purple too narrow.

(ix) The photochemical work suffers from the disadvantage of necessitating the data for absorption of visual purple to be obtained as a difference between absorption in an unbleached and in a bleached solution. This means that by-products in the formation and decomposition of visual purple, which well might be photochemically and physiologically active, do not necessarily affect the absorption curve. If relatively more stable than visual purple, they would remain unmodified in the bleached solution [Garten, 1907]. Progressive differences in the density between bleached and unbleached solutions may also prove difficult to compensate for, as found by Bayliss, Lythgoe & Tansley [1936].

Recent work by Hosoya & Bayerl [1933] and Hosoya [1934], confirmed by Hecht & Chase [1934], Bayliss, Lythgoe & Tansley [1936], and by Wald [1935] from a different angle, seems definitely to have established the reality of visual yellow, advocated long ago by Kühne [1879], Garten [1907], and others. The presence of this blue absorbing substance in varying amounts, coupled with the assumption that it is physiologically active, satisfactorily explains why in some animals the curve for the *b*-wave extends too far towards the violet end to fit the curve for pure visual purple. Smit [1934] also mentions that he has recorded very large responses at $0{\cdot}400\mu$ [cf. also Chaffee & Hampson, 1924]. The hump at $0{\cdot}460\mu$ at high intensities and the large mean variations in this region at low intensities can perhaps be similarly explained [cf. in particular Hecht & Chase, 1934].

Köttgen & Abelsdorff [1896] found two types of visual purple, of which the second type with a maximum absorption at $0{\cdot}540\mu$ was seen in fishes. Bayliss, Lythgoe & Tansley [1936] have described several new forms of V.P. with different absorption maxima which, however, seem to be typical of certain species of fish. They can also be extracted by digitonin and therefore cannot account for the difference between our results and those of Dr Lythgoe with the same experimental animal. In the blue and the violet the values of Dr Lythgoe fall between our two curves but towards the red our readings are relatively too big (see Fig. 8). There is the further difference in the results that our material definitely shows different types of response curves whereas, according to Dr Lythgoe, the absorption curves for V.P. are constant within a few p.c. By suitably selecting the series to be averaged we could obtain curves fitting the absorption curve for visual purple fairly precisely, particularly in the violet, but this result is not obtained with a sufficiently large material. Hecht [1924] points out that the human "visibility curves" of dark-adapted eyes also are shifted towards the red side compared with the absorption curve for V.P.

In order to explain the discrepancy, we have to choose between the possibilities mentioned above (see especially (vi) and (ix)) and the assumption that there are low threshold elements selectively sensitive to the long wave-lengths, e.g. the "green rods" of Schwalbe. The former alternative is suggested by the lability of the response curves and the possibility of visual yellow being formed at different rates in the elements responsible for the various component potentials, the latter by the fact that large responses in the red seem to have been generally obtained, not only by ourselves but also by Himstedt & Nagel [1901], Piper [1904], Brossa & Kohlrausch [1913] and Chaffee & Hampson [1924].

SUMMARY

The *b*-wave of the electroretinogram of dark-adapted frogs' eyes has been recorded with string galvanometer and directly coupled amplifier. Monochromatic stimuli 0·010μ apart in an equal energy spectrum have been used. The results are described in a section on "general observations" and in one concerned with "the measurements" of height and rate of rise of the *b*-wave.

In the former section it is shown that the *b*-wave is multiple and consists of several components of different latent period, size, and rate of rise and decay. At low intensities the various components of the *b*-wave may combine differently in response to a constant stimulus. This lability of the response has been termed "the switchboard effect".

In the latter section the measurements at high and low intensities are separately presented. The *b*-waves have been plotted against wave-length in terms of p.c. of the value at 0·510μ.

At high intensities broad, often asymmetrical curves with expanded maxima are obtained, and the *b*-waves show specific differences with respect to wave-length.

At low intensities most eyes give symmetrical curves around a maximum of about 0·507μ. They are either broad or narrow (see Fig. 8). The *b*-waves do *not* show specific differences with respect to wave-length.

Some eyes give asymmetrical curves or curves shifted slightly towards the red or the violet end of the spectrum. In some cases the maxima of *b*-wave and off-effect fall on different curves.

The results are compared with the absorption spectrum for visual purple (Lythgoe), and various sources of error tending to invalidate such comparisons are discussed. It is shown that visual purple cannot alone mediate the responses, obtained from frogs' eyes at low intensities. Other alternatives are pointed out. The specific differences with respect to wave-length at high intensities are held to be due to cones.

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