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THE ELECTRICAL RESPONSES OF LIGHT- AND DARK-ADAPTED FROGS' EYES TO RHYTHMIC AND CONTINUOUS STIMULI.

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PART I.

WORKING with the well-known phenomena of flicker and fusion, Schaternikow [1902] noted that the fusion frequency of a flickering light was lowered by dark-adaptation; and this finding has been amply confirmed by Lythgoe and Tansley [1929]. Some experiments by Piper [1911] indicated that the fusion frequency of ripples on the retinal action potential, caused by intermittent light, was lower in animals possessing chiefly rods (cat) than in those having chiefly cones (pigeon); but to our knowledge nobody has investigated the retinal response to intermittent light in light- and dark-adaptation with an eye which has both types of receptors present in considerable numbers. This consideration led us to undertake work along these lines with the frog's eye. The retina of this animal is well supplied with both rods and cones, and it was found to survive fairly well in excised eyes.

Well-marked differences in the behaviour of light- and dark-adapted eyes reacting to continuous and intermittent stimuli were noted, and these proved to be analysable in terms of the components of the retinal action potential [Granit, 1933]. Part I gives a brief description of the results obtained with continuous light. Part II contains an experimental analysis of the mechanism of "flicker" (intermittent ripples), made possible by the finding of a method for producing intermittent ripples, as it were, *in statu nascen.*

TECHNIQUE.

The records have been obtained with the permanent magnet string galvanometer and directly coupled amplifier previously used by one of us [Granit, 1933]. The stimulus was a concentrated filament lamp with a condenser lens, both inside a metal case with a circular opening of

10 mm. diameter at a distance of 35 cm. from the eye. The intensity of the light was about 1800 metre candles, as measured directly with a Lummer-Brodhun contrast photometer at the distance used. In a few cases neutral tint filters were used (Tscherning glasses); but all records published or described below have been obtained under conditions of maximal stimulation.

Eyes of large Hungarian frogs (*Rana esculenta*) or of *R. temporaria*, which is common here, were used indiscriminately as they were found to behave apparently identically. The frogs to be dark-adapted were placed in the photographic dark room from one to several hours; then the eyes were quickly excised below a dark red lamp and put on a specially constructed ebonite holder inside a shielded and earthed light-proof box. The eyes were supported from behind by a pad of cotton wool, soaked in Ringer solution. The wick of a non-polarizable electrode was taken to this pad. The ebonite holder contained a 4 mm. wide artificial pupil through the side of which the thin cotton wick of the corneal electrode was led. This artificial pupil was actually too large and served chiefly as a fixing device for the eye. As will be shown below, the differences between light- and dark-adapted eyes were too obvious to be explained by the diminished intensity resulting from the contraction of the pupil in the light-adapted state, and, besides, were of such a nature, in passing from dark- to light-adaptation, as would otherwise have indicated an increased intensity of stimulation in the light-adapted state. The quantitative work in Part II was carried out with a light-adapted eye and thus with a pupil of constant size.

The stimulus entered the box through a narrow blackened tube, serving as a light trap in the feebly illuminated room. In the light beam were placed: a circular rotating metal shutter, weighted by a metal rod, an episcotister with two 90° open sectors, and the edge of a prism reflecting part of the beam through other prisms to the camera. A slit in the circular shutter could be divided into parts so as to produce one or several flashes at various intervals. For approximately constant standard exposures of 2 sec. the shutter was operated by hand to the sound of a metronome. The episcotister was geared to a large wheel, usually rotated by hand, but operated by a load when comparable exposures were wanted in a series of experiments.

With eyes in the dark-adapted state 2 sec. exposures were taken at intervals of 3 min. Light-adaptation was obtained by allowing the stimulating light to shine all the time; then before the experimental observation the shutter was closed for a few seconds, during which the off-effect (see below) disappeared. The response to the same stimulus was thus obtained against a "background" of darkness just as with the dark-adapted eye. The interval of darkness was too short for the slowly reacting pupil to change in size.

Bulbs, thoroughly cleaned from adjoining tissue, were found to give reasonably constant responses for 30–40 min. and slowly diminishing ones for a considerably longer time. In the course of this work some 700 responses have been analysed, all of which have been from frogs giving the normal type of response at the beginning of the experiment.

Results.

Normal responses to continuous illumination.

As is well known from the comparative studies of v. Brücke and Garten [1907], Piper [1911], and others, the retinal action potentials of all vertebrate eyes are similar in outline. The first reaction to illumination is the negative dip, *a*, then follows a positive rise, the *b*-wave, which at high intensities fairly rapidly drops, to be succeeded by a slow secondary rise, the *c*-wave. Finally, on cessation of stimulation there is a positive off-effect or *d*-wave. These features, with the exception of a definite *c*-wave, may be recognized in the responses shown in Fig. 1, which are taken at fairly fast speeds of the film and with relatively short exposures.

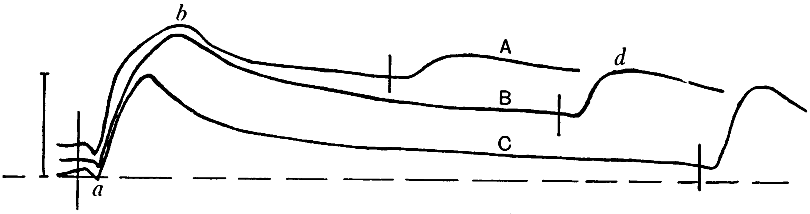

Fig. 1. The short vertical lines show the beginning and end of the stimuli: 1 sec. in *A*; 1·5 sec. in *B*; 2 sec. in *C*. Explanation in text. In this and subsequent figures the vertical line to the left shows calibration to 0·673 mv.

A is obtained after 4 hours' dark-adaptation. The potential remains fairly high after the *b*-wave proper and would have risen still higher into a typical *c*-wave, had the film been allowed to run further. In the frog's eye the *c*-waves are so slow that we have observed them only by inspection. The fact to be stressed is that this secondary rise disappears with light-adaptation [cf. v. Brücke and Garten, 1907, and several others later]. In the last response, *C*, the eye is completely light-adapted, and the curve slopes downwards towards a constant low level of potential, typical for the light-adapted eye. The *b*-wave has diminished. Another still more striking difference between light- and dark-adapted eyes is the increase in the rate of rise and in the size of the off-effect, which increase runs parallel with a shortening of its latent period. Thus *B* is taken 3 min. after *A* (the eye being in the dark meanwhile), and though the exposure is only 0·5 sec. longer than in *A*, this has definitely influenced the off-effect. In the thoroughly light-adapted eye the off-effect changes less rapidly with the length of the exposure. Even with short exposures in the light-adapted eye there are fairly brisk off-effects, while they can hardly be seen at all with the dark-adapted retina.

The initial *a*-wave may or may not be bigger in the light-adapted eye. Its size will depend upon how the negative *a* and the positive *b* balance against one another. In certain responses (see Fig. 2, this time after massage), which to all appearance are normal, the *a*-wave may be preceded by a small positive hump, indicating a shorter latent period of the positive *b* compared with the normally shorter latency of the negative *a*. The *a*-wave certainly increases with the intensity of the stimulus, as does the off-effect. With very strong stimuli Einthoven and Jolly [1908] observed *a*-waves of 0·3–0·6 mv. and off-effects up to 1·0 mv.

It is known from previous work with the frog's eye, as well as with other vertebrate eyes, that a diminution in either area or intensity simplifies the response into a positive one without the details described above [cf. a review by Kohlrausch, 1931]. As to the differences with light- and dark-adapted eyes, our work chiefly confirms earlier observations summarized by Kohlrausch.

In the retinal action potentials of frog's eyes certain features which indicate a more complicated picture have been described by Chaffee, Bovie and Hampson [1923]. They found in some eyes definite small humps on the *b*-wave. In rare cases we also have seen such humps. It should be observed, however, that these authors used excised eyes with opened bulbs and considerable amplification. Under such circumstances the electrodes are near enough to the optic nerve to enable the amplifier to pick up the discharge in the nerve. Before we conclude that such phenomena are retinal more definite evidence appears to be needed.

The components of the retinal action potential.

The retinal action potential of the vertebrate eye (cat) has recently been split into components [Granit, 1933]. A positive component, P I, could be easily removed with narcotization of the animal and was found to be chiefly responsible for the *c*-wave. The initial *b*-wave appeared as part of another positive component, P II, which then continued hidden under the *c*-wave. The *a*-wave was found to be the initial phase of a negative component, P III, which in normal responses continued below the positive components, and, with its relatively quick return towards the base line, elicited the off-effect by algebraical summation with the slower after-discharge of the positive components. The three components were found as three stages in progressive narcotization, and P II, besides, could be removed selectively through asphyxiation of the animal.

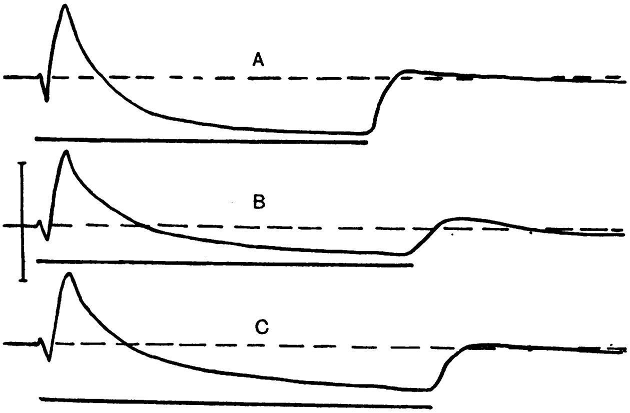

Fig. 2. Unbroken horizontal lines show duration of the stimuli: 1·8 sec. in *A*; 2 sec. in *B*; 2·1 sec. in *C*. Broken horizontal lines show respective base lines of the galvanometer. Explanation in text.

Apart from the time relations the only difference between the frog's and the cat's retinal response is seen in those features, *a*-wave and off-effect, which were shown to be dependent chiefly upon the negative P III. Both *a*-wave and off-effect are bigger in the frog's eye. We shall now see whether this is sufficient reason for assuming that the analysis established with the cat's eye is invalid for the frog.

The slow secondary rise, as we saw, can easily be removed by light-adaptation. Similarly in the cat's eye this rise disappears with light-adaptation [Granit, unpublished observations, confirming Kahn and Löwenstein, 1924, with the rabbit's rod-eye]. It is, however, not so easy to remove selectively other phases of the response of the frog's retina as it is with the warm-blooded mammalian eye. Instead of the quick, reversible changes produced by ether or asphyxia in the cat's eye, there appear in the cold-blooded retina very slow irreversible alterations in the general type of response, indicating that two components are affected simultaneously.

A definite step forward was taken when, utilizing an old observation by Waller [1909], we began to massage the eyes. After massage Waller obtained a pure negative deflection, with a fairly quick initial drop possessing the latent period of the *a*-wave, and a very quick rise towards the base line on cessation of stimulation. We have not succeeded in obtaining such negative waves entirely free from positive humps, but relatively pure negative responses may at times be had in a few minutes by this method. The characteristics of such potentials are illustrated by Figs. 2 and 3. *A* in Fig. 2 is the response of a well light-adapted eye after massage. It shows very clearly that a large negative component may be hidden under the positive deflection of the normal response and that its rise at the end towards the base line is very fast. It also shows that the large rise at "off" may take place entirely on the negative side of the base line. The eye was then given 5 min. in the dark, after which response *B* was obtained. The positive initial rise is unaltered, but the negative deflection is now considerably smaller and rises more slowly towards the base line. The eye was light-adapted once more for 2 min. and the response, *C*, again gives a larger negative deflection with a brisk rise towards the base line. Throughout this experiment the *b*-wave is practically constant and the changes with light- and dark-adaptation are reflected only by the behaviour of the negative wave.

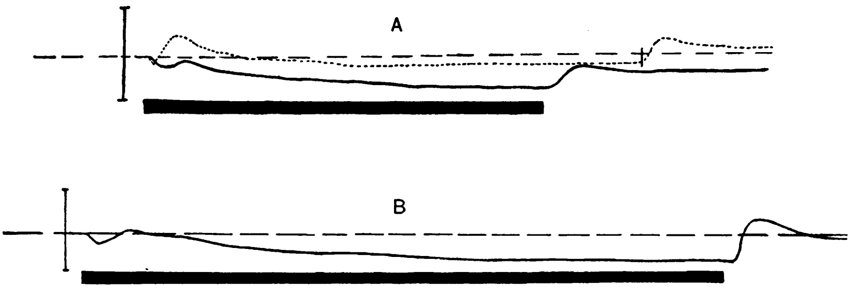

Fig. 3. Stimuli for curves drawn in full shown by thick black lines: 2·6 sec. in *A* (the dotted line is the response to a stimulus of 3·2 sec. ending at the short vertical line); 4·3 sec. in *B*. Explanation in text.

It can be shown in another way that the rise at "off" is relatively independent of the *b*-wave. In Fig. 3*A* the upper dotted response is obtained immediately after massage of a light-adapted eye. Later the response has assumed the shape traced below in full. Despite considerable diminution in the size of the *b*-wave the amount of rise at cessation of stimulation remains equal in the two responses. The total reaction is merely somewhat slower. That the *b*-wave and the off-effect may alter independently has also been shown by Nikiforowsky [1912] and Tirala [1917]. We have also seen above that with light-adaptation the *b*-wave diminishes and the off-effect increases (cf. Fig. 1). The last

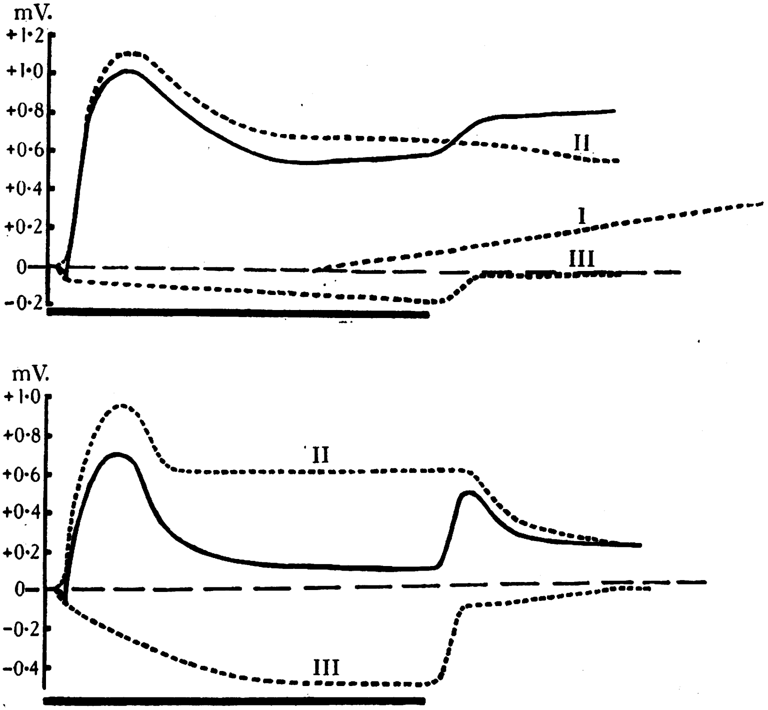

Fig. 4. Analysis of the components of the retinal action potential (drawn in full) in dark-adaptation (upper set of curves) and in light-adaptation (lower set of curves). Components drawn in broken lines. Thick black lines indicate a stimulus of 2 sec. duration. Explanation in text.

response of Fig. 3 shows a considerable negative deflection concurrent with a small *b*-wave. In such responses the negative may or may not rise above the base line at "off"; however, we have reason to believe that a pure negative deflection would show a quick initial swing towards the base line at cessation of stimulation, but that it would remain below it and gradually approach it asymptotically in what we have termed a "negative remnant." Signs of such a negative remnant can be seen in all the responses of Figs. 2 and 3, best marked in the upper curve drawn in full in Fig. 3, and also, at times, in normal responses. The long negative remnants may be, partly at least, pathological. That the negative deflection itself is a normal component of the retinal response is shown by Kohlrausch's observations with coloured lights and the pigeon's eye [1918]. Partly negative responses may be obtained by altering the wave-length of the stimulating light.

The phenomena described above indicate that the negative component reacts in such a fashion as to necessitate a large and rapid off-effect. This would be of positive sign in cases where at the end of stimulation a positive component is disappearing at a slower rate. It is particularly convincing to find the negative component to change with the state of adaptation so as to account for the most striking difference between light- and dark-adapted eyes, *i.e.* the increased off-effect and its rapid rate of rise (cf. Fig. 1). Thus the chief difference, not only between the retinal responses of cats and frogs, but also between the frog's retinal action potentials in the light- and dark-adapted state, follows from the change in the negative P III, as could be predicted on the basis of the analysis of the cat's retinal response [Granit, 1933]. Considering that all vertebrate retinæ are built on a similar plan this, indeed, was to be expected. In Part II normal off-effects will be analysed in a different way, confirming the conclusions reached above.

Our views on the components of the frog's retinal response are summed up schematically in Fig. 4. As complete isolation of P II and P III is difficult to obtain, the analysis can hardly pretend to be precise. On the other hand, in constructing the diagrams of Fig. 4 to fit our observations with normal and deficient responses we were struck by finding how very narrow were the limits within which alterations could be made. The figure needs no further comment.

DISCUSSION.

Before proceeding to an experimental analysis of the changes with intermittent light, to which Part II will be devoted, there is one fact of general significance in the theory of light- and dark-adaptation which these experiments have emphasized. This is that light- and dark-adaptation involve a great deal more than a mere change in the amount of visual purple available in the rods. Through all these experiments we have been using the same stimulus, both for producing light-adaptation and for stimulation, and this stimulus has been far above the thresholds for both rods and cones. Why then do not the cone effects appear superimposed upon the rod effects in dark-adaptation? The most obvious change with light-adaptation is an increase in the negative P III, which increase, if present, would be bound to appear in a dark-adapted eye upon the smaller P III of that state of adaptation. In terms of current theories on the physiology of the retina, working exclusively with over-simplified concepts as to the functional properties of rods and cones, our results are inexplicable. Obviously light-adaptation involves a fundamental change in the reaction of the eye as a whole. During continued illumination the eye is being "set" in a different fashion; the change might well be elicited by way of the paths activated through the initial reaction to light in the cones. A cone eye seems to be "set" in this fashion from the beginning, to judge from some results obtained by Meservey and Chaffee [1927]. These authors studied the responses of cold-blooded eyes possessing chiefly cones (turtle, horned toad, chameleon) and compared them with the responses of frogs' eyes. The off-effect was found better marked in the cone eye, which also appeared to lack a *c*-wave [but see Kohlrausch, 1918, for warm-blooded cone eyes]. The *b*-wave appeared to rise more rapidly in the cone eye.

PART II.

THE RESPONSES TO INTERMITTENT ILLUMINATION IN LIGHT- AND DARK-ADAPTED EYES.

Fig. 5 *A* shows the retinal action potential of a thoroughly dark-adapted eye (4 hours in the dark room). It is noticeable that despite the considerable potential developed and the rapidity of its development there are nevertheless at the most only traces of intermittent waves during the course of interrupted stimulation. The eye was then illuminated for 5 min. and after the off-effect had disappeared the record *B* was obtained. The briskness of the waves that now appear is striking and again indicates a fundamental change in the reaction of the retina. The string in both cases is at the same tension, as shown by the calibration to the left of *A* (0·67 mv.). The fusion frequency in *B* is at about 14 flashes per second. With less thoroughly dark-adapted eyes than the one responsible for curve *A* values between 6 and 8 flashes per second may be obtained.

The response *C* begins with the off-effect after some minutes of light-adaptation. Then intermittent stimulation follows before the off-effect has had time to drop. Several features in this record should be noticed: (i) that the first effect of the first flash upon the off-effect is to cause a large negative dip followed by a spontaneous rise before the next flash appears; (ii) that as the rate of intermittent stimulation increases the whole curve swings downwards, and again swings upwards at the end of the curve when the stimuli are spaced farther apart; (iii) that the peaks of the upstrokes are very sharp; and (iv) that there are small ripples superimposed upon the rounded tops of two long flashes in the middle of the curve. The fusion frequency is at about 18 flashes per second. In this experiment the eye was next dark-adapted again for 15 min., and the fusion frequency fell to 7; after 8 min. of renewed light-adaptation it once more rose to 18.

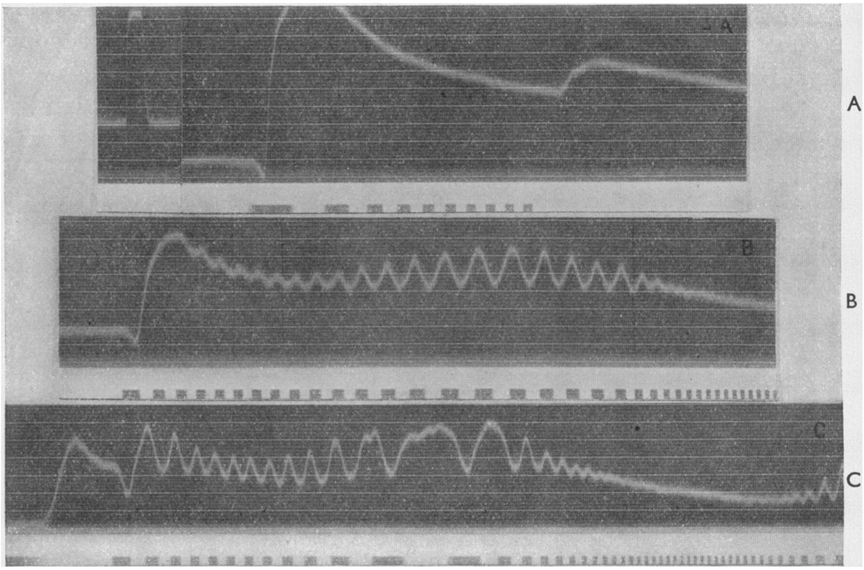

Fig. 5. Responses to intermittent light. Time marking given by tuning fork (1/100th sec.). Stimuli, directly photographed, shown below tuning fork record. Explanation in text.

Already in 1908 Einthoven and Jolly had noticed that particularly large *a*-waves were obtained when a second stimulus followed soon after interruption of the first. Piper [1911] seems to have overlooked this observation, though he was aware of the fact that the first effect of a new stimulus, when small "gaps" in a continued illumination were produced, always was the typical negative dip (frog's eye) initiating reactions to light.

Below we have undertaken a systematic study of this and other phenomena relating to the behaviour of the off-effect. The method has been to superimpose flashes upon the off-effect of a continuous stimulus of approximately constant duration. From the point of view of "flicker" the theoretical basis for such a procedure should be evident. An illuminated eye is, as it were, prepared to discharge its off-effect as soon as the stimulus is interrupted. If the flashes are sufficiently far apart the off-effect will be discharged. These are the conditions with which we are dealing in interrupting the illumination so as to cause intermittent waves.

As will be seen below the phenomena are complicated, and we shall therefore begin by stating briefly what the experiments show. The analysis will be carried out for the light-adapted eye before comparisons are made with the dark-adapted retina. It will be demonstrated that the negative component, P III, plays a decisive rôle in the production of intermittent ripples, that at rates of intermittent stimulation near the fusion frequency the off-effect is all-important, but that with slower rates of stimulation and very brisk flicker a *b*-wave plays an increasingly bigger part. Finally, spontaneous continuation of rhythmic activity after cessation of stimulation will be shown to occur under certain conditions.

RESULTS WITH FLASHES SUPERIMPOSED UPON THE OFF-EFFECT.

(*a*) *Flashes of constant duration at various intervals after cessation of the initial stimulus.*

Fig. 6 shows the effect of a flash of about 40σ superimposed upon the off-effect of a light-adapted retina at various intervals after cessation of stimulation. The vertical line through all the curves marks the end of a 2 sec. stimulus, the small vertical lines on the individual curves show the onset of the flash. The off-effect has gradually diminished during the experiment, but this does not interfere with the general trend of the result. This is easily confirmed by trying the various intervals in haphazard order, though for a rapid survey involving several observations it is convenient to run them in series. In every case the first effect of the flash is to cause a negative dip which is small at short and at long intervals, and largest when at the top of the off-effect. Farther out this

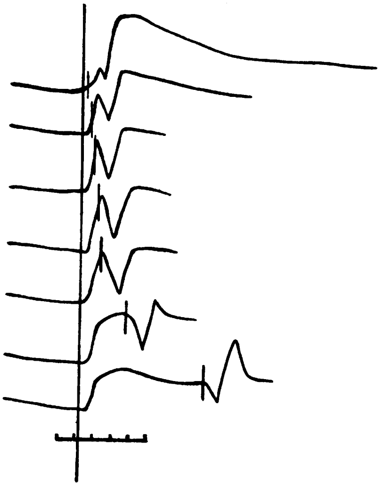

Fig. 6. Time marking shown = 1/10th sec. Explanation in text.

flash also causes a brisk positive peak which clearly is above the level of the off-effect. Though the film is run at slow speed one further effect is obvious enough: this is the lengthening of the latent period of the negative dip at short intervals.

If the amount of negative potential caused by the flash at different intervals after the 2 sec. stimulus is given as a percentage of the average uninterrupted maxima of the off-effects of one whole series, values are obtained which, plotted against time as abscissæ, fall along curves of the type shown by the lines drawn in full in Figs. 7 and 8. As zero time is taken the point at which the uninterrupted off-effect would reach its maximum. The average curve for development and decay of the off-effect itself is given in dotted lines in the same figures. The curves in Fig. 7 are obtained with a very active eye giving large negative dips; Fig. 8 represents a less active eye, but the negative dip is traced farther out to a point where it has reached the normal size of the *a*-wave in this particular eye during light-adaptation. It is thus shown to change gradually into a normal *a*-wave, a conclusion substantiated by other observations below. To the left of the starting point of these curves no negative dips are obtained. These early flashes cause merely an initial retardation of the rate of rise of the off-effect. Just after illumination there is, as it were, an "absolute refractory period" for *a*-waves, then follow a "relatively refractory period," a "supernormal phase," and a return to the normal value for *a*-waves. These terms are used in a purely descriptive sense, not implying any analogy with peripheral nerve.

(*b*) *Flashes of variable duration at various intervals after cessation of stimulation.*

In the experiments illustrated in Fig. 9 the off-effect proved to be very constant. Its normal course is illustrated in broken lines in most of the responses traced. The lines drawn in full show the responses themselves. Again every curve begins with the last phase of a 2 sec. constant stimulus. A higher speed of the film is used than in Fig. 6. The first response (1) is obtained with a 40σ flash following the interruption of illumination at an interval of 40σ. This, if repeated, would amount to intermittent stimulation at a rate of 12 ½-flashes per second. Values in well light-adapted eyes vary between 12 and 20 for the fusion frequencies. The first effect of the flash is the typical negative dip; but the important point demonstrated by this experiment refers to the rise following the negative. The off-effect continues after the interrupting negative dip and reaches its "intended" value. Thus the effect of the

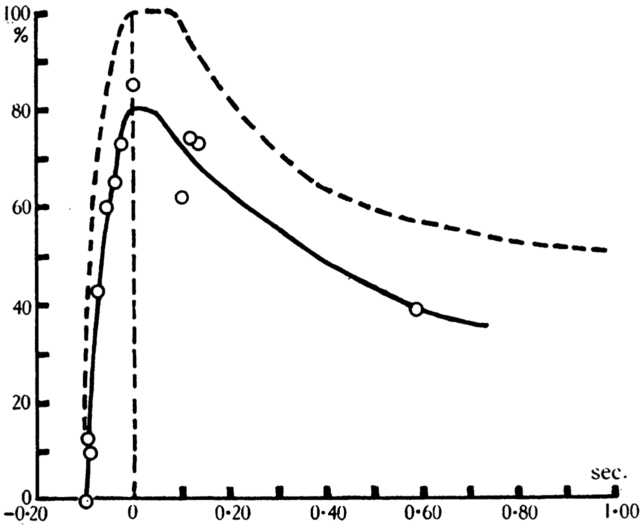

Fig. 7. Average latent period of off-effect (dotted curve) is 40σ. Explanation in text.

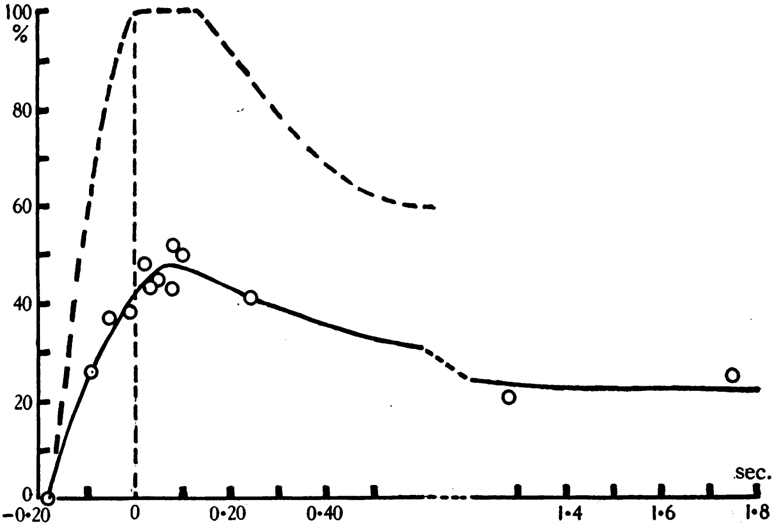

Fig. 8. Average latent period of off-effect (dotted curve) is 50σ. Explanation in text.

flash is a purely negative swing. The additional stimulus of the flash does not increase the amount of positive potential that would have been obtained without it; it only delays its rate of rise.

As soon, however, as the flash is lengthened, in curve 2 to 110σ, it definitely adds to the amount of positive potential that the off-effect would have given alone. The size of the negative dip is but little influenced by the increased duration of the flash. But it is definitely broadened and the rate of rise of the off-effect is delayed, though its latent period (from the end of the flash) has shortened. In curve 3 the 40σ flash is at an interval of 230σ from the cessation of the illumination. The rise above the off-effect is still more marked. In curve 4 the flash is at the same interval, but greatly lengthened. The negative is uninfluenced but the positive swing above the off-effect has increased with the duration of the flash precisely as in the first pair of responses at the shorter interval.

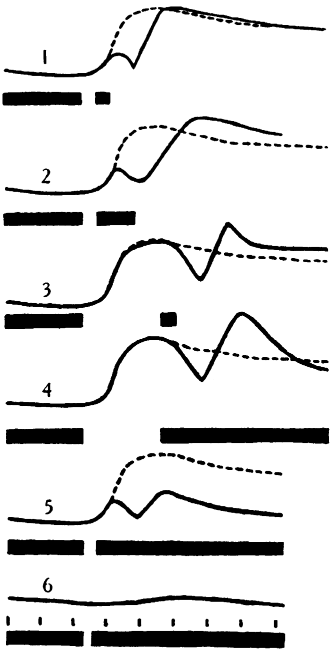

Fig. 9. Time marking shown = 1/10th sec. Stimuli indicated by thick black lines. Explanation in text.

Considering the negative dips of this figure and Fig. 6 one would like to state that the negative effect of flashes upon an off-effect has something of an all-or-none character in the way it tends to bring the string back to much the same level irrespectively of the locus of the flash within the off-effect. Seeing, however, that in addition to the off-effect an extra amount of positive potential appears, provided the flashes are sufficiently long or sufficiently late in the off-effect, one realizes that the actual depth of the negative dip need not be a correct measure of the level of negativity reached. Farther out on the off-effect the negative dip may be counterbalanced by a positive swing of short latent period and involving more than a mere resumption of the off-effect.

Curve 5 shows that with a sufficiently long flash it is even possible to obtain a small positive rise at as short an interval as 38σ, and this rise can hardly be an off-effect. In the last response with a long flash at the very short interval of 22σ both negative and positive are considerably flattened out and the stimulus acts as if directly continued. These, however, are not the conditions in which we are interested.

We want to know to what extent a positive swing which is not an off-effect can play a rôle in producing ripples in the retinal action potential in flicker. To this end we plot as ordinates the amount of rise above the off-effect, elicited by short flashes, against time as abscissæ (Fig. 10). In order to exclude slight variations during the course of the experiment the difference between the total positive swing and the height of the off-effect is given in percentage of the *b*-wave of the 2 sec. stimulus (the positive peaks are below shown to be *b*-waves). Zero time in this figure is the point at which the off-effect is maximal (about 230 σ from cessation of stimulus), and which is shown by the broken vertical line in Figs. 7 and 8. The curve drawn in full is based on observations from the experiment which provided the negatives plotted in Fig. 8. The flashes are 35σ long. The broken line shows the average curve from five experiments with flashes of longer duration (240σ). It rises more steeply than the line drawn in full, in accordance with the fact that longer flashes cause larger positive swings. Together both curves show that with flashes at the rates necessary for small flickering ripples in the retinal action potential, that is, just below the fusion frequency, a positive swing which is not an off-effect does not enter into the question. The true positive is effective only

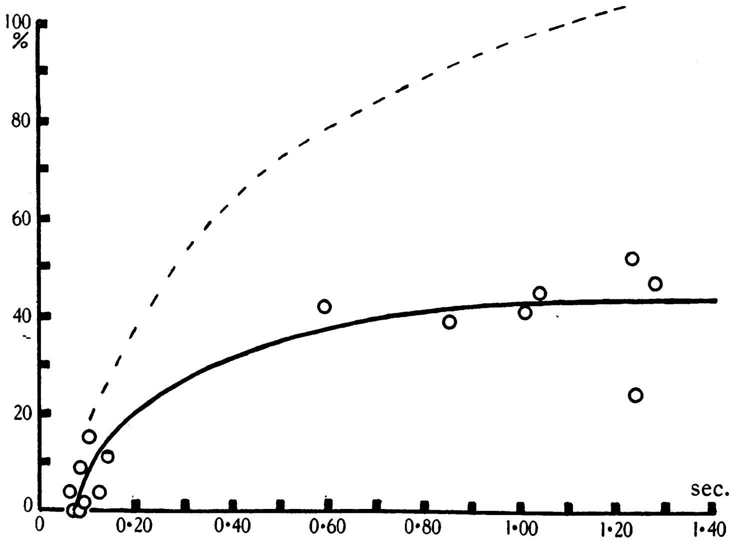

Fig. 10. Explanation in text.

when rates of intermittent stimulation are used which allow the off-effect to reach its plateau height. Now, the very shortest time measured in our experiments from the cessation of the constant stimulus to the top of the off-effect is 120σ. Usually it is much longer, say, about 200σ. Within this time brisk flicker may occur before any other positivity than the rising off-effect has had a chance to develop. This conclusion will be substantiated by other evidence below (section (*g*)).

Just as the negative dips caused by short flashes on the off-effect gradually diminish in size along the off-effect and become ordinary *a*-waves, so the positive peaks gradually increase in size and, after some time, late on the off-effect, appear as ordinary *b*-waves. In a purely descriptive sense the curves of Fig. 10 show the "refractory period" and gradual increase in size of the *b*-wave.

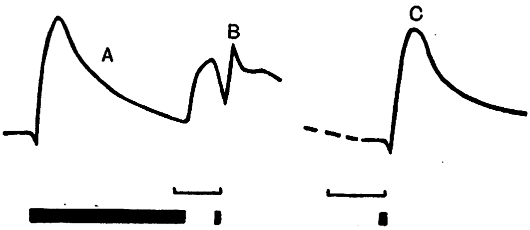

Fig. 11. Time markings shown = 0·5 sec. Explanation in text.

But the *b*-waves do not change merely in size as the off-effect passes. They also become more rounded. Thus, in Fig. 11, *A* shows the potential of the continuous 2 sec. stimulus; *B* the positive peak elicited by a flash early in the off-effect. Curve *C* shows the effect of the same flash 10 sec. after cessation of a stimulus similar to *A*. The off-effect has then disappeared. The motor running the camera has picked up speed during this long interval as shown by the broadening of the flash and by the lines marking an interval of 0·5 sec. But the positive peak, *C*, is broadened far more than can be accounted for by the increased speed of the film. This effect upon the shape of the positive peaks is more marked with shorter flashes such as are used in intermittent stimulation.

(*c*) *The effect of two successive flashes upon the off-effect.*

In curve 1, Fig. 12, there is again the typical effect of a single flash (50σ); in curve 2 the first flash is followed by another one (35σ) at an interval of 35σ. The negative dip is slightly increased in size, but still more obvious is the absence of ripples corresponding with the flashes.

The rate of rise of the positive swing following the flashes is slowed. In curve 4 the pair of flashes is shifted farther inwards on the off-effect. The broadened negative and the delayed rate of rise of the positive swing is more marked than in curve 2. In curve 5, however, where though the interval between the flashes is again 35σ they occur earlier still, there are definite ripples corresponding with them. In Fig. 14 this effect is shown by original photographs. The first flash of curve 5, Fig. 12, is at an interval of only 25σ from the end of the constant stimulus. In curve 4 the corresponding interval is 40σ. In curve 6 this interval is 30σ but the flashes are only 20σ apart.

It is clear then that double flashes at short intervals apart well out on the off-effect merely tend to bring about a return of the conditions set by a constant stimulus, that is, an eye prepared to discharge its off-effect afresh together with an additional *b*-wave. In order to obtain ripples during this phase of the off-effect a slower rate of intermittent stimulation must be used, such as with the first two flashes upon the off-effect shown by curve *C*, Fig. 5. Then, exceedingly brisk flicker may appear, as both the increased negative dips and the positive peaks above the level of the off-effect, described in the previous sections, have time to develop.

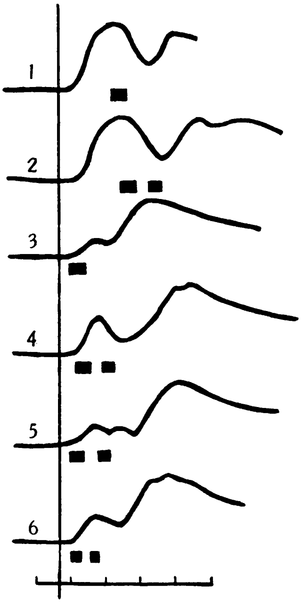

Fig. 12. Time marking shown = 1/10th sec. Explanation in text.

In order to obtain flicker at fast rates the off-effect must be interrupted by a small negative dip from which it can recover without a very much delayed rate of rise. Then a second early flash can interrupt it a second time (curve 5, Fig. 12). This condition is automatically brought about as the rate of intermittent stimulation increases. The optimal conditions for ripples at fast rates thus tend to "reset" themselves by bringing the eye back to the state at which the off-effect is held spring-like ready to go. And now we understand why with flicker on an off-effect the total level of potential can be made to swing up and down with the rate of stimulation as in curve *C*, Fig. 5. At slow rates off-effects and *b*-waves together push the string upwards in the picture, whereas at fast rates the negative dips quickly cut down the off-effect and the *b*-waves and sum to bring about a low potential near the base line, on which ripples appear as interrupted off-effects. How under such conditions two negative flashes sum to keep the string down may be seen in curve 4, Fig. 12.

The small ripples occurring after the double stimuli in curves 2, 4, and 6 in Fig. 12 cannot be explained as artefacts. Below it will be shown that, although it is possible to derive the details of retinal flicker at various rates of stimulation from the behaviour of the *a*-wave, the off-effect and the *b*-wave, yet, as soon as a definite rhythmic response is produced, this tends to persist for some time as a definite rhythmic state with peculiarities of its own. At times this state is produced after flashes upon the off-effect which themselves have not been able to set up ripples.

(*d*) *Factors determining fusion.*

In a previous section (*a*) it has already been pointed out that, at short intervals between the constant stimulus and the flash, the latent period of the negative dip becomes lengthened. This means that the negative dip cannot appear before a certain small amount of off-effect has had time to rise. Flashes that are too early merely retard the initial rate of rise of the off-effect; slightly later flashes have a relatively long latent period, so that the actual negative dips at short intervals tend to occur in much the same place relative to the off-effect. Thus in a series of twelve observations with short flashes at intervals between the end of the 2 sec. stimulus and the flash varying between 25 and 125σ, the intervals between the onset of the off-effect and the onset of the negative dips varied only between 40 and 90σ. A range of variation of 100 in the former case thus corresponded to one of 50 only in the latter set of values. Too rapid flashes, relative to the latent period, therefore keep the string down without causing ripples.

In the last instance then, the latent period of the off-effect and its rate of rise determines the fusion frequency. This is clearly seen when for some reason or other the eye becomes less active, as happens in every experiment with excised eyes after some time. Then the latency of the off-effect increases, its rate of rise decreases, and the fusion frequency diminishes. But it is particularly instructive and convincing to demonstrate the effect of the lengthened latency and the decreased rate of rise of the off-effect by dark-adapting the eye or by decreasing the strength of the stimulus with filters.

(*e*) *Comparison between dark- and light-adapted eyes.*

In Fig. 13, *A* and *B* are the responses of a dark-adapted eye and show the slow, late off-effect. A calibration to 0·67 mv. is shown to the left in the figure. The eye is then light-adapted and the sensitivity of the instrument is increased so as to make the two responses, *C* and *D*, in this state of adaptation, practically equal to *A* and *B*. The flashes in *A* and *C* occur after an interval of 120σ, in *B* and *D* after an interval of 270 and 290σ respectively. In the dark-adapted eye the first effect of the flash, inasmuch as it has any effect at all, is to cause a negative dip, but both this and the rise following are less marked than in the light-adapted eye. Curve *A* in particular, being the first response of an eye left for a long time in the dark-room, shows that the negative dip requires an off-effect as a background against which to appear. The results are so obvious that further comment seems unnecessary.

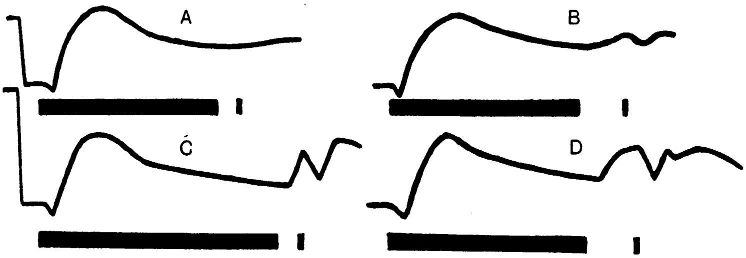

Fig. 13. Stimuli shown by thick lines. *A*, initial stimulus lasts 1·3 sec. and is followed at an interval of 120σ by a flash of 35σ; *B*, corresponding values for stimulus and interval are 1·3 sec. and 270σ; *C*, corresponding values 1·6 sec. and 120σ; *D*, corresponding values 1·3 sec. and 290σ.

It is easy to see why flicker in the dark-adapted eye requires slow rates of intermittent stimulation. Brisk flicker requires a brisk off-effect. We are thus brought back, in our analysis of intermittent stimulation, to the same difference between light- and dark-adapted eyes which we learned to recognize when using steady illumination. Just because the mechanism of flicker in light- and dark-adapted eyes is the same, the intermittent waves and the fusion frequency decrease in the latter state of adaptation.

(*f*) *Spontaneous rhythms.*

As indicated by curves 2, 4, and 6, Fig. 12, as well as by the response *C* of Fig. 5, small rhythmic waves in no direct relation to the stimuli themselves may appear after rhythmic stimulation. In the beginning we believed them to be artefacts, though, on the other hand, we were aware of the fact that Einthoven and Jolly [1908] described spontaneous rhythms in the retinal action currents from frogs, and that Adrian and Matthews [1928] recorded in the optic nerve of the conger eel rhythmic grouping of impulses with continuous stimulation and found the phenomenon to be similar to the waxing and waning of impulses obtained with intermittent stimulation [cf. also Adrian, 1932]. Application of strychnine to the eye seemed to make the retina especially prone to set up spontaneous rhythmic discharges through the nerve, and this tendency was particularly well marked when the whole retina was illuminated. In the beginning we ended most experiments with application of strychnine under various conditions of area, intensity, and dose of the poison, but did not succeed in obtaining spontaneous rhythms. Probably the time of the year is of some importance, as Adrian and Matthews obtained this effect most easily in the summer.

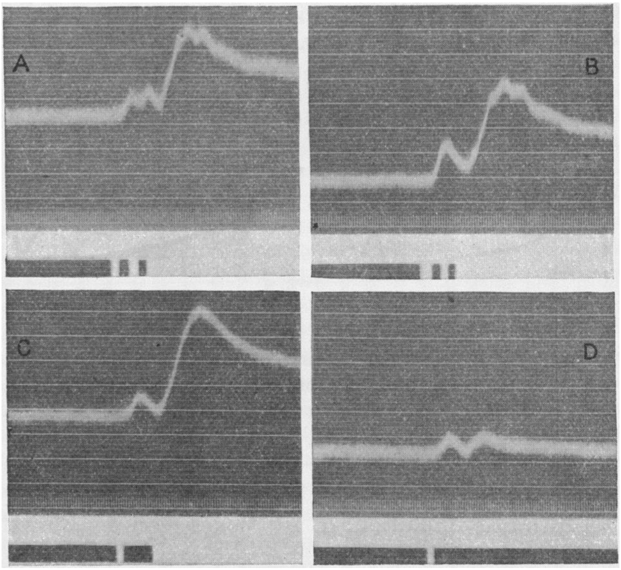

Fig. 14. Spontaneous rhythms upon off-effects interrupted by flashes. As in Fig. 5. Explanation in text.

However, spontaneous rhythms have often appeared under conditions illustrated by the responses of Fig. 14. Starting a flicker with two flashes at short intervals from the cessation of the 2 sec. stimulus leads to continued ripples on the off-effect at much the same rate, between 13 to 14 flashes per second in response *A*. The second response, *B*, shows that the flashes can appear too late on the off-effect to cause anything but a negative dip, at this interval apart, and yet be followed by spontaneous ripples, gradually damped out as the off-effect passes. The conditions illustrated in *C* are less favourable to the setting-up of spontaneous rhythms. And with experimental conditions of the type illustrated in response *D* they have not appeared. The off-effect must be given a chance to develop. At times there is some indication of a rhythm even on an uninterrupted off-effect, though we find that in eyes where spontaneous rhythms tend to occur, a short interval and one or two flashes distinctly favour the appearance of them. It is as if a taut string were sharply plucked.

(*g*) *Experiences with deficient responses.*

This point need not detain us long, as in every respect the observations with deficient responses have confirmed conclusions reached in previous sections. We shall therefore only summarize the results:

(i) If the positive components are largely removed in such a fashion as to leave a mainly negative response with a brisk off-effect towards the base line, a flash upon this off-effect gives the typical negative dip, this time practically down to the original level of negativity reached by the steady stimulus. The whole effect may take place on the negative side of the base line and shows that the negative dip merely represents a resumption of the previous value of P III. Hence it increases when the off-effect increases.

(ii) P II is necessary for obtaining the positive peaks above the level of the off-effect, just as it is necessary for obtaining the *b*-waves. These sharp positive peaks above the level of the off-effect diminish *pari passu* with the loss of the positive component initiated by the *b*-wave.

(iii) Provided that a brisk off-effect is left in a largely negative response, intermittent ripples are obtained even though the initial *b*-wave has become very small and rounded. The fusion frequency in such cases is lowered, which may, however, be merely because of the fact that in such cases the negative is less brisk than in an eye giving a response normal in every respect. At slower rates the sharp peaks of the *b*-waves are lacking and do not appear until the rate is slow enough to show them as rounded second tops on the quicker initial upstrokes caused by the interrupted off-effects. Such records emphasize in a most convincing way the double nature of the upstrokes in retinal flicker, set forth above.

(*h*) *Comparison with an eye dominated by rods.*

Piper [1911] held that in eyes with a large negative *a*-wave and a correspondingly large off-effect (frog) retinal flicker was chiefly determined by *a*-waves and off-effects and to a lesser extent by *b*-waves, as we have proved above to be the case, whereas in eyes with small *a*- and *d*-waves (cat) the ripples consisted only of repeated *b*-waves. Creed and Granit [1933], working with the dark-adapted cat's eye, were prepared to admit that the *a*- and *d*-waves played a rôle in flicker, but did not find Piper's evidence conclusive. They took the view that the response to intermittent light could not be reconstructed from data provided by measurements of the latent periods of the various waves obtained with single isolated flashes and brief interruptions of a steady light (Piper's method). This criticism was based on the facts that an isolated *b*-wave tends to take its own course independently of whether it is elicited by one or several flashes [Piper, 1911; Creed and Granit, 1933], and that in the cat's eye the latency of the intermittent ripples is longer than that of the initial *b*-wave. Moreover, Creed and Granit found it unlikely that various vertebrate eyes should behave in a fundamentally different fashion with respect to intermittent stimulation. Finally, they produced some evidence to the effect that in the cat's eye also interaction between *a*-waves and *b*-waves must be assumed in order to account for certain features of the response to intermittent stimulation. A definite negative dip, as the first effect of intermittent flashes, could not be seen, but the latency of the upstroke was lengthened in the intermittent positive peaks as compared with the initial value, and this was interpreted as due to a negative swing temporarily balancing the upstroke.

In the frog's eye the latent periods change in a complicated fashion with the rate of stimulation, thereby emphasizing the difficulty in basing conclusions on identification of the latent periods under various conditions. Above, most of our measurements of latencies have been disregarded, as our conclusions have been definite enough without them. But in view of the work of Creed and Granit one set of measurements might be mentioned. If with ripples near the fusion frequency the interval from the beginning of the flash to the upstroke following the negative dip is measured, a value is obtained which corresponds to the one measured by Creed and Granit in the cat's eye. This they called the latent period, obviously enough, as the upstroke was the first measurable effect of the flash. The flash length at the fusion frequency was found to be about 44 p.c. of this "latent period" independently of how the experimental conditions were varied as to area and intensity. In the light-adapted frog's eye we have obtained a similar constant by averaging thirty determinations of the flash length at the fusion frequency at various intensities together with the corresponding "latent periods." The value works out as 40 p.c. with a mean variation of 9·4 p.c. of 40. The result indicates a similar mechanism for flicker and fusion in the eyes of cats and frogs. On the other hand, it is quite probable that the relative parts played by P II and P III in flicker are in favour of P II with the cat's eye. Its off-effect is small, and removal of P II leads to disappearance of the intermittent ripples [Creed and Granit]. Again, the fact that a few isolated flashes lead to a single *b*-wave without ripples is understood when one realizes that with stimuli of short duration the negative P III is small; it is perhaps too small to interact so as to produce ripples. Moreover, in the cat's eye the off-effect becomes better marked when the eye is left for some time in the light.

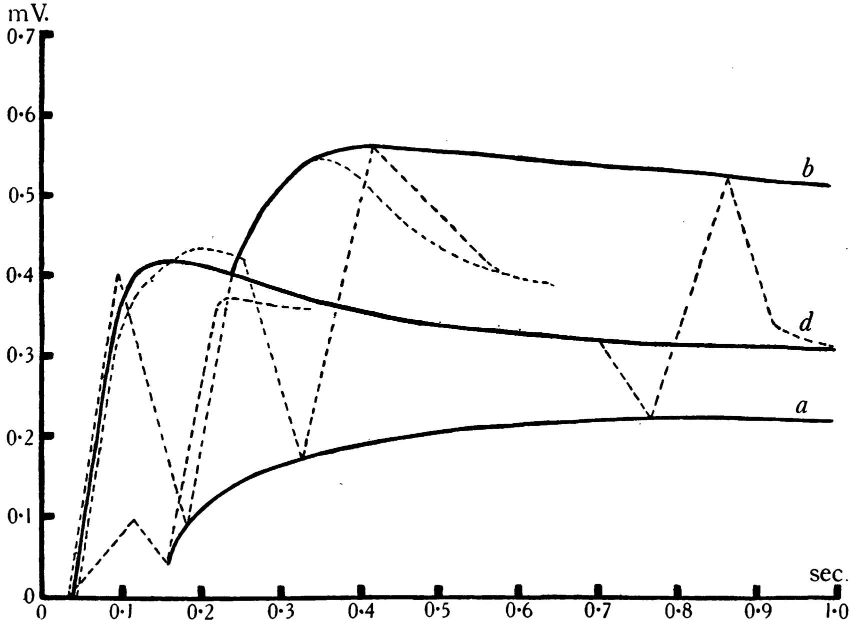

Fig. 15. Explanation in text.

Discussion.

Certain facts concerning the origin and development of the rhythmic reaction to intermittent stimuli have emerged from this analysis with unmistakable directness. These are conveniently summarized by Fig. 15. The figure represents data obtained in a typical experiment with single flashes upon the off-effect and shows the levels of potential reached by the off-effect (*d*-wave), by the negative dips (*a*-wave), and by the positive peaks (*b*-waves). Curve *d* is the average off-effect. Curve *a* gives the level of the negative dips occurring along the off-effect, as indicated by the broken lines showing the effects of actual flashes. Finally curve *b* represents the height of the positive peaks as again shown by the broken lines. The latter curve begins, as we have seen above, at the point where the off-effect is about to turn downwards. The evidence with both normal and deficient responses agrees in demonstrating that the *a*-, *b*- and *d*-curves express the behaviour of the waves in the retinal action potential, known for descriptive purposes as the *a*-, *b*- and *d*-waves. It is evident that Fig. 15 summarizes the results shown in Figs. 7, 8, and 10 in somewhat different plottings.

Certain phenomena are perhaps more obvious in this summarized representation of the facts. Thus, for instance, while the *a*-curve given in percentage of the maximal off-effect rises and falls with the latter (Figs. 7 and 8), it here changes very much less in absolute height above the base line. The experiments with deficient responses showed that the supernormal negative dips express a tendency of flashes on the off-effect to make the negatives approximate the value of P III, attained at the end of the steady stimulus. The smallness of the negative dips early in the off-effect means only that a flash of the same strength as the previous stimulus cannot add to the negative component. There must be a certain amount of recovery from negativity, this recovery being the off-effect. There is therefore no real "supernormal phase" with *a*-waves on the off-effect. On the contrary, the *a*-curve of Fig. 15 shows that the lowest levels of negativity are reached by the earliest flashes. On the other hand, the more complete the recovery (larger off-effect), the larger can the negative dips become. As soon as the *b*-wave appears above the off-effect, two processes of opposite sign are balancing against one another, and it is impossible to draw any conclusions as to the absolute size of either of them without knowing the latent periods and rates of rise of the individual components.

In contradistinction to the quick recovery of P III (off-effect) stands the slow recovery of P II, the earliest signs of this component appearing about 200σ after cessation of the 2 sec. stimulus (Figs. 10 and 15). The analysis of the components (Fig. 4) shows that at cessation of illumination P II must remain at plateau level for much the same period. Probably therefore the rise of the *b*-curve indicates the point where P II begins to drop from its plateau height and hence is prepared to rise again to a new stimulus of the same strength as the previous one.

Flicker at all but the fastest rates is obviously a process involving interaction between two opposite potentials in the retina. Though fast flicker can be obtained by interrupting momentarily the quick recovery of P III alone, it is, on the other hand, probable that very fast negative components can be obtained only in eyes with some P II present. At least in the cat's dark-adapted eye no flicker appears with a pure negative response [Creed and Granit, 1933], and interaction between P II and P III must be assumed in order to account satisfactorily for a number of observations with intermittent light. As soon as flicker has been started it might well continue as a process *sui generis*, to judge from the fact that spontaneous rhythmic reactions sometimes appear upon intermittent stimulation. The curious sharp peaks of the *b*-waves on the off-effect and the delayed rate of rise of the off-effect, when interrupted by a long flash, may also be expressions of specific types of interaction between P II and P III.

Even more than the results with continuous light, the observations with intermittent illumination stress the fundamental change in the activity of the eye after light-adaptation. The dark-adapted eye is a sluggish organ, registering grossly the total amount of light. With the appearance of the large negative P III during light-adaptation, the retina has become a very rapid and delicate differential instrument recording the slightest change in the amount of luminous flux reaching the eye. This can be prettily demonstrated by passing filters quickly through the light beam. But the gradual alteration in the type of reaction, when a dark-adapted eye is left in the light, is most conveniently followed by observing the briskness of the intermittent ripples caused by intermittent stimulation and by noting the fusion frequency. In the introduction we mentioned that the equivalent sensory phenomenon, the fusion frequency as perceived by a human subject, changes with the state of adaptation just as the retinal fusion of flicker, studied above. This suffices to show that the effect, traced to the negative component of the retinal action potential, involves the physiology of our visual sensations, and thereby emphasizes the significance of possessing for a starting point in retinal and visual problems a formal analysis of the complex electrical reaction to light in the retina [Granit, 1933]. Obviously the current concepts as to the processes underlying light- and dark-adaptation must be revised in order to conform to the facts described above. This analysis will probably also shed light upon several other functions involving rapid and delicate retinal reactions, such as brightness discrimination and visual acuity. But the discussion of sensory phenomena will be postponed till a later occasion, when simultaneous records of impulses in the optic nerve should be available. The hypothesis that P III is associated with an inhibitory process [Granit, 1933] can then be directly tested.

SUMMARY.

The retinal action potentials of light- and dark-adapted frog's eyes have been recorded with a string galvanometer and a directly coupled amplifier. Part I describes the typical responses to continuous light and discusses their relation to the components of the retinal action potential [Granit, 1933]. Part II presents an analysis of the reaction to intermittent stimulation in terms of the various waves and components of the retinal action potential.

The chief changes during light-adaptation of an originally dark-adapted eye are: disappearance of the *c*-wave, diminution in the size of the *b*-wave, and a considerable increase in the size and rate of rise of the off-effect (*d*-wave) together with a shortening of its latent period.

Largely negative responses, produced by massage of the eye, show that the negative component, P III, on light adaptation increases in size, and on cessation of stimulation returns towards the base line with an increased rate of rise and thereby causes a large and rapid off-effect to take place wholly on the negative side of the base line. This effect can occur without concomitant changes in the *b*-wave and in P II.

Removal of P I (*c*-wave) and the change in P III suffice to explain the main differences between the responses in light- and dark-adapted eyes. Fig. 4 shows the components, P I, P II, and P III, of the retinal action potential of the frog's eye in light- and dark-adaptation. The increase in P III and its rapid return at "off" account for the increased off-effect of the light-adapted eye.

In the discussion to Part I it is shown that the increase in P III with light-adaptation makes untenable the widely held view that light-adaptation merely involves a shift from rod- to cone-vision following removal of visual purple. On the contrary the whole type of reaction of the eye has altered.

On the idea that an eye reacting to interrupted light is an eye prepared to discharge its off-effect at every interruption of the illumination, the responses to intermittent light have been analysed in the light-adapted eye by superimposing one or two flashes of short duration upon the off-effect of a constant 2 sec. stimulus. The first visible effect of such flashes is a negative dip, the size of which varies with the size of the off-effect as shown in Figs. 7 and 8. The negative dip is followed by a positive swing.

At short intervals between the end of the constant stimulus and the flash, the positive swing merely consists of an off-effect which has been momentarily interrupted by the initial negative dip. At longer intervals

the positive swing adds a sharp peak above the level of the off-effect. This peak increases with the interval and with the duration of the flash (see Fig. 8).

The negative dip is an *a*-wave, the positive peak above the level of the off-effect is a *b*-wave. As the off-effect passes they gradually become the typical *a*- and *b*-waves of the normal response. When P II is removed by massage the positive peaks and the *b*-waves disappear with P II. P III alone is needed for the *a*-waves and the negative dips, together with the off-effect, which the latter interrupt. The results with largely negative responses further show that the increased negative dips upon the off-effect express a tendency of a flash upon the off-effect to inhibit the latter and re-introduce the large negative P III attained at the end of the previous 2 sec. stimulus.

Intermittent ripples on the retinal action potential are produced by *a*-, *b*- and *d*-waves (off-effect). At fast rates of intermittent stimulation fast "flickering" ripples are produced by small *a*-waves interrupting the off-effect produced by P III returning towards the base line. At slow rates of intermittent stimulation the intermittent waves become very brisk owing to increased negative dips followed by positive peaks (*b*-waves) above the level of the off-effect.

In some eyes one or two suitably timed flashes upon the off-effect elicit a spontaneous rhythm which is gradually damped out.

In the dark-adapted eye the intermittent ripples are small and fuse at very slow rates of stimulation, less than 6 or 8 flashes per second. In the light-adapted eye the fusion frequency may reach 21 flashes per second and generally soon goes above 12.

Brisk intermittent ripples and a high fusion frequency presuppose a large P III and a correspondingly large and brisk off-effect. On the small, slow off-effect of the dark-adapted eye the positive peaks and negative dips, caused by single flashes, are much smaller than in the light-adapted eye.

The dark-adapted eye cannot follow quick changes of the illumination. It is a sluggish organ registering grossly the total amount of light. When the large and rapid negative component, P III, appears during light-adaptation, the retina becomes a very delicate and rapid differential instrument, as shown particularly by the brisk reaction to intermittent stimulation.

It is pointed out that this fundamental change in the behaviour of the eye is also demonstrated by the sensations. The fusion frequency, as perceived by a human subject, decreases with dark-adaptation just as does the fusion frequency of the retinal action potential.

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