

EXCITATION AND INHIBITION IN THE OFF-EFFECT OF THE RETINA

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(Received 1 July 1937)

THE retina differs from other sense organs by signalling cessation of stimulation with an off-effect, a new rise of positivity in the electroretinogram, and this phase of the retinal response is accompanied by a discharge of impulses through the optic nerve [Adrian & Matthews, 1927; Granit, 1933]. When a flash is thrown on the off-effect the latter is partly cut down by a large negative wave, first seen by Einthoven & Jolly [1908] and studied in detail by Granit & Riddell [1934] and Granit & Therman [1935] who showed that it was due to reactivation of the negative component P III of the retinal response (negative inhibitory P III) and was accompanied by inhibition, and a rapid diminution of the frequency of the impulses in the optic nerve. Here, therefore, was on record evidence to the effect that excitation and inhibition were accompanied by potentials of opposite sign in a structure belonging to the central nervous system (cf. Gasser's similar results with the spinal cord, e.g. Erlanger & Gasser [1937]), a problem later developed by Eccles [1936] in such an interesting manner with the aid of experiments on the superior cervical ganglion.

The whole off-effect could not, however, be removed in this way, suggesting that it might contain two components, one inhibitable, the other one non-inhibitable. A very important contribution to this problem was made by Hartline [1935] who recorded the impulses from individual fibres in the optic nerve and found that some of the fibres which discharged at the onset of stimulation also discharged at "off" whereas another group of fibres discharged *only* at cessation of stimulation. The discharge in the latter type of fibre was easily inhibited by re-illumination as with a flash on top of the off-effect. It is hardly necessary to emphasize how well these results harmonize with those just

reviewed. In addition they suggest that one part of the off-effect is more intimately connected with the positive excitatory component P II, another part with the negative inhibitory component P III of the retinal response.

We are now in a position to appreciate an observation made by Granit & Riddell [1934] which at that time was not clearly understood. After removal of the positive excitatory component P II of the electroretinogram the off-effect could be almost completely inhibited by a flash on top of the off-effect that remained. In such cases the electroretinogram was a practically pure negative potential rising rapidly towards the base line at "off". The flash momentarily re-established the negative potential reached during stimulation. In a normal electroretinogram only about 50 p.c. of the off-effect would have been removed. Why then this high degree of inhibition after removal of positive excitatory P II? Could it possibly mean that one part of the retinal off-effect is more intimately connected with the positive excitatory component P II and that this part had disappeared with the disappearance of P II? This fraction would then have to be non-inhibitable by a flash on the off-effect.

Below we have developed a method by means of which it is possible to remove in a simple manner an inhibitable as well as a non-inhibitable part of the off-effect. We shall show that the latter actually is more intimately connected with the positive component P II causing the initial *b*-wave of the electroretinogram, the former with the negative component P III.

TECHNIQUE AND PROCEDURE

The experiments were carried out with the same recording apparatus, used in several earlier contributions to similar problems: a string galvanometer and a directly coupled amplifier. Our preparation was the excised opened bulb of the frog, continuously illuminated with an adapting light, interrupted every now and then to give the off-effects to be experimented upon. From another source of light a $\frac{1}{10}$ sec. flash was made either to precede the off-effect or to fall just on top of it. The effect of this test light upon the off-effect has been studied. All experiments conform to this very simple type. Both lights fill the whole bulb.

Two different devices were used for the stimuli: (I) In a number of experiments spectral light from the Tutton monochromator, previously described [Granit & Munsterhjelm, 1937], illuminated the eye and the test-light flashes were delivered with a Compur shutter in the beam of a standard incandescent bulb made monochromatic with the aid of an Ilford "spectral filter". In such cases both lights were fitted to the

same spectral region. The Ilford "spectral filters" are made to cover relatively narrow spectral bands. We have not attempted to determine their transmission (see e.g. Wright [1937] for such measurements). We shall see below that our immediate concern is neither wave-length nor intensity as such, but relative size of *b*-wave and off-effect. (II) In another group of experiments a single light source was used, a Zeiss carbon arc with an electromagnetic device automatically regulating the distance between the carbons. By means of a block of prisms, arranged as described by Graham & Granit [1931], this light was divided into two separate equally bright beams which were united again to fall on the opened eye of the frog. Tscherning neutral tint filters were placed in the beams to give the relative levels of intensity wanted for adapting light and test light. In most experiments the full strength of the arc was used for the $\frac{1}{10}$ sec. flash with the eye adapted to 1/100th of this intensity. However, other levels and differences of intensity also were tested.

In order to reproduce the results to be described it is not necessary to use particularly high intensities. In fact, we shall see later that too strong flashes (test lights) introduce various complications. Nor is it necessary to limit the observations to any particular state of adaptation. But it is advisable to use an intensity above which the off-effect does not fluctuate. We generally started with a dark-adapted eye, left for 5 min. in the adapting light before beginning the experiment. This light was interrupted about once in a minute to give the off-effect to be experimented upon.

RESULTS

"Refractoriness" of off-effect to a preceding b-wave

If part of the off-effect be due to elements reacting with a positive initial *b*-wave of P II, that part should show the same kind of "refractoriness" to a previous *b*-wave as would be evident when one *b*-wave follows immediately after another. Fig. 1 shows diagrammatically what happens when a number of identical flashes follow one another at identical intervals. The diagram is based on the work of Piper [1911] and of Creed & Granit [1933] who showed that it takes some time before, in a series of flashes (flicker), the individual small positive *b*-waves can break through the large *b*-wave caused by the first flash. The elements reacting with positive P II are, as it were, in a state of refractoriness caused by the first flash. The term "refractoriness" is throughout this work used in a purely descriptive sense.

Accordingly, if part of the off-effect is due to positive P II in elements capable of reacting with a *b*-wave, that part should disappear when a

b-wave is made to precede the off-effect. The latter should diminish. In order to test this proposition the $\frac{1}{10}$ sec. flash is thrown in about 200–500 msec. before the off-effect is about to take place. The flash must be made strong enough to give a definite *b*-wave when superimposed upon the steady potential kept up by the permanent adapting illumination. In Fig. 2, A shows the off-effect control, B the *b*-wave elicited by

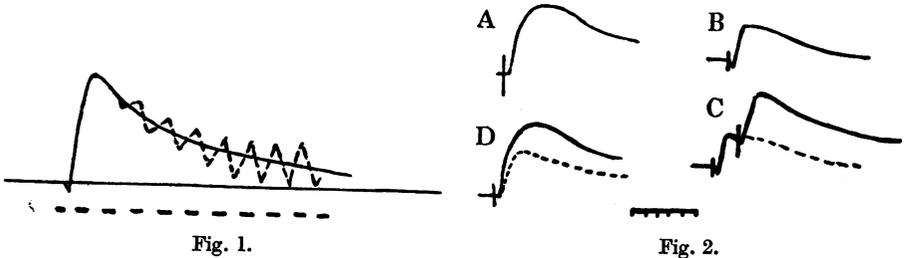


Fig. 1. Diagram showing the effect of later flashes as compared with the initial *a*- and *b*-waves caused by the first flash of the series.

Fig. 2. A, off-effect alone, off-control; B, preceding *b*-wave alone, *b*-control; C, combined effect of preceding *b*-wave plus off-effect, broken line showing course of *b*-control; D, off-control drawn in full; off-remainder, as reconstructed from curve C, shown by broken line. Time interval, 200 msec.

the flash superimposed upon the adapting light. When the flash a few minutes later is thrown in about half a second before the adapting light is interrupted to give the off-effect, record C is obtained. The latter is a combination of the preceding *b*-wave plus off-effect. From this record we have to subtract the amount of potential that the *b*-wave would have given alone, that is, curve B, the *b*-wave control. The course of the latter change is shown by the broken line in the same figure, curve C. The residual off-effect is replotted in curve D below the off-effect control. The broken line shows the reduced off-effect. Apparently the preceding *b*-wave has succeeded in removing about 40 p.c. of the off-effect.

The off-effect therefore is not independent of the potential change of the same sign known as a *b*-wave. It is, in fact, partly influenced by a preceding *b*-wave as if that part of the off-effect itself had been a *b*-wave. Questions of relative intensity and interval between *b*-wave and off-effect will temporarily be left aside while we continue to follow the main trend of the experimental analysis. In the experiments to follow the $\frac{1}{10}$ sec. flash will be put in within about 500 msec. before the adapting light is turned out to give rise to the off-effect.

The inhibitable part of the off-effect

In Fig. 3, A shows the *b*-wave elicited by the $\frac{1}{10}$ sec. flash superimposed upon the adapting light. The same flash is then thrown in on top of the off-effect, giving the record B, in which the off-effect control is drawn in broken lines. There is now to be seen the typical negative notch, which, as we know, is accompanied by inhibition in the optic nerve. To what part of the off-effect does this negative notch belong? Is it still there after removal of that part of the off-effect which above was found to be influenced by a preceding *b*-wave?

An answer to these questions may be obtained by the simple expedient of throwing the flash in twice, first some 200–500 msec. before the off-effect is about to take place and then a second time on top of the off-effect. The

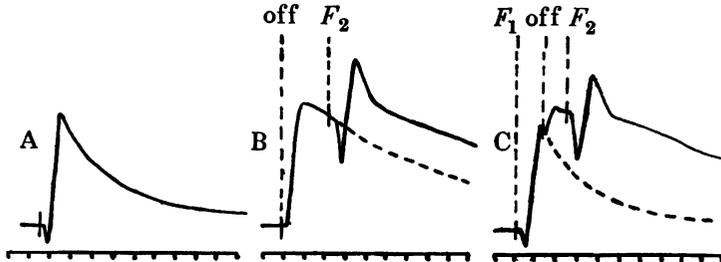


Fig. 3. A, *b*-wave control given by preceding flash F_1 alone; B, off-control with flash F_2 superimposed on top of it; off-control continued as broken line; C, combined effect of F_1 plus off-effect plus F_2 , broken line showing course of *b*-wave control. Time interval, 200 msec.

record thus obtained is shown as C in Fig. 3. The off-effect has been cut down by the preceding flash as shown by the flash control drawn in broken lines in the record. The inhibitory notch is as big as in curve B where the flash is falling on the off-effect control. The amount of inhibition, measured as negative P III, has therefore not been influenced by the removal of a large fraction of the off-effect. The inhibitable part of the off-effect is constant. This presupposes that the flash on the off-effect is properly timed [Granit & Riddell, 1934].

The difference between the flash control and the off-effect potential in curve C shows the amount of off-potential left by the preceding flash. If the inhibitory notch had been touching the broken line it would have meant complete momentary removal of the off-effect. However, there is a fraction left, about 12 p.c., which the two flashes together have not succeeded in removing. How much is left depends upon the relative size of off-effect and flash as well as upon state of adaptation. The inhibitory

notch is small or absent in a completely dark-adapted eye, stimulated for a short time with a high intensity [Granit & Riddell, 1934]. If the flash is very strong, relative to the size of the off-effect, it causes a relatively larger positive *b*-wave after the negative phase. This *b*-wave interrupts the negative notch at some point not directly obtainable from the record. Therefore the lowest point on the negative notch need not necessarily represent the maximum amount of inhibitory P III elicited under given conditions. It merely represents the point of balance between two opposite potentials affecting the same measuring instrument. In order to obtain the negative notch alone it is necessary first to remove the positive component P II of the electroretinogram.

This was done by Granit & Riddell [1934] who found—as has already been mentioned—that, when the electroretinogram after massaging the eye had been made negative and the off-effect was a swing-back towards the base line, a flash on top of this off-effect re-established the state of negativity prevalent during illumination. Recently Therman [1937] has shown that the positive component P II of the electroretinogram can be removed even better and sometimes completely by increasing the concentration of potassium in a Ringer or isotonic glucose solution pipetted directly into the excised opened bulb. The eye then reacts to illumination with a very brisk and large negative component P III swinging rapidly back towards the base line at “off”. In this eye *the whole off-effect is inhibitable*. A flash causes a negative notch reaching the level of negativity from which the off-effect rose. A flash, thrown in just before the off-effect is about to appear, elicits no positive response, only a small negative *a*-wave. It has no influence on the off-effect of the type studied in the previous section. Thus, *when the b-wave due to positive P II disappears, so also does that part of the off-effect (non-inhibitable off) which showed refractoriness to b-waves, and the remaining part of the off-effect is completely inhibitable*.

Conclusion

We have on purpose left out all complications in order first to present the main results of this investigation into the nature of the off-effect. These are that the off-effect consists of at least two elements, parts or components: (I) One is part and parcel of the positive component P II, responsible for the *b*-wave, and disappears when it falls into the wake of refractoriness left by a preceding *b*-wave. After removal of P II it likewise disappears. This we call the *non-inhibitable off-effect*. (II) The other one does not require the positive component P II and is not in a similar manner influenced by a preceding *b*-wave. Only the negative

component P III is needed for this element of the off-effect to appear. It reacts with a deep and rapid negative wave ("negative notch" of Granit & Riddell [1934] when a flash is thrown on top of it. This negative notch coincides with an inhibition of the discharge in the optic nerve [Granit & Therman, 1935]. After removal of all positive reactions to light by treating the retina with a sufficiently strong solution of K⁺-ions [Therman, 1937] it is found that the flash on the off-effect re-establishes the level of negativity reached by P III during illumination. On account of these properties this part of the off-effect has been called the *inhibitible off-effect*.

The inhibitible and non-inhibitible retinal off-effects probably are retinal equivalents to fibres with similar properties [Hartline, 1935]. The fibres reacting only or chiefly at "off" stop their discharge upon re-illumination of the eye. Our results indicate that during illumination the corresponding retinal elements develop negative P III thereby preparing them for the discharge that takes place at "off". The fibres reacting at both "on" and "off" probably are connected with the elements responsible for the non-inhibitible off-effect which is so sensitive to a preceding *b*-wave.

Complicating factors

Under this heading we are collecting a number of observations which, though incapable of invalidating the main line of arguments presented above, yet show that we must reckon with phenomena of interference between the components of the electroretinogram as complicating factors. Thus, for instance, we shall show that strong preceding *b*-waves not only remove part of the off-effect—non-inhibitible off—but also may modify the remainder.

In the first instance one would naturally expect a strong preceding *b*-wave to remove a greater fraction of the off-effect than a weak one. Within limits this is so. The *b*-wave caused by the preceding flash may be diminished by either reducing the intensity of the stimulus or by shortening its duration. In both cases the off-effect emerges less and less influenced by the flash. This therefore is according to expectation. But unexpected changes begin to make their appearance when the preceding *b*-waves are made larger than the off-effects. This, of course, means that the light eliciting the *b*-wave is a great deal stronger than the adapting light upon which it is superimposed. In order not to have to use excessively strong stimuli it is advisable to adapt the eye to an intensity which is only just sufficiently strong to give an off-effect at about 0.10–0.20 mV.

Fig. 4 shows a typical example. The eye is continuously illuminated by wave-length 0.650μ from the monochromator and the interruption of this light gives an off-effect control varying somewhat in size as shown by the several lines of curve A. It is perhaps unnecessarily small, 0.10 mV. Curve B illustrates the flash control, curve C the combination

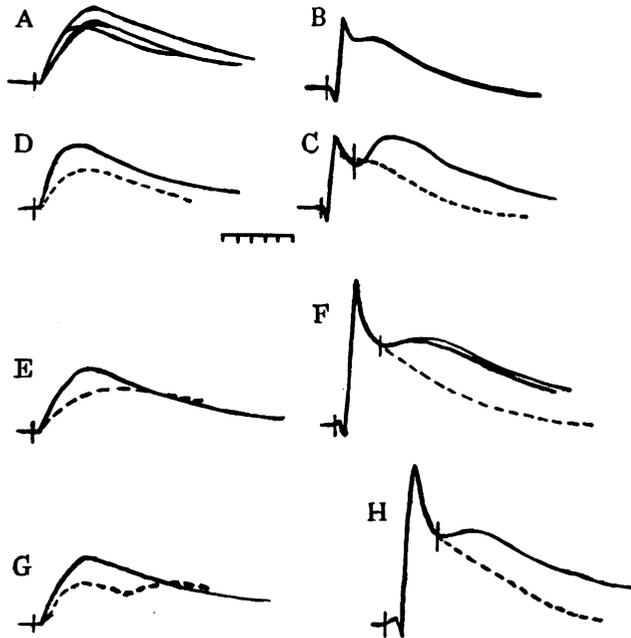


Fig. 4. A, a number of off-controls; B, *b*-wave control (Note: diphasic *b*-wave); C, combined effect of *b*-wave plus off-effect, course of *b*-wave control shown by broken line; D, average off-control drawn above off-remainder reconstructed from C. The pairs E and F, G and H correspond to the pair D and C, the preceding flash in the two latter pairs having been increased in strength as shown by bigger *b*-wave control drawn in broken lines in F and H. Note: with increasing strength of preceding flash the off-remainder becomes diphasic. Time interval, 200 msec.

flash plus off-effect, and curve D the reduced off-effect plotted below the average "off" control. The Ilford spectral red is used for the flash. So far the figure is similar to Fig. 1. Curves E and F show a similar set of records from the same experiment when, somewhat later, the *b*-wave has been made bigger by increasing the strength of the flash. The two records E and F correspond to C and D, F being the combined effect of *b*-wave plus off-effect with the *b*-wave control shown in broken lines, E the off-effect control with the reduced off-effect remainder in broken lines. The off-effect

control is now about 45 p.c. of the preceding *b*-wave. Despite this the percentage reduction is roughly constant, that is, the amount of non-inhibitable "off" is not influenced by the size of the preceding *b*-wave when it is increased beyond a certain limit. But the rate of rise of the remainder is reduced and there is an indication of a second phase rising above the level of the control. A further increase in the intensity of the flash does not make the *b*-wave very much bigger but definitely splits up the reduced off-effect into two phases as shown by the records G and H.

Sometimes the influence of a strong preceding *b*-wave on the off-effect is even more striking. The *b*-wave and off-effect controls may be reasonably normal and yet, when the *b*-wave is made to precede the off-effect, the string suddenly stops dead at a certain plateau level of positivity where

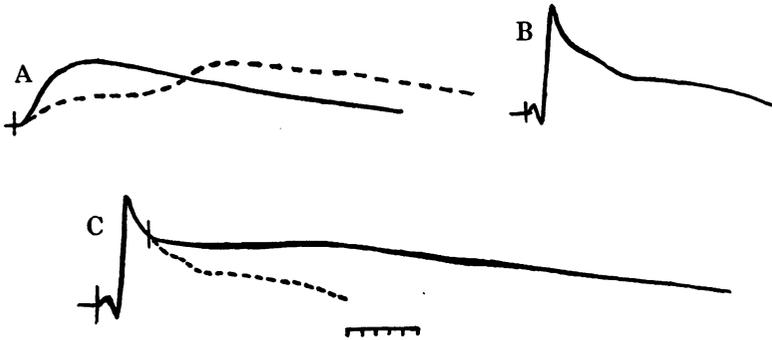


Fig. 5. B, *b*-wave control; A, off-effect control drawn in full, off-remainder as reconstructed from C shown in broken lines; C, combined effect of preceding flash plus off-effect. Time interval, 200 msec.

it remains for a considerable time. The off-effect is so much changed that one has the impression that the preceding *b*-wave somehow has succeeded in activating a new slow element of positivity of the kind that one sometimes sees when recording from the retina with localized electrodes [Granit, 1937]. Some specimens are shown in Fig. 5. At the moment we have no definite explanation of these phenomena to offer.

The *b*-waves of Fig. 4 (F and H) are interesting inasmuch as there is a small rise of positivity before the *a*-wave changes into the positive phase. A similar positive phase preceding the *a*-wave has previously been seen by Granit & Riddell [1934] and recently by Jolly [1936]. We take this opportunity to point out that initial irregularities of this fast type are often seen when for some reason or other retinal detachment has taken place with consequent formation of a small fold suitably localized relative to the electrodes.

Interval between preceding flash and off-effect

When trying to work out curves illustrating the effect of the interval between the preceding flash and the off-effect on the amount of off-effect removed we first believed that the curve had an optimum at certain short intervals between 250 and 500 msec. But later we came to the conclusion that such optima only were apparent. To begin with, off-effects tend to vary in size more than *b*-waves, thereby simulating irregular optima in the curve. But in most eyes there is in addition a slow change taking place during the time the retina is continuously illuminated. This effect is illustrated in Fig. 6.

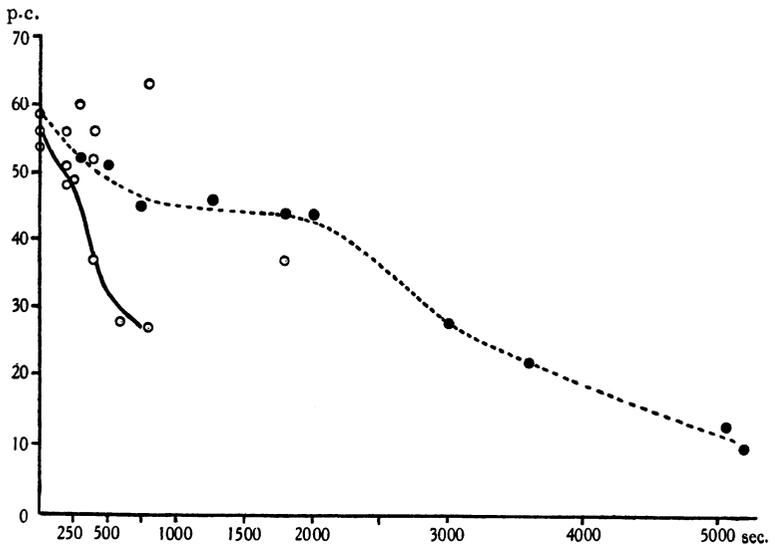


Fig. 6. Ordinates: percentage off-effect removed by preceding flash. Abscissae: interval between flash and off-effect. Circles, values obtained early in the experiment; filled circles, later values.

The interval between preceding flash and off-effect is shown on the abscissa, the removed percentage of the off-effect on the ordinate. In order to secure as many observations as are plotted in this figure one may have to keep the eye illuminated for a couple of hours. During this period the time constants of the retinal reaction gradually alter. The flash succeeds in influencing the off-effect at increasingly longer intervals. For this reason it was found advisable to divide the values into two groups: those obtained in the beginning of the experiment which in the figure are given as open circles, and later values that are given as filled circles.

Towards the end of the experiment the influence of the preceding flash on the off-effect is traceable for 5-6 sec.

The removed percentage of the off-effect has been calculated in the same way as above: the *b*-wave caused by the flash control has been subtracted from the combined curve, flash plus off-effect, and the off-effect remainder compared with the "off" control. The difference in height between "off" control and "off" remainder has been given in percentage of the former. We have also tried other ways of plotting and estimating the amount removed aiming at including the fact that at short intervals the off-effect is delayed. But the method adopted in Fig. 6 was found to present the results in the simplest and most straightforward manner.

Off-effect preceding b-wave

If the *b*-wave and a certain fraction of the off-effect arise in the same structure, not only should that part of the off-effect be removed by a preceding flash, but vice versa, *b*-waves elicited during or after the off-effect should be smaller than usual and gradually increase in size as the interval between off-effect and *b*-wave lengthens. Experiments directed towards this end have already been performed by Granit & Riddell [1934] and have given the expected result (see e.g. their Fig. 10, p. 15): the *b*-wave gradually increases in size as the interval between end of stimulation and re-illumination increases. The curves given by them were interpreted as illustrating the gradual recovery of the component P II, an interpretation which is still true in the light of our present knowledge. However, they did not realize that part of the positive P II belonged to the (non-inhibitable) off-effect, as the present results indicate, but referred the whole recovery to a recovery from the depression of positivity at the end of illumination. Probably both factors enter into play: the *b*-waves on top of the off-effect are small both because the off-effect has used or is utilizing some of the elements reacting with *b*-waves of the rapid type [Granit & Munsterhjelm, 1937] as well as because of a gradual recovery of all the *b*-elements from the after-effects of illumination.

DISCUSSION

We are aware of the need for some modification of the analysis of the frog's retinal action potential as given schematically by Granit & Riddell [1934] in order to include the results presented in this paper. However, we shall not attempt to introduce the necessary modifications now. The analysis is still good enough as a diagram illustrating in a general way the course of the three fundamental components of the

retinal response. Since it was published the multiple nature of the *b*-wave and the off-effect [Chaffee *et al.* 1923; Granit & Munsterhjelm, 1937] has been definitely established and we now obtain in this laboratory with localized electrodes slow and fast *b*-waves and off-effects in routine experiments with the electroretinogram. The important work of Hartline [1935] and the results presented in this paper have to be kept in mind when the details of the analysis at some later stage are filled in.

In this discussion we only want to draw attention to two aspects of our results. One is the connexion with the phenomena of "rebound" and inhibition in the central nervous system, especially interesting in view of the dual nature of the off-effect. As a first approximation one is bound to believe that the difference between the inhibitible and non-inhibitible off-components is quantitative rather than qualitative. Some structures, the most inhibitible ones, have in a higher degree than others a capacity for charging themselves with inhibitory P III when stimulated with light. Without being too definite with regard to the nature of the inhibitory block one can assume this blocking mechanism to be more or less highly developed or to act directly or indirectly upon the locus where the impulses are generated. The retinal results do not in the first instance support the view that the inhibitory mechanism could be identified with the subnormal phase and positive after-potential of peripheral nerve [see Gasser, e.g. in Erlanger & Gasser, 1937; Eccles, 1936]. Inhibition in the retina seems to be independent of excitation and is best developed when there is least reason to assume that impulses have been passing through the structures concerned.

Another point worth emphasizing here is the general usefulness of this procedure with one flash before and an identical flash just on top of the off-effect in the analysis of the properties of the off-effect, when a large number of variables such as wave-length, intensity, area, state of adaptation, etc. have to be studied. The method has already been successfully applied by one of us (P. O. T.) in an analysis of the influence of a number of biologically active substances on the electrical response of the eye. We have suggested that the inhibitible and non-inhibitible retinal off-effects have their equivalents in corresponding fibres of the optic nerve, studied by Hartline [1935]. If this be so—as we have every reason to believe—our method will become an easy and valuable substitute to the exceedingly laborious dissection of individual fibres in the nerve, whenever, after introduction of any of the above mentioned variables, the relative distribution of inhibitible and non-inhibitible elements in the off-effect has to be measured. The advantage of precision,

offered by the unitary analysis, is in such cases hardly compensated for by the labour involved, there being in addition some risk for chance factors like size and toughness of the fibres determining the end result.

SUMMARY

The retinal electrical responses of excised, opened frogs' eyes have been recorded with the aid of a string galvanometer and a directly coupled amplifier.

The retina has been kept illuminated by one light, every now and then interrupted to give an off-effect. A $\frac{1}{10}$ sec. flash from another lamp has been made to precede the interruption of the adapting light or to fall just on top of the off-effect.

When the flash precedes the off-effect the latter is reduced by a certain amount, dependent upon the relative strength of the two stimuli, indicating that some of the elements reacting at "off" also had responded to the flash and therefore were incapable of reacting with an off-effect immediately after the flash had been delivered.

The flash falling on top of the off-effect produces the well-known negative "notch", that coincides with inhibition in the optic nerve. The depth of this inhibitory notch is independent of whether a flash has preceded the off-effect or not. This is held to signify that the elements reacting to re-illumination with inhibition are independent and not identical with those that could be put out of function by a preceding flash.

The significance of the interval between flash and off-effect as well as certain complications arising when the flashes are very strong are illustrated by suitably designed experiments.

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