THE COMPONENTS OF THE RETINAL ACTION POTENTIAL IN MAMMALS AND THEIR RELATION TO THE DISCHARGE IN THE OPTIC NERVE.

BY RAGNAR GRANIT (Helsingfors).\(^1\)

(From the Physiology Laboratory, Oxford.)

Part I. Isolation of components in the retinal action potential of the dark-adapted decerebrate preparation.

Our knowledge of the retinal action currents, discovered by the Swedish physiologist Holmgren [1882] in 1865, has proceeded hand in hand with the development in electrophysiology in general. The history of this striking progress in electrical recording is briefly summarized in the literature relating to retinal action currents. Since Gotch [1903], working in this laboratory, with the aid of the sufficiently fast capillary electrometer, obtained the first curves embodying all the features of the process, and since v. Brücke and Garten [1907] and Piper [1911] in extensive series with the string galvanometer had shown the responses to light to be fundamentally alike for various vertebrate eyes, the main features of the retinal action currents have been common knowledge to all physiologists. Valve amplification was used at an early stage for the investigation of retinal action potentials by Chaffee, Bovie and Hampson [1923]. Unfortunately they used excised opened bulbs, although the method was particularly well suited for the study of intact animals, a feat attempted as early as 1876 by Dewar and McKendrick [Dewar, 1876]. With their slow Thomson galvanometer the latter authors even succeeded in obtaining responses from the human eye, but it remained for Hartline [1925] to prove by systematic comparisons with the string galvanometer that the deflections obtained from intact animals were identical with those given by the bulbs. Hartline also recorded some fairly good retinal action currents from the human eye.

The retinal action currents have generally been held to be composite effects. In view of the complex structure of the retina and the equally complex appearance of the potential change accompanying stimulation by light, interference phenomena between potentials differing in sign,

\(^1\) Fellow of the Rockefeller Foundation.
strength and time relations would certainly offer a reasonable explanation of the effect in terms of simpler components. Several such solutions have been propounded [see e.g. Kohlrausch's review, 1931], the best known being those of Einthoven and Jolly [1908] and of Piper [1911]. Evidently it is theoretically possible to resolve a complex curve in an infinite number of ways. And, though a many-sided experimental experience may make certain solutions more probable than others, yet a final decision can only be reached when the composite curve has been split into components by biological means. Such an attempt forms the subject of this paper.

The work has been based on the assumption that an organ like the retina where cells have become differentiated for specific purposes may show selective sensitivity or selective resistance to certain agents. It then becomes of paramount importance to find a preparation sufficiently stable and yet sufficiently sensitive to serve for the analysis. Frogs were tried but soon discarded in favour of the Sherrington decerebrate cat preparation [cf. Hartline, 1925]. This proved very satisfactory, provided that no operations were carried out around the bulb. In the best animals the first positive deflection, the b-wave, remained constant within 4-5 p.c. for several hours. The secondary rise varied more. Some thirty animals were used and the number of photographed responses approached 800.

**ANIMAL TECHNIQUE.**

Cats were decerebrated under deep anaesthesia by a "backward" section generally carried down to the base of the skull. A dilated pupil was thus obtained. In a few cases atropine had to be given to immobilize the iris. This did not seem to influence the action potentials. The modified decerebration technique, described by Bazzett and Penfield [1922] and used by Hartline [1925] in order to prevent interference with anastomotic connections at the basis cranii, was not found (in ten preparations) to possess any advantages over the ordinary clean section. The nictitating membrane was removed and the lids tied apart. The carotid of the side to be recorded from was temporarily occluded during the operation.

The preparation was placed in a shielded and earthed box, the inside of which was painted black. The head of the animal was fixed in a specially constructed clamp and adjusted with one eye towards the opening of a tube leading to the stimulus and entering the box. This was placed on a heated table of the type used in this laboratory: 2 to 3 hours were allowed to elapse before any records were taken. The box had then been
COMPONENTS OF RETINAL ACTION POTENTIAL. 209

closed for 1½ hours or more, except for the moment when the corneal electrode was applied. This was done about half an hour before the first records were taken. Faint light was used during this procedure.

Apparatus.

Contact with the cornea and the decerebration wound was made through cotton wicks leading to U-tubes filled with Ringer solution into which dipped silver-silverchloride wires. The leads were taken to the input of a directly coupled amplifier containing two valves coupled against one another in what is generally known as a "push-pull" arrangement. A small permanent magnet string galvanometer (Edelmann's small model) was placed in a bridge between the anode circuits and balanced to zero by potentiometers in the grid circuits. This coupling scheme, for which I am indebted to Dr H. K. Hartline of the Johnson Foundation, University of Pennsylvania, gives a circuit practically free from drift. A different type of directly coupled amplifier was used by Chaffee, Bovie and Hampson [1923].

The valves were 41 M.X.P. Cossor with indirectly heated filament, high mutual conductance, and low amplification factor. The actual amplification is only about five times. The use of valves was dictated, not so much by any necessity of amplifying the retinal currents, as by the advantage of having a potentially worked device such as the grid of a valve. This renders the resistance in animal and electrode negligible. Valves also make it possible to use the fairly insensitive permanent magnet string galvanometer. Further advantages are the cheapness of this galvanometer and the fact that the magnet and the case of the string remain at a practically constant temperature during the experiment. There is thus no need for continuous adjustment of string tension followed by calibration. As a matter of fact the calibration current was turned on only once or twice an hour, depending on the nature of the work. A disadvantage of amplification in this particular type of work is that not only action potentials derived from the retina but also those caused by movements in the eye muscles, lids, etc., are amplified. However, it can be seen from the records published below that good preparations are fairly stable.

The string was generally kept aperiodic or just periodic with a rising time not exceeding 20µ. The fastest b-waves recorded rose in about 50µ to their maximal value. The deflection to 1 mv. was rarely over 40 mm. through animal and amplifier. A platinum string 2 micra thick and with a resistance of 3100Ω was used throughout the work.

14—2
Since the permanent magnet string galvanometer has not been used very much as a measuring instrument on account of its low sensitivity, it may be useful to mention the disadvantages connected with this apparatus. The adjustments for string tension and focus are crude. The microscope and the magnet are not well balanced on the small upright to which they are attached. Hence the apparatus is very sensitive to mechanical disturbances. The string cannot be precisely centred in the magnetic field and the proportionality range is low. The latter fact is of little concern when the string can be kept slack. The range of proportionality was tested and found to be satisfactory within the limits of deflection used in this work, where not only was the string fairly slack but the optical magnification was considerable (approximately 600 times).

The stimulus was supplied by a Primus projection lamp (1000 c.p.) with reflector. The light beam was led through a system of lenses and screens and focussed to a narrow opening in a diaphragm. An adjacent minute slit let part of the light through a series of prisms to the camera. A Compur photographic shutter at the focussing point interrupted stimulus- and signal-beam simultaneously. The light reached the box through a system of concentric tubes of which the innermost possessed a ground glass disc with an area of 1661 sq. mm. on which an image was formed.

Though several intensities were used the typical effects are well illustrated by the highest intensity, 14 millilambert, and a 100 times lower intensity. The high intensity will be referred to in the text as I, other intensities as fractions of I. The intensity was varied by means of Wratten filters and was measured by a Lummer-Brodhun contrast photometer and a lamp standardized by the National Physical Laboratory. These were obtained when the apparatus had been used for nearly 5 months, so that the intensities given do not take into account the ageing of the lamp.

The cat's retina.

Some eyes were fixed in "Susa" and stained in Heidenhain's iron-haematoxylin. In accordance with older observations [cf. e.g. Chiewitz, 1889] the retina was found to contain very few cones. Also the convergence of receptors towards ganglion cells was found to be considerable, between 30-60 cells in the external nuclear layer per ganglion cell, in sections from various parts of the retina. In the human periphery the corresponding figures vary between 20-80, the latter value in the outermost parts of the retina [Chiewitz].
The normal action potential.

Some typical specimens are shown in Fig. 1. A, B and D are at the highest intensity, C is at I/100, E is at I/10. A is taken at a slower speed with the stimulus lasting 11 sec. and 5 sec. cut out from the film. The secondary rise is the largest noted in these experiments. A suitable terminology for designating the various phases of the retinal action potential was suggested by Einthoven and Jolly [1908]. Thus the initial fast negativity is called a, the first positive rise b, the slow secondary rise c. The off-effect (Gotch's term) was lettered a' by Einthoven and Jolly. Below, d is used [Day, 1915].

The three high-intensity curves show typical variations in the relative size of the waves. B, again, is typical of the large majority of the high-intensity responses. The a-wave is only present with high intensities and with large areas. The same holds for the off-effect or d-wave, which is not always present and is hardly ever more than a retardation in the falling part of the c-wave [cf. similar observations by Piper, 1905, 1911; Kohlrausch, 1918]. The very simple character of the response at low intensities should be noted; likewise the fact that occasionally, as in D, the base line may be re-attained for a moment after the b-wave at high intensities. The a-wave is not an abnormal phenomenon due to excessively high intensities of stimulation. It is a perfectly normal phenomenon and can, for instance, be seen in the human response curves published by Hartline [1925]. Though 14 ml. as a maximal intensity is not very high for the light-adapted human eye, it is a strong stimulus for a dark-adapted nocturnal animal with a wide-open pupil.

The effect of area.

This question will be treated in greater detail in Part II, where some measurements of the latent period of the action potential will be published. Here it is only necessary to emphasize the fact that a diminution in area affects the action potential just as a diminution in intensity [cf. Kohlrausch's review, 1931]. Thus the response for small areas of about 1–2° of visual angle differs from that for areas over about 5° in lacking secondary rise, the a-wave and the d-wave. They resemble the low-intensity curve C of Fig. 1.

Fröhlich [1928] and Fröhlich, Hirschberg and Monjé [1928] contend that the ordinary complicated responses depend upon the fact that the leads are not localized to the point of stimulation. Kohlrausch [1918, 1931] has convincingly shown that this hypothesis is untenable.
It may be added that the alleged proofs published by Fröhlich, Hirschberg and Monjé refer to experiments in which the area stimulated was not only kept just in front of the electrode but was also diminished in order to obtain strict localization. Inasmuch as the responses then became simplified, this result might just as well be ascribed to the diminution in area as to the fact that the lead was at the point of stimulation. In my experiments the corresponding lead was taken from the decerebration wound on the skull and yet the responses became simplified by merely diminishing the area. No localization of the electrode is therefore necessary. The results to be described below put Fröhlich's theory definitely out of court. But the manner of leading off naturally is of some importance, particularly with a large excised eye as used by Fröhlich in some cases (Cephalopod), where probably his explanation holds.

Fig. 1. A, intensity I; 5 sec. cut out. B, intensity I. C, intensity I/100. Standard area at 70 mm. in this and all following records except D and E, which are taken with the same area at 370 mm. at respectively I, and I/10. The potential developed in D and E is unusually low even for this retinal area, though normal in appearance. The deflection to ½ mv. is marked on one of each of the records of a series with the same animal, in this and in all illustrations following. Time is marked in all records in 0·5 sec. In addition time is marked by the shadow of the Rayleigh wheel giving 100 and 20° when visible in reproductions of original curves.
COMPONENTS OF RETINAL ACTION POTENTIAL. 213

Removal of c-wave.

The c-wave varies a great deal in height from animal to animal. It is extremely slow as is well shown by Fig. 1, curve A. It generally reaches its maximum in about 2 sec. or slightly less, and may last for several seconds after stimulation.

In order to remove it ether was chosen as a differentiating agent and was found to be very satisfactory. It was administered by way of inhalation. Fig. 2 (Plate I) shows three ether experiments in which the high-intensity stimulus was employed. In the upper pair of curves it can be seen that narcotization has removed the rising component of the c-wave in 14 min., and that the fast components are unaltered. The string remains at a practically steady low potential. In the next pair of records, of which the upper one again is the control, the picture after 14 min. of narcotization is similar, but the base line has shifted a little towards the end of stimulation. It is important to note that in this record the off-effect is enhanced by narcotization. This, in fact, is very often found. The third pair of records shows a case where 9 min. of anaesthesia has left the fast components unchanged, but greatly diminished the secondary rise. Whereas in some animals the rise may be completely removed by narcotization without any measurable effect on the fast initial components, there are certainly cases where part of the secondary rise is left at a stage of anaesthesia when a diminution of the b-wave is already noticeable.

It can be shown that the remaining part of the c-wave, in cases where something does remain, is not identical with the component that has been removed. The procedure is to analyse the effect of narcotization at two intensities. In Fig. 3 the upper pair of responses shows the control curve and the potential elicited by the stimulus I after 29 min. of narcotization. The lower pair shows the corresponding curves at 1/100. The ether effect on the responses is very different. Whereas the c-wave in the high-intensity record has diminished by 54 p.c., it is only diminished by about 8 p.c. at the low intensity. High-intensity records were taken before (the one published) and 2 min. after the low-intensity record, and showed that the lack of effect with the lower intensity did not depend upon insufficient narcotization or recovery from the anaesthesia. The explanation is clearly that the rising component, which is such a marked feature of the high-intensity response, is minimal at the 100 times lower intensity. Other experiments showed it to be absent or below the instrumental threshold at still lower intensities. The low-intensity curves were unchanged at a stage of anaesthesia showing high-intensity responses
affected as in Fig. 2. In this manner, then, it is possible to determine the time course and magnitude of the slow secondary rise, and, in fact, to isolate it by subtraction.

It is evident that the purely descriptive term, c-wave, has a double sense in terms of components. The c-wave is not homogeneous at high intensities. With certain animals nearly the whole of the secondary positive potential can be removed with ether at high intensities. It is then not identical with the corresponding component at low intensities which is unaffected. The component that is so easily removed by narcoti-

![Graphs A, B, C, D](image)

Fig. 3. Full description in text. Cross marks artefact following slightly increased off-effect.

zation will henceforth be termed the first process, P I. At times most of it disappears in 5 min. when there is still reflex activity left in the animal. Sometimes P I is more resistant.

**Final effects of narcotization.**

Further narcotization begins to affect the b-wave, which diminishes in height. When this happens the latent period, so far constant for a given intensity, begins to lengthen, and generally the rate of rise of the b-wave also becomes slower. This effect can be noted at all intensities. If the original intensity has been high the positive slow remainder after removal of P I often becomes replaced by a slow negative deflection. The final result is always that the whole response becomes negative, provided the intensity has been high enough to elicit a negative effect. A large number of such negative responses have been published by previous
COMPONENTS OF RETINAL ACTION POTENTIAL. 215

workers [see below and Kohlrausch, 1931]. The early investigators, whose technique involved elaborate dissection, saw practically nothing but the apparently very resistent negative responses with mammalian eyes [see e.g. Holmgren, 1880; Dewar and McKendrick, 1873]. To the work of Kühne and Steiner [1881] we owe the knowledge that the response to white light should be chiefly positive [cf. also Dewar, 1876].

The negative response is influenced by ether just as is the b-wave. During continued narcotization its latency begins to lengthen, the potential diminishes and the rate of fall becomes slower. These effects can often be noticed before the b-wave has disappeared. The most

![Fig. 5](image-url)

Fig. 5. Progressive effect of anaesthesia recorded at I/10. A is control before narcotization. B, after 21 min. of anaesthesia. C, after 31 min. of ether. Slack string, b-wave of A giving 0.417 mv.

marked alteration, however, found already at an early stage of anaesthesia, is that the return to the base line after cessation of the stimulus becomes sluggish. Fig. 4 (Plate I) shows part of a typical negative response of this type. All this makes it extremely difficult to intercept with the camera a negative wave at a moment when it can be proved to be uninfluenced by ether. An early negative wave was photographed in the experiment shown in Fig. 5. The intensity is I/10, the uppermost curve showing the normal response. The next curve shows a stage of anaesthesia where the positive remainder after removal of P I is already affected. The final negative wave in this case falls rapidly and also rises fairly rapidly at the cessation of the stimulus. It is larger than most of the negative waves seen in these experiments.
If the narcotic be removed at this stage of final negativity, and, if necessary, the animal be given some artificial respiration, complete recovery is generally possible. All the effects are thus reversible. Often the secondary rise is enhanced some time after recovery has taken place. Likewise when ether is first given there may be a stage of very short duration when P I is temporarily increased. Similar effects are also at times observed with the b-wave.

The final stage in progressive narcotization is complete disappearance of the response. This stage may mark an irreversible change. It has not been possible to revive the retina once it has ceased to respond.

By comparing ether effects at different intensities of stimulation it is again possible to separate into components the curve remaining after removal of P I. Thus, at low intensities, removal of the total positive remainder shows that this was practically the only component present, since no effect whatsoever or a minute negative response is all that is left at a stage of anaesthesia when there is a large negative deflection at high intensities. The positive remainder after removal of P I reacts uniformly and simultaneously to ether at all intensities of stimulation and will, therefore, be designated by the term P II. The negative wave will be termed P III. All three processes, P I, P II and P III, react in a similar manner to ether, though a certain amount of selective resistance to the narcotic makes it possible to remove them in three characteristic steps. The normal action potential to weak stimuli consists of an almost pure P II. This makes the cat a very suitable animal for an analysis of the kind attempted in this paper, for any effect on P II can always be checked by using a low-intensity stimulus.

**Removal of P II.**

The fact that it is difficult to intercept a negative wave at a stage of anaesthesia when its latency can be proved to be unaltered may depend upon deficient selectivity of the narcotic with regard to P II and P III. It is, therefore, necessary to try some other means of removing P II from a high-intensity curve in order to determine the shape and latency of the negative wave, particularly with reference to the initial brief negative deflection. It is also necessary to test the analysis obtained from the ether results by some other method.

A case in point is illustrated by Fig. 6 (Plate II). A is a normal response to I. Then the carotid is occluded for 2 min. and B is obtained. The a-wave is slightly accentuated, the b-wave has become very small. It is now both followed and preceded by a negative deflection. P I is enhanced by the
COMPONENTS OF RETINAL ACTION POTENTIAL. 217

asphyxia. The off-effect is definitely larger having been only a retardation in the post-stimulatory fall in the previous normal response. The animal has moved just before the light goes off. Troublesome movements generally occur when the carotid is occluded. The off-effect represents, however, a real increase, as has been confirmed in several similar cases, and may be seen in curve $H$ in the same figure. $C$ shows the initial part of the response to the same stimulus 2 min. later. Then the carotid is released, and 14 min. later a new record $D$ is taken showing full recovery and persistence of the enhanced P I. The carotid is finally occluded once more, and a record $E$ is taken 5 min. afterwards. Apparently then some collateral compensation has taken place, since the record is normal in form but shows diminished deflections. $F$ shows a normal response to the same intensity in another experiment. Then the animal was curarized and $G$ was taken showing one further stage in the process begun above by arterial occlusion. Curare may affect the $b$-wave to some extent—I have seen it do so—but the curve may still be of the normal type [Kohlrauschn, 1918]; there was, however, a serious leakage in the tube leading to the pump of the respiration apparatus, and this must be the explanation of the almost complete removal of the $b$-wave. The $b$-wave is only a retardation in the negative deflection initiated by the $a$-wave, and the initial negativity $a$ runs almost directly on into the large negative P III. The latency is constant, about 20$\alpha$ in both records.

Curare was used to induce asphyxia without troublesome movements in the animal. But it was deemed unnecessary to continue these experiments since the question appears to have been settled by Kohlrauschn [1918], who used a curarized rabbit (rod-eye). The results obtained by arterial occlusion may just as well be brought about by stopping the respiration in a curarized rabbit. Kohlrauschn accidentally noted a response of the type shown in $B$, $G$ and $H$, and then found that the respiration apparatus did not function properly. Adjustment of the artificial breathing brought the response back to normal again. His deficient curve shows no $b$-wave, an enhanced secondary rise and an increased off-effect. It looks, in fact, precisely like curve $H$ in Fig. 6 (Plate II)$^1$.

The deficient response $H$ was the result of an unsuccessful operation. Probably the carotid was occluded too long, since this in some animals leads to irreversible disappearance of $b$. In this case the animal gave the same type of response for some time. In other similar cases there either appeared later a small $b$-wave or the response diminished in amplitude

$^1$ In later experiments, some of which are mentioned in Part II, asphyxia after occlusion of the carotid sometimes completely removed P II.
fairly rapidly. When in such cases ether was given, removal of the secondary rise left a pure negative remainder. In order to find out by some other means whether the second phase of P II, which in high-intensity curves is covered by P I, is lacking in responses of this type, the 100 times lower intensity was used. Curve I gives the response to this stimulus, adjusted to the same light signal as H. Of the considerable negative practically nothing is left at this intensity, and of the large secondary rise there is but a fraction. This wave has a very long latency just as P I. The fraction left also corresponds to the amount of P I that the ether analysis (cf. Fig. 3) showed may be present at this intensity. P II at this intensity is much larger and quite different as clearly shown by Fig. 1, curve C. The negative P III also behaves with respect to intensity as was to be predicted by the results obtained with ether. Evidently then P II in addition to the b-wave contains a second positive phase, which is practically pure at low intensities. At these intensities P I and P III are small or absent. The response H is a combination of P I and P III.

Components in relation to stimulus.

By using ether it was possible to obtain P II alone at low intensities, P III alone at high intensities, and also to produce the response P II + P III. By interfering with the oxygen supply it was possible to produce the response P I + P III. It has not been possible to obtain P I alone. Thus it follows that P II and P III are very directly related to the stimulus. It does not seem probable that the one elicits the other. As to P I, it may, of course, merely be a matter of finding the right procedure in order to obtain it alone, but so far it seems as if P I required P III. It does not require P II to judge from the large P I in the response P I + P III.

P III in relation to a- and d-waves.

The evidence so far obtained allows certain conclusions to be drawn regarding the place of the a-wave and the off-effect in the analysis of the composite effect. The ether experiments (cf. Figs. 2, 3) often show an enhanced off-effect after removal of P I. Thus P I is not necessary for the off-effect to appear. On the other hand, removal of P II at high intensities regularly increases the off-effect as confirmed by Kohlrausch's similar findings. Therefore P II cannot cause the d-wave. This also follows from the low-intensity curves which contain a large P II and practically nothing else. They never show an off-effect. Thus the component necessary for the d-wave to appear is the negative P III and, in addition, either P I or P II to serve as a background against which the return to zero of P III
at the end of stimulation can set itself off. For the alternative explanation that P III actually rises above the base line at the end of stimulation there is no evidence. The purer the negative waves the more definitely are they monophasic. Since P I and P II drop at the cessation of the stimulus, it follows that the absence of either counteracting fall must make the off-effect caused by the return to zero of P III more marked. P II drops faster than P I. Therefore, the removal of P II must cause particularly large off-effects which actually is the case. Kohlrausch [1918], using Piper's analysis, gives the same explanation of the large off-effects after removal of P II by interfering with the artificial respiration. The experiments reported above settle the fact that the off-effect depends upon the behaviour of P III.

The negative waves obtained after ether in general possessed too long latencies to have started before the positive P II, though, on the other hand, the effect of the narcotic upon the latency made it probable that, if a negative could have been obtained at a sufficiently early stage of anaesthesia, it might well have started before the positive wave. The experiments on removal of P II by interference with the blood supply gave the additional information wanted. A series of records was obtained showing the initial a-wave gradually running on into the large negative P III. This time the latency was not altered, but remained at about 20\(\sigma\), the value of the latent period of the a-wave. Not only, therefore, is it unnecessary, but it is also unreasonable to assume a separate twitch-like initial a-wave when all the evidence shows it to be closely related to the large negative P III. Thus it is influenced by area and intensity just as P III, and is lacking in the pure P II responses.

Previous analyses will be mentioned below, but in this connection some of the facts relating to negative waves deserve to be pointed out separately. Thus Piper [1911], confirmed by Kohlrausch [1918], found that vertebrates (fishes, amphibians, reptiles) which had large a-waves also had good off-effects, whereas those (certain mammals as rabbits, cats, dogs) which gave small and inconstant a-waves also had small or inconstant off-effects. These two, the a- and the d-wave, must therefore be ascribed to the same process, which above was shown to be P III. The same conclusion was reached by Einthoven and Jolly [1908]. Waller [1909], confirmed by Jolly [1909], obtained fast monophasic negative waves by giving frog's eyes massage. Waller found these negatives to possess a shorter latency than the positive deflections. The return to zero was also fast. These facts he incorporated in his analysis [cf. also Einthoven and Jolly, Piper, Kohlrausch].
Nikiforowsky [1912] obtained negative waves by cooling frog’s eyes. He, as later Tirala [1917], found that the d-wave cannot be due to the same process that causes the b-wave, above demonstrated to be P II.

The analysis of the composite effect.

The analysis of the retinal action potential in the dark-adapted cat’s eye may now be given with a fair degree of confidence. There are three processes numbered in the order of their disappearance during progressive anaesthesia. Their relative contributions to the composite effect varies with area and intensity as set forth above. Since an increase in area affects the response just as an increase in intensity, it is only necessary to solve for two intensities. Besides, the effect of area, owing to effects of shunting by the tissues, cannot be as reliable an index of what takes place as the effect of intensity. The low-intensity response is simpler and the components are therefore only given together with the composite curve in Fig. 8. The high-intensity components are also given separately in Fig. 7.

Since the high-intensity second process, P II, cannot be obtained separately at this intensity, it is evident that the rise which sometimes is observed after removal of P I may be solved from a rise in P III or a rise in P II itself. Isolated third processes have sometimes in the course of this work been found to rise slightly during continued illumination. If this rise in some animals were large enough it could account for the rise observed after removal of P I. That would necessitate a P II of the type a in Fig. 7. The same type of P II would be obtained if for some reason in certain animals P I were unusually resistent, though the evidence with low-intensity second processes does not support this view. The same experimental response, P II + P III, after removal of P I could also be obtained if P II itself rose slightly as shown by curve b in Fig. 7. The latter alternative finds some support in evidence to be presented in Part II.
COMPONENTS OF RETINAL ACTION POTENTIAL. 221

There the components will be evaluated in terms of processes in the optic nerve. The composite curve of Fig. 8 is constructed on the basis of a P II of the type b. The figure summarizes the results of the analysis carried out above and needs no further comment. It may, of course, be constructed to give a larger off-effect after removal of P I.

![Composite curve](image)

**Fig. 8.** Analysis of composite retinal action potential at two intensities, 14 ml, and 0.14 ml, and area of 1661 sq. mm. viewed at a distance of 70 mm. Components: broken lines. Composite curve drawn in full. The a-wave is broadened slightly out of scale to show its derivation more clearly.

**Previous analyses.**

In view of the fact that the retinal action potential for various vertebrate eyes contains the same phases and that histological evidence shows all such retina to be built on a similar plan, the analysis must be general in principle. It therefore becomes particularly interesting to compare it with the two most important analyses, i.e. those by Einthoven and Jolly [1908] and by Piper [1911]. Behind these two attempts there is not only a many-sided experimental experience [Piper], but also systematic analysing of response curves obtained under various experimental conditions [Einthoven and Jolly], [Kohlrausch, 1918]. The slow instruments used by the early workers in this field, often in connection with severe operations, made delicate work impossible. A notable exception is the very interesting contribution by Kühne and Steiner [1881].
Einthoven and Jolly recognized the three processes, or "substances" as they call them. P I is quite correct, P II lacks the slow second phase showing that they did not realize that the c-wave is not homogeneous. P III gives the negative as a twitch below the base line and the off-effect as a positive deflection. The intervening negative phase is lacking. It is important to note that they found that the a-wave and the d-wave had to be due to the same component. Piper improved upon this analysis by introducing the intervening negative phase of P III, and by not allowing it to end in a positive process. In fact, his negative wave is strictly monophasic and the off-effect as well as the a-wave are solved by precisely the same interference construction as above in Fig. 8. This negative wave had in the meantime been found by Waller [1909], who corrected Einthoven and Jolly's solution in this respect. He resolved d and what he believed to be a, as did Piper later, but made the mistake of using only two components, a positive and a negative. He also believed the fast a-wave to be abnormal. Piper further realized that the c-wave is not homogeneous, but contains P I and the second phase of P II. P I is identical with the corresponding "substance" of Einthoven and Jolly. P II differs from the analysis given above in that it rises rapidly to a maximum which then is retained during continued stimulation. It is a low-intensity P II added to high-intensity first and third processes. This P II was based on his experiences with the Cephalopod eye [1911] which, of course, is analogous only, not homologous with the vertebrate retina. It is possible that in some animals the fall of the b-wave is mainly due to P III rather than to a decline in P II. In the cat at high intensities this certainly is not the case. Evidence to be presented in Part II makes it somewhat doubtful whether it can ever occur at such intensities. Still, Piper's analysis is essentially correct, though largely hypothetical, and the general interest taken in Einthoven and Jolly's and Piper's combined efforts to solve the retinal action potential has not only been fully justified by the work presented in this paper, but also by Kohlrausch's experiments with various colours. He there found that the short wave-lengths produced very small second processes followed and preceded by negative deflections, resembling, in fact, curve B of Fig. 6. The long wave-lengths, on the other hand, had a greater effect upon P II. Both lights together gave summed curves, which not only corresponded well with the theoretical composite curves but could also be explained on Piper's solution. Most important was the finding that partly negative deflections can be obtained in, for instance, a cone-eye like the pigeon's by stimulating with short wave-lengths. This rules out the supposition that a
COMPONENTS OF RETINAL ACTION POTENTIAL.

retina with negative deflections is pathologically changed. The perfect recovery from the negativity obtained above is also difficult to interpret on such a basis.

The discussion of the nature and localization of the components will be postponed till evidence relating to the optic nerve has been presented.

SUMMARY.

Leads from the cornea and decerebration wound have been taken to the input of a directly coupled amplifier with a string galvanometer in the output. The aim of the work has been to try to establish a biological analysis of the complex action potential of the retina. This has been done in two ways: by giving the animal ether and by interfering with the blood supply of the retina. Both agents were found to affect certain components selectively and in a reversible manner.

Narcotization removes in three characteristic steps definite components of the response to stimulation with white light. These components are indicated in Fig. 8 by Roman letters in the order of their disappearance and given separately for a high intensity in Fig. 7. Process I (P I) disappears rapidly during narcotization and the fast deflections are left unchanged. It is essentially a high-intensity component. Thus, at an early stage of anaesthesia, this component may be minute or even absent at high intensities, whereas the low-intensity response is almost or even completely unchanged. Therefore the slow phase of the composite effect is not homogeneous. The positive remainder after removal of P I reacts uniformly and simultaneously to ether at all intensities, diminishing gradually during continued anaesthesia. This component is termed P II. Finally only a negative, P III, is left provided the intensity has been high enough. The last stage is a gradual disappearance of P III. The ether analysis shows the response at low intensities to be a practically pure P II. Removal of P I need not affect it, and when the positive deflection is removed there is no negative left.

Asphyxia in the animal or occlusion of the carotid affects selectively P II. The selectivity may be demonstrated by testing with the practically pure P II at a low intensity. The high-intensity response contains P I and P III, and is a large negative deflection followed by a secondary positive rise.

Removal of P II in this manner shows the brief initial negative (a-wave) running on into the large negative P III of which it is therefore a part.

PH. LXXVII. 15
Removal of P I by ether often enhances the off-effect. Removal of P II by asphyxia regularly enhances the off-effect. The practically pure P II at low intensities never gives an off-effect. Therefore the off-effect depends primarily upon P III. Since, however, P III produces an off-effect only in the presence of either P I or P II it must be resolved by an interference construction from the rise of P III (cf. Fig. 8).

Part II. The latent period and the relation between the processes in retina and nerve.

Action currents from the optic nerve were first successfully recorded by Kühne and Steiner [1881], later by Ishihara [1906] and by Westerlund [1912]. The effect obtained resembles the retinal action potential, even the initial fast a-wave being present in the records of Westerlund. In none of the records published can a secondary rise (c-wave) be found. Fröhlich [1914] observed upon the retinal action current of the cephalopod eye oscillations which have been interpreted as caused by impulses in the optic nerve, but there are also other explanations to be considered [cf. Kohlrausch, 1931].

The actual impulses in the optic nerve were then recorded in an interesting work by Adrian and Matthews [1927 a, b, 1928], who used a capillary electrometer and an amplifier. They used the long optic nerve of the conger eel. Adrian and Matthews confirmed the general relation between intensity of stimulation and frequency of discharge, established by Adrian and his successive collaborators [cf. Adrian, 1928] for various sensory end organs and neurones. They also obtained the frequency-time curve of the retinal discharge. We now know that the frequency of the impulses discharged by the retina first rises rapidly at the onset of stimulation, then falls to a lower level during continued stimulation, and also that the off-effect of the retinal action potential has its counterpart in a renewed outburst of impulses at the cessation of illumination. Considering the slowness of the instruments used by the early workers it is possible that what they recorded was the integrated total frequency-time curve, obtained by Adrian and Matthews by plotting the impulses per unit time against time of stimulation. But it is also quite probable that the effect recorded was due to spread from the retinal currents. The latter view appears to be taken by Westerlund, and my own experiences with “integrative” recording controlled by
OSCILLOGRAPH RECORDS taken with large condensers in the amplifying circuit show that "integrative" records may be seriously distorted by retinal effects, at least when the leads are applied as will be described below.

Most important is the observation by Adrian and Matthews that the off-effect also is translated into impulses. This distinguishes the retinal discharge from that of other sensory end organs recorded by Adrian and his co-workers [Adrian, 1928]. Interesting work with the Limulus eye has recently been published by Hartline and Graham [1932], who succeeded in obtaining impulses from a single ommatidium. The ommatidium is a fairly complicated structure [Demoll, 1910; Versluys and Demoll, 1922-3], but is not connected with other ommatidia by way of internuncial neurones. However, its internal organization is complicated enough to make it appear questionable whether it can be assumed to be non-synaptic. The retinal action potential of several ommatidia looks like the isolated component P II of the cat's eye and appears to be related to the frequency of the discharge in the nerve [Hartline, 1932]. Further experimentation, no doubt, will show whether it is homogeneous or contains a hidden component of opposite sign and whether this eye gives an off-effect.

In this work the aim is to gather information as to how the components of the retinal action potential, isolated in Part I, are represented in the optic nerve. It has not been possible to accomplish this in a quantitative manner. The cat's optic nerve is rather unaccessible and easily damaged. In order to ensure satisfactory development of all three components of the action potential a great number of fibres must be activated which further complicates the task of recording. But the choice of preparation is fully justified by the fact that the retinal action potential of the decerebrate cat is easily split into components.

METHOD.

For retinal responses the technique has already been described in Part I. The "push pull" battery-coupled amplifier was used in most cases; in later work a new two-stage amplifier, also battery coupled, built on the principles set forth by Chaffee, Bovie and Hampson [1923], was used. With Mazda Pentodes 220, this system gives a base line free from drift and a total amplification of about 50. This is more than needed for work with eyes of decerebrate animals. The same amplifier and string galvanometer were used for obtaining records from the optic nerve with syringe needle electrodes [Adrian and Bronk, 1929], stuck into foramen opticum from the cranial side [Granit, 1932 a].
When impulses were recorded the animal in its well-insulated and shielded box was moved into another research room where a Matthews’ oscillograph with its amplifying system was set up for other purposes. A Cambridge string galvanometer could be worked alongside the oscillograph, and sometimes this string was also connected to the directly coupled amplifier described above. The stimulating and signalling system could not be shifted as easily as the preparation, and therefore a small lamp, run from an 8-volt accumulator and adjusted by means of lenses to illuminate a large part of the retina, was used in connection with the oscillograph. Records of the retinal action potential showed this illumination to be of the order of magnitude of the high intensities obtained with the other apparatus (cf. Part I). The electrodes were generally silver pins. The two leads were used in various positions relative to one another, but the best results were generally obtained when they were parallel and stuck in obliquely deep into the foramen opticum. The discharge recorded in this manner consists of regular or irregular oscillations dependent upon the degree of synchronization in the fibres concerned. Naturally this index of nervous activity is qualitative rather than quantitative, but some idea about the intensity of the effect can be gained by considering various aspects of the records. A test on artefacts was provided by the fact that the experiments ended with removal, sometimes accompanied by restoration, of the components of the retinal action potential.

The stimulating light was generally switched on by means of a key in its own circuit. This moment was recorded on the plate by a pointer attached to a magnetic short-circuiting device. But in some cases a photographic shutter was employed, and then the on and off of the stimulus were not recorded. In the former case the heating and cooling time of the filament entered into the latency of the on- and off-effects. This, of course, was not the case when the accurate device used with the apparatus described in Part I was used. However, when oscillograph and string galvanometer were worked together an absolute value for the latent periods was not needed, the purpose of this combination being to compare retinal and nerve responses relative to one another. Altogether some fifteen animals were used.

Retinal processes in relation to nerve discharge.

Adrian and Matthews [1927 a] also made a first attempt to study the mutual relation between retinal and nerve response. They were, however, seriously hampered by the fact that their condenser-coupled amplifier could only reproduce fast processes correctly; hence, as they
COMPONENTS OF RETINAL ACTION POTENTIAL. 227

point out, the comparison between retinal and nerve response had to be restricted to the initial and final phases of the two processes. They found that the impulses started during the a-wave and that the retinal nerve interval was roughly constant. The latter interval was measured from the beginning of the a-wave. They concluded that the initial negative deflection is closely associated with the initial outburst of impulses. That the off-effect in the retinal action potential was found to be correlated with an outburst of impulses has already been mentioned.

In view of the fact that the retinal action potential, as shown in Part I of this paper, is initiated by two processes of opposite sign, their evidence with regard to the a-wave hardly allows any conclusions as to which component is associated with the discharge in the nerve. Either the positive component P II, responsible for the b-wave, or the negative P III, responsible for the a-wave, or both, may be concerned with the initial outburst of impulses. P II may be present at the onset of P III, or P II, if alone assumed to set up impulses, might also start at a constant interval from the beginning of the a-wave. It is evident that no records of the complex retinal action potential give any information on this point. The latent period of the b-wave merely shows where the rise in P II has cancelled the fall in P III. This moment is probably of some significance in an interpretation of the retinal action potential, but it cannot throw any light upon the relation between the retinal processes and the discharge in the nerve. There was therefore no object in trying to determine the latency of the initial outburst of impulses relative to the beginning of the a- and b-waves, the less so as in the cat the a-wave is small and present only at high intensities. In so far as the onset of the discharge could be measured from records involving a certain amount of synchronization of impulses, it was found to be almost coincident with the onset of the b-wave, or to follow not later than 10\sigma after the first manifestation of a positive deflection, provided that the retinal potential was measured with the string connected to the amplifier.

Not only does indirect evidence support the view that the discharge in the nerve is associated with a positive component, but it also renders it difficult to assign a similar function to the negative wave. One difficulty deserves to be pointed out. The analysis of the retinal action potential (cf. Part I) indicates that the positive off-effect is a release of the positive components from the negative wave, following the return of the latter to zero. Now the retinal off-effect is definitely connected with a discharge of impulses through the nerve [Adrian and Matthews]. If now the a-wave, representing the beginning of the negative wave, is
assumed to set up a discharge, it does not seem possible to account for the fact that thereby the negative component is assumed to increase the frequency of the discharge both when P III increases (a-wave) and decreases (off-effect).

The only way in which at present these questions can be approached appears to lie in careful consideration of the various phases of the retinal action potential and how they may be brought to conform with the picture obtained from the nerve. The latter should at least give information on two points: (i) Is there a secondary increase in the discharge corresponding to the large secondary increase in the retinal action potential due to P I (c-wave)? (ii) How does removal of the positive components of the retinal action potential affect the discharge through the nerve?

Fig 9 (Plate III) shows what happens when a purely negative response is produced by interfering with the other waves by the methods described in Part I. Unfortunately, in this case a very small retinal response was obtained with a slack string without an amplifier, but the experiment has the advantage of illustrating both the effect of ether and asphyxia, and also recovery from negativity, in the same preparation. The condensers in the amplifying circuit of the oscillograph were 0·02 microfarad. A shows the retinal response at the full intensity; the secondary rise is small. C shows the corresponding oscillatory discharge in the nerve. The tendency of these oscillations is to diminish in amplitude during continued stimulation; the frequency may diminish but need not necessarily do so. Values between 100 and 150 oscillations per second are commonly found, the lowest regular frequency noted has been 80. Evidently the discharge consists of synchronized impulses [cf. Adrian and Matthews, 1928; Adrian, 1932]. The frequencies are higher than those noted by Adrian and Matthews in the eel’s eye with large areas. If the oscillations noted by Fröhlich are to be similarly interpreted, it is to be observed that the effect of a diminution in intensity primarily affects the amplitude of the oscillations, whereas the frequency decreases but little if at all. Thus B is the retinal response at a 100 times lower intensity and D the corresponding nerve response, illustrating the diminution in amplitude. Fröhlich found the frequency of the oscillations to increase and decrease with intensity of stimulation. The off-effect in the nerve records is small in this particular experiment, but again the increase in amplitude of the oscillations is noticeable. Thus with large areas in the dark-adapted cat’s eye the amplitude of the oscillations is the most definite index of a change in intensity of the retinal effect,
though by no means as good as direct counting of the frequency of the individual impulses where this can be done. It should be noted that there are no indications of a slow secondary rise in the amplitude at the high intensity though the retinal response \( A \) definitely rises during continued stimulation.

Then the animal is given ether heavily, and in 5 min. the retina has lost its positive components. \( E \) shows the slow negative deflection in the string. \( F \) is the nerve record taken immediately afterwards. No oscillations are visible. The anaesthesia is interrupted and 8 min. later the retina gives the response \( G \). The oscillations have returned as shown by \( H \). Then the carotid is occluded and the negative response \( I \) is produced in less than 1 min. This is larger than the positive response at the low intensity \( (B) \). Yet, again no oscillations are visible, though the nerve record, \( J \), is taken but a few seconds after the retinal response on the same plate.

Both methods of removing the positive components thus lead to the same result: the impulses disappear. Agents that block the passage of impulses also block the positive components of the retinal action potential. These two processes in retina and nerve respectively are thus closely interrelated. Whether this means that the impulses actually are set up by a positive component or only that the positive components and the oscillations in the nerve are two aspects of the excitatory process as expressed by physiologically and histologically different structures, is at present a secondary question, to be solved, if possible, with different methods.

It is further evident that of the two positive components \( P \, II \) undoubtedly is concerned with the discharge through the nerve. The impulses appear long before there is any sign of the slow secondary rise \( (c\text{-wave}) \) of \( P \, I \), and, even though the latter is small in this experiment at the high intensity, there is nothing at all to indicate an equivalent rise in the amplitude of the discharge. The off-effect in the cat's retinal action potential is generally only a retardation in the drop of potential following cessation of stimulation, yet this is nearly always accompanied by a corresponding increase in the amplitude of the oscillations. In Part I it was pointed out that the off-effect on the response \( P \, II + P \, III \), from which \( P \, I \) had been removed, at times was found larger than in the complex response owing to the fact that in the latter it was compensated for by the fall in \( P \, I \) at cessation of stimulation.

The isolated negative response cannot be shown to set up impulses. This may be accounted for by a block caused by ether or asphyxia, an
explanation difficult to exclude, but not altogether satisfactory. Observations mentioned in Part I showed that an alteration in the amount of potential developed by any component was preceded by a change in latent period. It was further found that a short asphyxia, as in this experiment, did not change the latent period of the negative component. Thus, by this method P III is obtained in as nearly normal form as possible, as indicated by the relatively large negative deflection I (Fig. 9). Considering that the impulses disappear and reappear with P II it is difficult to assume that a negative wave, which hardly is influenced at all by the same amount of asphyxia (1 min. occlusion), should be responsible for the discharge in the optic nerve.

Fortunately the analysis of the retinal action potential demonstrates the nature of the process behind the negative wave, provided that we know the function of P II. This, as we have seen, is to set up impulses or to be a link in the chain of events leading up to the discharge through the nerve. The analysis gives the further information that the off-effect is a release phenomenon, caused by the return to zero of the negative wave. The evidence may be found in Part I. This would be merely an algebraical fact—and is probably so with respect to the response P I + P III (see below)—if we did not know that P II is concerned with the discharge and that the off-effect also is accompanied by impulses. Knowledge of all these facts leads to the conclusion that, when P II is released from the negative P III, it is actually being released from something which not only did not produce impulses, but actively inhibited them. P III can act in this manner only if in some way it is connected with an inhibitory process. The off-effect must then be a true "post-inhibitory rebound," the term signifying a discharge following as a release from an inhibition. Further evidence bearing on these conclusions will be presented in a subsequent paper by Dr R. S. Creed and the author.

In order to stress the significance of possessing the correct analysis of the retinal action potential and what is chiefly an application of this knowledge to the present problem, the following facts might be pointed out: the b-wave and the off-effect are both positive in the retinal action potential, and both are concerned with the discharge through the nerve. In addition the off-effect has also been shown to depend upon the negative wave (cf. Part I). All these facts are taken account of by the above deductions which also are in accordance with the fact that the retinal action potential arises in the synapses (see below).

As to the slow secondary rise given by P I it has already been
COMPONENTS OF RETINAL ACTION POTENTIAL. 231

mentioned that previous work with the optic nerve has failed to give any indication of a corresponding increase in the frequency of the impulses in the optic nerve. Likewise in this work it has been noted that, whereas a small positive off-effect, visible only as a retardation in the drop of potential at cessation of stimulation, has been accompanied by a definite increase in amplitude of the oscillations, nothing of the kind has been observed with c-waves of considerable magnitude. An especially large secondary rise was found in the experiment illustrated in Fig. 10 (Plate IV). A and B show the retinal action potential at two speeds of the plate. C shows the response obtained from the nerve. This was taken with large condensers in the amplifying circuit, combined with a grid leak such as to cause a constant potential applied to the input of the amplifier to drop in the output to half its full value in half a second.

The question arises whether the initial deflection in the nerve and the large off-deflection are artefacts from the retina, or whether the discharge is partly monophasic, in which case the height of the deflection would roughly indicate the frequency of impulses out of phase. Both slow changes ultimately develop into the typical synchronized discharge. Independently of whether the amplitude of the synchronized oscillations alone or these together with the slow rise are interpreted as indicative of the intensity of the effect in the nerve, it is evident that the large secondary rise in the retina has no equivalent effect in the nerve. In this respect it clearly differs from the positive effects in the retina at on and off. Whichever index is used, the off-effect, though only a retardation in the drop of potential at cessation of stimulation, is followed by a marked increase in the intensity of the effect in the nerve. The small secondary rise, to be seen in this nerve record, could not be obtained in the other experiments of the same series. Yet, in one the illumination was allowed to act for over 2 sec. Similarly after removal of P I with ether no definite change can be found, though for such experiments the method is hardly sensitive enough. In some records there has been a small early secondary rise in amplitude which, however, has persisted after removal of P I [cf. Hartline and Graham, 1932]. This rise may be given by P II (cf. Fig. 8, Part I).

The latent period.

A theoretically very important experiment on the latent period was made by Adrian and Matthews [1928]. These authors confirmed an observation, previously made by Ishihara [1906], that an increase in area shortens the latent period just as does an increase in intensity. Actually the latent period of the optic nerve response was measured, but
since the retinal nerve interval was found roughly constant, the process responsible for the shortening of the latent period could be ascribed to the period preceding the retinal response. This latter latency was measured directly by Ishihara. But Adrian and Matthews also added an interesting analysis of this fact by proving that the spatial effect on the latent period could be obtained when areas some distance apart on the retina were stimulated. The latency of the nerve discharge for four lights acting simultaneously was found to be shorter than that for each spot alone. In addition they proved that stimuli so far apart as not to interact could be made to do so by application of strychnine to the retina. This not only excludes any explanation based on scatter of light but also shows that the lateral connections between cells in the retina react to strychnine just as similar connections in the central nervous system. In an eye lacking the internuclear neurones of the vertebrate retina the spatial effect should be absent. Adrian and Matthews' analysis of the spatial effect may therefore be held to have been significantly verified by the observation by Graham—kindly communicated to the author from work in course of publication—that in the eye of Limulus, which lacks internuclear neurones, the influence of area on the latent period is absent, though the intensity effect is present.

The spatial effect is of such fundamental importance because it localizes the process responsible for the action potential to a point in the retina which is synaptic or post-synaptic. The first lateral connections are lying around the synapses between receptors and bipolar cells. Thus, since the synaptic effect is to precede the response, the action potential cannot be localized to the rods and cones themselves, but must be produced at the point where the receptors are joined to the bipolar cells or later. There cannot therefore be more than one synapse between the retinal action potential and the impulses in the optic nerve.

This experiment by Adrian and Matthews has been confirmed with the flicker method and the human eye [Granit, 1930], but it was thought desirable to repeat it with the action potential of the cat's eye as well. Four circular discs, 6 mm. in diameter (about 56° of visual angle), were placed symmetrically as outer tangents to an imaginary circle 19 mm. in diameter (nearly 3°) at a distance of 370 mm. from the cat's eye. The intensity was 11.2 millilamberts. The average latency of response to the individual lights in nine determinations was found to be 67 ± 2.3σ mean variation. All four together gave 59 ± 2.0σ as an average of five determinations. The decrease in latency is thus 11.9 p.c. of the value obtained with the "singles." The latencies were measured with a magnifying glass
placed over a scale reading 0·1 mm., the speed of the film averaging 8–9 scale divisions for 10σ.

In order both to compare this effect with the corresponding variation caused by an increase in area and to find out how much could conceivably be accounted for by scatter of light, the area was varied in the same experiment between limits of 4·5 and 46 mm. in diameter, other conditions being equal. Three readings were taken for each area. The latent periods are plotted in Fig. 11 against log area. The relation is well

![Graph](https://via.placeholder.com/150)

**Fig. 11.** Ordinates: latent period of b-wave of retinal response (no a-wave visible). Abscissae: log area of stimulus. The readings are marked by lines corresponding to ordinates of 2σ. Explanation in text.

represented by a straight line, the one drawn being calculated from the data by the method of least squares. From this graph or from the equation could be read the decrease in latency corresponding to a quadrupling of the area stimulated, given by the four spots together over the area of each single stimulus. The effect evaluated as above is 15·4 p.c. as compared with 11·9 p.c. obtained with separated stimuli. Thus about 77 p.c. would have to be accounted for by scattering of light. This appears an improbable figure, even if the strychnine experiments were not there to disprove this hypothesis.

Intensity influences the latent period in a similar manner (Einthoven and Jolly, 1908; Ishihara, 1906; Adrian and Matthews, 1927 a;
Granit, 1932 b]. But within the range of intensities used in these experiments it has always been possible, by diminishing the area, to reach a value of the latency, which could not be compensated for by an increase in intensity. This means that the individual receptor-bipolar synapse at any intensity must have a longer latency than any recorded. The value obtained is largely dependent upon the amount of spatial summation which occurs, that is, upon the area stimulated. The negative initial wave, as pointed out above, also complicates the picture. It is therefore misleading to compare, as Hecht [1931] does, the results obtained by Adrian and Matthews [1927 b] on the latent period of the eel's eye with those obtained by himself with the visual end organs of certain invertebrates. Adrian and Matthews did so themselves, but in a later paper [1928], the third in their series, they gave clear proofs that a synaptic factor entered into the latent period of the vertebrate retina. This is not taken into account in Hecht's theoretical considerations, and it may be unnecessary to consider it in the receptor organs which he used.

The latent period in a synaptic structure has been shown to be the time necessary for building up an excitatory state to threshold value [Eccles and Sherrington, 1931], in our case, partly against adverse inhibition. But it is difficult to deduce any information from the latent period of the retinal action potential when it is not even known whether the whole of P II and of P III refers to opposite processes in the same neurones. The presence of an off-effect indicates that part at least of the inhibitory effects is influencing neurones in the act of building up excitation.

Summarizing conclusions.

In the following summary the term "component" (sc. of the retinal action potential) is often substituted for "process," as has been done throughout this paper. But it is important to realize that when two components, measured by the amount of potential produced, are equal, this does not necessarily imply, for instance, that the processes eliciting those potentials are of equal strength. Not only does the picture obtained with the galvanometer depend upon the manner of leading off from the preparation and upon the sensitivity of the instrument employed, but it is also at present impossible to determine whether, to take an example, the frequency of the impulses is proportional to the deflection of the galvanometer with a pure P II or whether it is some more complicated function of the recorded potential. The same holds for P III. A more suitable preparation than the decerebrate cat would appear to be needed for a
study of these questions. It should further be recalled that the traditional way of representing the retinal action potential reverses the sign of the potential with respect to the retinal layers.

The complex retinal potential arising in the retina on stimulation with white light has been found to develop in that part of the sense organ which histologically is a "true nervous centre" [Cajal]. It appears after a latent period involving synaptic interaction, and hence cannot have arisen distal to the locus where the first synapses occur. The complex effect is an algebraical sum of three components, the properties of which may be summarized as follows:

The first process, P I (see Figs. 7 and 8, Part I, pp. 220 and 221), rises slowly after a long latency and falls in a similar manner. It is positive in the usual representation of the retinal action potential. P I is easily removed by ether. Before it begins to diminish it may, however, pass through a temporary stage of enhancement. Likewise after removal of the narcotic it is often temporarily enhanced in good preparations. Slight asphyxia often favours P I. In the dark-adapted cat the first process is only present with large areas and high intensities of stimulation. Observations by Kohlrausch [1918] on the c-wave of the complex potential indicate that P I in nocturnal animals is more marked in the dark-adapted eye, in diurnal animals in the light-adapted eye. In order to account for these facts Kohlrausch suggests that this wave appears whenever an eye functions under conditions most appropriate for the particular retina in question [Kohlrausch, 1931]. The dependence of P I upon a large area and a high intensity also suggests that this component appears when the retina is especially active. This component is not at all or only slightly concerned with the discharge of impulses. But it might well represent some process of importance for the maintenance of a continued discharge. Kohlrausch's results appear to exclude pigment or rod and cone movements as possible sources of P I. Its reactions to ether and asphyxia indicate a process of central origin (retinal synapses, cell bodies), perhaps akin to the slow changes recorded by Adrian and Buynendijk [1931] from a central structure (cf. also Birsch-Hirschfeld [1900] for histological changes in the retina after illumination).

The second process, P II, rises rapidly as the positive b-wave of the complex response, then falls fairly rapidly at high intensities, less rapidly at low intensities, and continues hidden by the first process under the c-wave of the complex action potential. It is the only process that can be detected at all intensities capable of giving a detectable response
R. GRANIT.

and is of the same sign as the potentials recorded from non-vertebrate eyes. This component is associated with the production of impulses. P II is selectively affected by asphyxia and can also be removed with ether during prolonged narcotization. It thus reacts to ether and asphyxia as the negative potential recorded by Gasser and Graham [1932] in the spinal cord which they believe is connected with summation. The fall in the b-wave and the similar phenomenon observed by Adrian and Matthews in the frequency of the impulses probably represents, partly at least, a process of adaptation but is also dependent upon.

The third process, P III: this is of negative sign and therefore by algebraical summation influences the amount of potential in the complex response. P III first appears as the a-wave of the composite potential, its further course is hidden, but by its return to zero at cessation of stimulation the positive off-effect is elicited as a release phenomenon. The off-effect has its counterpart in a renewed discharge through the optic nerve. This is held to imply that when P II is released from the negative P III it is being released from a process in some way concerned with the inhibition of impulses. By definition the off-effect is then a "post-inhibitory rebound." P III is the most persistent of the components of the retinal action potential. A potential wave of opposite sign to the one Gasser and Graham [1932] believed to be concerned with summation was also noted by them in the spinal cord. They suggest that it might be inhibitory in character. The eye performs several functions in which an inhibitory process should be useful [cf. Graham and Granit, 1931], but there is little reason to discuss them as long as this process presents a number of problems, accessible to experimental approach, which should first be solved.

Vision and retinal processes.

The previous pages should have made it evident that our knowledge of retinal physiology still is at the stage when even quite elementary facts have to be established about the nature of the processes concerned. Little can therefore be gained by theorizing extensively about the significance of this work for the subject of vision. It is not even possible at present to express the retinal action potential in terms of frequency of impulses. A hypothesis has been offered by Kohlrausch [1931], but we are clearly far from the stage at which elaboration of theories may prove fruitful.

But it is worth while to make one generalization: this is the necessity of realizing that the retina as a sense organ cannot be identified with
the rod-cone receptor system. The synaptic apparatus continuously modifies the primary response determined by the properties of the receptors. Special methods have to be developed to prove in casu that a measured function is due exclusively to, say, the photochemical processes in the receptors. Thanks largely to the work of Sherrington and his collaborators [see e.g. Creed, Denny-Brown, Eccles, Liddell and Sherrington, 1932] the physiology of the synaptic reactions has now been developed to a point when real significance may be derived from the fact that such reactions are present in the retina. A case in point is the question of area stimulated. In Part I it was pointed out that the action potential obtained with a small area and a high intensity differs, not only in amount, but also with regard to form and time relations, from the potential obtained when the area is large. Thus the reaction behind the potential has been organically changed to a low-intensity process by a diminution in area stimulated. Probably the number of active units also enters into the total effect in a manner determined by the electrical conditions in the tissue, but more important is the fact that they do so in a purely physiological way, making up a characteristic total reaction by way of processes of interaction at the synapses.

Considering the complications present already at the "sub-sensational stage," studied above, more work with the retina rather than with sensations would appear to be necessary for the establishment of a retinal physiology on a sufficiently broad and unprejudiced basis. Quantitative correlations between sensory phenomena studied through the medium of sensations and certain assumptions as to the nature of the photochemical mechanism of the receptors elaborated, for instance, by Hecht, can hardly give more information about the processes concerned than purely empirical equations.

**Summary.**

Of the three components of the retinal action potential only one, P II, can be shown to be associated with the discharge of impulses through the optic nerve. P III appears to be related to an inhibitory process. P I does not appear to be concerned with the discharge of impulses, or, if so, to a very small degree. These statements are summarized in greater detail on pp. 223 and 234.

Prof. Sherrington has placed at my disposal the facilities of this laboratory; for this and for his kind active interest in the work I am very grateful.
The electrical recording system was tried out in some preliminary experiments in the Physiological Institute at Helsingfors, where Prof. Y. Renqvist kindly provided me with assistance. I also wish to acknowledge the valuable cooperation of Dr J. C. Eccles and Dr G. L. Brown in some preliminary experiments with the oscillograph.

I wish to tender my thanks to the Rockefeller Foundation for a generous grant towards apparatus and to the Cristopher Welch Trustees for a grant towards the expenses of photographic material.

REFERENCES.

Adrian, E. D. (1932). *J. Physiol. 75*, 26 P.
Granit, R. (1932 a). *J. Physiol. 76*, 1 P.
Fig. 2. Three deflections to full I during initial stage of light anaesthesia together with controls taken before narcotization. Full description in text. Artefact marked by cross is probably movement in animal's eye.

Fig. 4. Negative deflection to full I obtained after prolonged narcotization.
Fig. 6. Full description in text. Deflection in bottom record, to save space, adjusted to light signal of foregoing record. Arrow marks end of stimulation.
Fig. 9. String galvanometer records of retinal response and oscillograph records taken from the optic nerve. Time: tuning-fork, 100 per sec. Signal is seen causing escape at on and off of light, picked up by oscillograph. Explanation in text.
Fig. 10. A and B: string galvanometer records of retinal action potential. In A stimulus lasts about 1.3 sec., in B about 2.5 sec. C: oscillograph record from optic nerve, stimulus lasting about 1.3 sec.
COMPONENTS OF RETINAL ACTION POTENTIAL. 239