On page 387, Vol. 13, No. 3, January 20, 1930, the numbers in the last column of Table 1 should be divided by 10 to give the true values of $k'$.

In the next to the last line, under Table 1, for value of $C = 0.337$ read value of $C = -0.337$. 
THE DARK ADAPTATION OF THE EYE OF LIMULUS, AS
MANIFESTED BY ITS ELECTRIC RESPONSE TO
ILLUMINATION

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In a previous paper (Hartline, 1928) there has been reported the result of a study on the electric action potential of the arthropod eye, with respect to the dependence of its form and magnitude upon the stimulating light. It is well known that the electrical responses of photoreceptors to illumination also depend upon a number of other factors which are not concerned with the stimulus, but which exert their influence upon the condition of the photoreceptor tissue; such factors are temperature, adaptation, oxygen supply, etc. It is the purpose of this paper to deal with one of these factors, namely, the condition of adaptation of the eye, and in particular to follow the course of dark adaptation as manifested by the behavior of the action potential.

Material and Method

The electric response to a light-stimulus manifests itself by a change in the potential difference existing between the corneal surface and the back of the eye. The form of these electric responses, and the apparatus and methods used in obtaining and recording them have been fully described (loc. cit.).† With the introduction of the following

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† Briefly, the method is as follows: The animal is securely fixed, by means of pins and plaster of paris, in a light-proof moist-chamber. A water-immersion microscope objective focuses a very small image of the filament of an electric lamp upon the cornea of the animal’s eye. The droplet of immersion fluid is balanced isotonic saline (i.e. ordinary seawater in the present experiments), and furnishes contact for one electrode. The other electrode makes contact on the
modifications the method used is identical with that described in the paper cited.

1. The electrical measuring system has been improved by a vacuum tube circuit in connection with the string galvanometer. Vacuum tube circuits are now so generally used in physiological technique that details of apparatus need not be described, excepting to state that the grid bias was sufficient to render the system completely potential-operated, thus eliminating polarization effects at the electrodes and changes due to fluctuation in resistance of the preparation. The entire unit was calibrated carefully to insure linearity of deflection with impressed potential.

2. The single brief flash of stimulating light was obtained by a rotating-disk shutter. The apparatus consists of a disk from which is cut a sector of known angle, rotating in front of a slit placed in the path of the light on its way to the eye. The duration of each flash is determined by the angular aperture of the sector and the speed of revolution of the disk. The disk is driven by a motor whose speed is regulated to any desired value, within 1 per cent, by means of a stroboscopic method. An automatically operated electro-magnetic shutter serves to single out one flash of the succession set up by the rotating disk. The operation of this shutter is accomplished by means of a commutator, cut in the form of a coarse screw in a bakelite drum, and mounted on the shaft carrying the disk. A flexible steel wire, with a platinum point, engages the screw, and when released is carried over the metal contact embedded in the groove of the screw. Contact is made over only part of a revolution, and the magnetic shutter is thereby activated, the disk and commutator being properly adjusted so that only a single flash of light is allowed to pass. The tip of the steel wire is carried on to the end of the screw, and must be re-set before another exposure can be made. This device made it possible to obtain a range of exposures from 0.0005 to 0.4 second.

The material employed in this study was the compound eye of young *Limulus polyphemus*. The action potential of this photoreceptor has been described in the previous paper (*loc. cit.*), and was seen to possess a simplicity of form which other arthropod eyes, as well as vertebrate retinas, do not show. The response to illumination is a simple wave of potential, rising sharply to a maximum, and subsiding more slowly toward the original base-line. It shows no other element, regardless of whether the illumination is continued or is only a momentary flash. In addition to this simplicity of form, the *Limulus* eye,
in the intact animal preparation,* survives and gives consistent results over a period of many days; this is indispensible in the study of such a slow process as dark adaptation.

RESULTS

The recovery of sensitivity, in the dark, by a photoreceptor which has been subjected to prolonged illumination is familiar to all; it is termed dark adaptation, and the course of the process has been investigated quantitatively by many experimenters. The criteria which have been used in detecting this recovery of sensitivity are many and diverse; the electric response furnishes yet another.

Upon being returned to darkness after an exposure of several minutes to a bright light, the eye of Limulus shows only a feeble response in the first minute or two, but as time goes on its response to the same stimulus becomes greater and greater, until it finally reaches the maximum value it can have for that stimulus. The exact course of the process is shown in Fig. 1.

In this experiment the eye was exposed to the brightest available light (ca. 85,000 meter candles) for 10 minutes; this light was then turned off, and at successive intervals thereafter the eye was stimulated by a flash of the same light, 0.002 second in duration, and of constant intensity. The magnitude of the maximum of the ensuing potential wave was measured, and is plotted as ordinate against time as abscissa. It is seen that the response elicited by a stimulus of constant energy increases rapidly during the first few minutes in the dark, and then more slowly, approaching a limiting value asymptotically.

The precautions observed in making these experiments are not unusual; the temperature must be carefully controlled, the intensity and duration of the stimulating flash must be regulated properly. The initial illumination must be carefully controlled as to its brightness; its exact duration, however, is not so important, as the eye is very nearly completely light-adapted in 10 minutes. Preliminary experiment has shown that the stimulating flashes themselves have little effect upon the condition of adaptation of the eye, consequently

* The intact-animal preparation has been used throughout this study; excised eyes give strictly comparable though less consistent results, and last only a few hours.
Fig. 1. The course of dark adaptation in the eye of Limulus. Ordinates: Magnitude of maximum of potential wave ensuing from stimulation by light. Abscisae: time in darkness. Initial adapting illumination, ca. 85,000 meter candles; area of eye illuminated, ca. 0.1 mm.²; duration of initial illumination, 10 minutes; cessation of this initial illumination marks the instant \( t = 0 \). Stimulating flashes of same brightness as initial illumination; duration of stimulating flashes, 0.002 second.

Circles are experimental points; smooth curve is theoretical, given by equation

\[
\mu = \frac{1}{E_\infty - E} + C
\]

where \( E_\infty \) is mean of two experimental values, obtained after ca. 2 hours in darkness \( \mu \) and \( C \) are obtained graphically from a plot of experimental values of \( \frac{1}{E_\infty - E} \) against \( t \).

The experimental points are measurements of individual action potentials, obtained from the same preparation within a period of about 3 hours.

I have four additional experiments similar to this, from three other animals, and numerous other less extensive experiments which are all fitted, to within the limits of experimental error, by the same type of theoretical curve.
three or four points on the curve, particularly if they are widely spaced in time, may be determined after each period of initial illumination.

**Fig. 2.** Relation between intensity of stimulus and magnitude of response, at various times during dark adaptation. Ordinates: Magnitude of potential wave ensuing from stimulation by light. Abscissae: logarithm (base 10) of intensity of stimulating flashes. Duration of flashes = 0.002 second. Each curve obtained after a time \( t \) had elapsed, during which the eye was in darkness, subsequent to an initial illumination, as in Fig. 1. Values of \( t \) belonging to each curve given at the right. Intensity corresponding to \( \log I = 0.0 \), ca. 85,000 meter candles. Area illuminated ca. 0.1 mm.²

The experimental points are measurements of individual action potentials, obtained from the same preparation within a period of about 15 hours.

Intensity of stimulating light was controlled by the use of photometrically calibrated Wratten "Neutral Tint" filters, as described in the previous paper.

This is the only experiment of its type which I have performed.

Two or three such "runs" thus serve to determine satisfactorily the curve of the process. Repetition of such an experiment, particularly
if made within 10 or 12 hours, yields results identical to within 3 to 4 per cent.

Now, since the magnitude of response depends not only upon the condition of adaptation, but also upon the energy of the stimulating flash, it should be possible to compensate for the lowered sensitivity of the early minutes of dark-adaptation by increasing the strength of stimulus. It would then be possible to determine the intensity required to produce a constant response, at various times during dark adaptation. In order to do this, it is necessary to perform the experiment shown in Fig. 2. In this experiment the eye was stimulated, after a given, constant time of dark adaptation, by a flash whose energy was varied (the exposure was held constant at 0.002 second; intensity only was varied). In the figure is plotted the logarithm of the intensity of the stimulating flash as abscissa, against the magnitude of the response as ordinate. Each curve was obtained after a constant interval of dark adaptation had elapsed—the uppermost curve represents the most sensitive condition of the eye, after 2 to 4 hours in darkness; the lowermost curve was obtained after only 1 minute of dark adaptation. It is now possible to compare intensities of stimulus necessary to produce a given response after various times of dark adaptation; they are given by the abscissae of points on the various curves, whose ordinates represent the magnitude of the chosen response.

The first point of importance to be noted is that it will make no difference what value is chosen for the required response, the experiment shows that (within the limits of the method and material) all members of this family of curves have been obtained from a single curve by a shift along the axis of abscissae. It is with the amount of this shift—the same for all ordinates—that we are concerned.*

The reason for wishing to obtain the data in this form is two-fold. In the first place, it is an experimental fact, readily verified, that two records of action potential, obtained under different conditions of

* This would be even more evident had the uppermost curves in this particular experiment been carried further, using still lower intensities. The tailing off of the lower portion of the curve is always present; for the completely dark adapted eye it has been abundantly figured in the previous paper (loc. cit.).
stimulation and adaptation, but rising to the same maximum value, may be superimposed, and found to agree throughout their entire length, except for chance minor variations.* The data in their present form are consequently independent of a certain arbitrariness which was introduced in choosing the magnitude of response as a criterion of study. In the second place, this is precisely the form in which the data on the dark adaptation of the human eye have been obtained. In this case, the intensity of light which is just visible is determined at various times during dark adaptation; this threshold stimulation presumably represents a constant effect in the sense organ. Now the data on dark adaptation of the human eye have been analysed by Hecht (1920), and since these data on the Limulus eye are now in the same form, they may be subjected to the same analysis.

Hecht makes use of the idea that dark adaptation represents the regeneration of a photosensitive substance in the sense organ. According to his conception of the photoreceptor system a substance $S$ is broken down by light into certain products; these products then re-unite, independently of light, to reform $S$. The system may be represented schematically by the reversible system

$$ S \xrightarrow{\text{light}} P + A $$

$$ S \xrightarrow{\text{"dark"}} P + A $$

The initial exposure to a bright light decomposes most of $S$, with the consequent accumulation of the products of its decomposition, $P$ and $A$. When the light is turned off, the recombination of $P$ and $A$ is no longer opposed by photolysis, and $S$ is regenerated, with the consequence that the sensitivity, which depends on the concentration of $S$, increases from a low value in early dark adaptation to its original value, when $S$ is entirely regenerated.

This scheme has been developed and used by Hecht in an extensive series of papers; the application of it to the process of dark adaptation in several different animal forms is to be found in a recent paper

* I have investigated this only for the responses greater than 0.1 mv.—it is possible that the smaller responses—especially those obtained in the first few seconds of dark adaptation—deviate from this finding. It is furthermore necessary to compare only curves obtained at the same temperature.
(Hecht, 1927); the present treatment, however, is taken directly from
his earlier paper dealing with the processes in the human eye (loc. cit.).

To account for the data quantitatively, it is necessary to assume a
relation between the concentration of $S$ and the intensity of light
necessary to produce a given response. This assumption, which is
by no means entirely arbitrary, is that the concentration of $S$ is
given by a linear function of the logarithm of the intensity. Since the
condition of complete dark adaptation, when 100 per cent of $S$ is
present, requires the smallest intensity, the assumption becomes

$$[S] \propto -\log I$$

Choosing some one ordinate—say 0.5 mv.—the abscissa of the point
on the 2 hour curve represents 100 per cent of $S$; the amount of $S$ at
any time, $t$, during dark adaptation is then represented by the abscissa
of the point having this same ordinate, on the curve corresponding to
$t$. The difference between the two abscissae represents—and, since
the plot is logarithmic, is proportional to—the amount of $S$ which is
absent at that time, or, what is the same thing, the amount of products
de decomposition which are present. On the basis of Hecht's assump-
tion, then, the distance that a given curve has been shifted to the right
from the 2 hour curve may be taken proportional to the concentration
of products of photolysis which are combining to regenerate the photo-
sensitive substance. A plot of this distance against time represents
the course of the reaction, and would be expected to follow the known
laws governing chemical kinetics. This indeed appears to be the case,
and, as in the human eye and the photoreceptors of several lower
animals, the order of the reaction is two; that is, the rate of recom-
binination is proportional to the second power of the concentration of
the combining substances. It is for this reason that Hecht has
written two members on the right hand side of his schema (1).

The integrated form of the bimolecular isotherm is

$$kt = \frac{1}{x} + C$$

where $t$ is the time of dark adaptation; $x$ is the concentration of $P$
(and of $A$); $C$ a constant of integration, and $k$ is a constant of
proportionality.
In the case of the present data, $x$ is to be calculated from (2) according to the equation

$$x = m (\log I - \log I_\infty), \quad E = \text{const.},$$

where $I$ is the intensity required to produce the response $E$ at the time $t$, $I_\infty$ is the intensity required to produce the same response when $t = \infty$ (2 hours), and $m$ is a factor of proportionality which according

<table>
<thead>
<tr>
<th>(minutes)</th>
<th>$\log_{10} I$</th>
<th>$\log_{10} I - \log_{10} I_\infty$</th>
<th>$\frac{1}{\log_{10} I - \log_{10} I_\infty}$</th>
<th>$k'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\infty$ (2-4 hours)</td>
<td>3.290</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>32</td>
<td>3.625</td>
<td>0.335</td>
<td>2.98</td>
<td>0.83</td>
</tr>
<tr>
<td>16</td>
<td>3.870</td>
<td>0.580</td>
<td>1.72</td>
<td>0.87</td>
</tr>
<tr>
<td>8</td>
<td>2.362</td>
<td>1.072</td>
<td>0.93</td>
<td>0.74</td>
</tr>
<tr>
<td>4</td>
<td>2.778</td>
<td>1.488</td>
<td>0.67</td>
<td>0.85</td>
</tr>
<tr>
<td>2</td>
<td>1.280</td>
<td>1.990</td>
<td>0.50</td>
<td>0.82</td>
</tr>
<tr>
<td>1</td>
<td>1.670</td>
<td>2.380</td>
<td>0.42</td>
<td>0.83</td>
</tr>
</tbody>
</table>

First column: time in darkness (cf. Fig. 2). Second column: abscissae ($\log_{10} I$) of points on various curves of Fig. 2, having an ordinate = 0.5 mv. Third column: $\log I - \log I_\infty$ (cf. text). Fourth column: $\frac{1}{\log I - \log I_\infty}$. Fifth column: $k'$ (cf. text), calculated according to equation

$$k' t = \frac{1}{\log I - \log I_\infty} + C.$$

Value of $C = 0.337$ obtained by graphic extrapolation from a plot of $\frac{1}{\log I - \log I_\infty}$ against $t$.

It is evident that $k'$ is, within the limits of the method, a constant. An additional fact is worthy of notice. It is seen that the upper
parts of the curves of Fig. 2 are linear, and, as has been remarked, quite
closely parallel. It is consequently clear that, in making the analysis
of the data, one could just as well have taken the difference in the
ordinates of points having the same abscissa as the difference in
abscissae of points having the same ordinate. This follows from a
simple proposition of geometry, that the direction in which a straight
line cuts a family of parallel straight lines does not affect the ratio of
the segments. This procedure just mentioned merely changes the
scale, i.e., the units in which concentration is measured. Equation (4) becomes

\[ x' = m' (E_m - E), \quad I = \text{const.} \]

where \( m' \) is different from the \( m \) of (4).

In the light of this experimental fact the original assumption becomes
much simpler, viz., that the magnitude of electrical response, provided
it be sufficiently large, is a linear function of the concentration of \( S \).
Indeed, Hecht's assumption originally was based upon the fact that
the photochemical effect in photoreceptors is, in general, proportional
to \( \log I \)—which is an experimental fact also for the larger electric
responses. It may seem, then, that the treatment given has been
rather indirect; it must be clearly understood, however, that this
simpler alternative is subject to the limitation that small responses,
where the response is no longer linear with \( \log I \), must not be included,
whereas the first treatment given is general.

This experiment which measures the ordinates at the same abscissa
is much simpler to perform than the other; it is, indeed, the experiment
of Fig. 1. Here the difference between the value of the response after
prolonged dark adaptation, and that of the response at any time \( t \),
is proportional to the concentration of products of photolysis. The
curve itself represents the course of the dark adaptation reaction, the
points being experimental values of response, the smooth curve being
theoretical, calculated according to (5) and (3).

The constancy of \( k' \), in Table I, and the close fit of the curve to the
experimental points in Fig. 1, are results that would be predicted
according to Hecht's conception of the photosensory process, a con-
ception which was reached by fundamentally different methods from
that employed in this study. This close agreement may be taken as
indicating that the electrical responses of the eye to illumination are intimately related to the fundamental photoreceptor process, and, indeed, constitute a sensitive measure of the decomposition and regeneration of the photosensory substance.

SUMMARY

1. The phenomenon of dark adaptation of the eye of Limulus is reflected in the behavior of the action potentials obtained upon stimulation by light. The method of obtaining and recording these action potentials has been described in an earlier paper.

2. By determining the intensity of stimulus necessary to produce an electric response of a given magnitude (as to maximum action potential), at various times during dark adaptation, a quantitative analysis of the process may be made. This analysis is identical with that of Hecht for the dark adaptation of the human eye.

3. The results of this analysis indicate that the process of dark adaptation in the Limulus eye may be represented by a chemical reaction of the second order—the recombination of products of photolysis to renew the depleted supply of photosensitive material. This is in complete accord with Hecht’s conception of the photosensory process, and is in quantitative agreement with the results obtained by other methods, in several different animal forms.

4. The experimental relation between strength of stimulus and magnitude of electric response reduces the assumption originally made by Hecht to account for the data on the human eye to an equivalent form; that the magnitude of electric response, provided it be sufficiently large, is directly proportional to the concentration of the photosensitive material in the sense organ.

BIBLIOGRAPHY

